Are There any Toxic Effects of Food Additive Tricalcium Phosphate on Pregnant Rats and Their Fetuses?

Gıda Katkı Maddesi Olan Trikalsiyum Fosfatın Gebe Sıçanlar ve Fötusları Üzerinde Toksik Etkisi Var mıdır?

Research Article

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ABSTRACT

The aim of the study was to evaluate the maternal and fetal developmental toxicity of food additive Tricalcium phosphate (TCP) in Wistar rats. Two doses of TCP (E341) 175 mg/kg bw/day (TCP I group) and 350 mg/kg bw/day (TCP II group) were administered to rats during gestation days (GD) 0-20. The possible toxic effects of TCP exposure were investigated by biochemical, histopathological and morphological examinations. All dams were observed for maternal body weights, food and water consumptions and subjected to caesarean- section on gestation day GD20. According to maternal haematological analysis, lymphocyte, monocyte and neutrophil percentages were changed in TCP I and TCP II groups. Biochemical analysis showed that while enzyme activities of aspartate aminotransferase, alanine aminotransferase and levels of urea and creatinine did not change among groups; alkaline phosphatase activity increased in TCP I group. There were histopathological changes in maternal liver, kidney, heart, brain tissues and fetal liver, kidney and placenta tissues in treatment groups.

Key Words
TCP, histopathology, placenta, E341

ÖZET

Bu çalışmanın amacı bir gıda katkı maddesi olan Trikalsiyum fosfat (TCP)'ın Wistar sıçanlarda maternal ve fötal toksisitesini değerlendirmektir. 0-20. gebelik günlerinde (GD) TCP'nin (E341) iki dozu olan 175 mg/kg vücut ağırlığı/gün (TCP I grubu) ve 350 mg/kg vücut ağırlığı/gün (TCP II grubu). TCP maruziyetinin olası toksik etkileri biyokimyasal, histopatolojik ve morfolojik analizler ile araştırılmıştır. Bütün gebe sıçanlar maternal vücut ağırlığı, yem ve su tüketimi açıdan incelenmiş ve GD 20'de sezeryan yapılmıştır. Maternal hemotolojik analizlere göre, lenfosit, monosit ve nötrofil yüzdeleri TCPI ve TCPII gruplarında değişmiştir. Biyokimyasal analizler aspartat aminotransferaz, alanin aminotransferaz enzim aktiviteleri ve üre, kreatin seviyelerinde değişiklik olmadığını; TCPI grubunda alkalin fosfataz aktivitesinin arttığını göstermektedir. TCP uygulama gruplarında maternal karaciğer, böbrek, kalp, beyin ve fötal karaciğer, böbrek ve plasenta dokularında histopatolojik değişiklikler tespit edilmiştir.

Anahtar Kelimeler
TCP, histopatoloji, plasenta, E341

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INTRODUCTION

Phosphate, which is an essential nutrient for all living organisms, should be consumed by ingestion in the diet, as it cannot be synthesized. Inorganic phosphate salts have been used safely as food ingredients and also for commercial applications in producing cleaning products, toothpaste, fire extinguishers, textile processing and horticulture fertilizers. The four major classes of cations forming inorganic phosphate salts with the ortho and condensed phosphate anions are monovalent cations (sodium, potassium and hydrogen), divalent cations (calcium and magnesium), ammonium and aluminum [1]. Tricalcium phosphate $Ca_3(PO4)_2$ is included in divalent cation containing inorganic phosphates.

Tricalcium phosphate (TCP) is a food additive in white powder form which is also known as E341. The molecular weight and density of TCP is 310.18 and 3.14 respectively. Besides, it is used as anticoagulant, nutritional supplement, calcium intensifier pH regulator, flour anticoagulant, additive for milk powder, sweet, pudding, flavoring, and meat, refined aid agent for animal corn oil, yeast food [2]. It makes the ceramic in possession of exquisite porcelain quality, high whiteness, good transparency, gentle luster [3]. According to the manufacturer company (Polen Flour and Food Additive Industry, Istanbul, Turkey), E341 as a food additive is not only composed of TCP but also includes heavy metals in ppm levels such as arsenic, lead, cadmium, mercury and copper [4].

A large amount of toxicity data exists for all four classes of inorganic phosphates. Acute oral LD_{50} (mg/kg) of TCP is >5000 for rat [5]. Although teratogenic potentials of most of the divalent ortho and condensed inorganic phosphates were tested in pregnant rats, mice, hamsters and rabbits using standard protocols, the reports about teratogenic potential and maternal/fetal toxicity of TCP are very rare.

Exposure to xenobiotics during pregnancy may adversely affect normal development of the fetus. The dose causing maternal toxicity may lead to secondary developmental effects. In toxicology studies, the toxic alterations within the maternal organism cannot be expected to occur on the embryo or fetus [6]. The effects observed on developing fetus may be due to the alterations in placental function including wealth of proteins for the transport and metabolism of xenobiotics and endogenous compounds [7].

According to FDA, most inorganic phosphates, including TCP, do not represent a health hazard when added directly to food or used at an acceptable daily intake dose (ADI) which is 70 mg/kg bw/day [8]. In most developing countries, bread and other bakery products containing E341 are consumed more than three times a day due to low purchasing power. Considering this situation, in the current study the two dose selection was higher than ADI.

The present study has conducted to determine the potential maternal and fetal toxicity of E341 in Wistar rats treated at higher dose than ADI dose during gestation considering the results of the previous study [4] in which some morphometric alterations in placental and fetal bone measurements were reported after E341 treatment.

MATERIALS AND METHODS

Chemicals

The test chemical, E341 (TCP) was obtained from a bakery inputs producer company in Turkey in white powder form. It was stored in a dark place, at room temperature. The microbiological and typical analysis of E341 (TCP) is shown in Table 2 (Polen Flour and Food Additive Industry, Istanbul, Turkey).

As E341 (TCP) is not soluble in water, TCP was dissolved in corn oil and was prepared just before the treatment in order to ensure the freshness.

Animals and Experimental Design

The Wistar rats, including 20 males and 40 females, were obtained from Experimental Animals Production Center of Başkent University, in Ankara, Turkey. Animals were reared on a basal diet with water *ad libitum* and maintained in an air-conditioned room at 22.4±1.6 °C, in a relative humidity of 47.2±1 and 12-h light/dark cycle. Virgin female rats were mated overnight with male rats. The day when sperms were first detected in the vaginal smear was considered to be the day 0 of pregnancy. At the beginning of the experiment, the pregnant rats, weighing 145±8 g

Table 1. Composition of pellet diets

Nutrients (%)		Raw materials (%)		Macro and Micro elements (%)	
H_2O	12	Trifolium	20-30	Calcium	1.0-1.8
Raw protein	23	Red Dog	20-30	Phosphore	0.9
Raw celulose	7	Corn	15-20	Sodium	0.5-0.8
Raw ash	8	Wheat	0.10-30	Lysine	1.0
Ash not dissolved in HCl	2	Barley	0.10-15	Methionine	0.3
NaCl	1	Soy	0.25-35	Mangane	10
		Fish powder	0.5-10	Zinc	4
		Fish oil	10-15	Vitamins (A, B1, B2, B12, E, K)	2
		Toxin binding	2		

and 10 weeks of age were separated randomly into 4 groups. Each group contained 10 pregnant rats which were observed two times a day for general wholesomeness and care. Rats in control group were fed only with standard diet. Standard rat diet was purchased from Dokuz Tuğ Yem A.Ş., Ankara, Turkey (Table 1). Rats in corn oil control group were treated only with corn oil by oral gavage (Oil Control Group). TCP I group rats were administered with 175 mg/kg bw/day E341 (TCP Group I) and TCP II group rats were administered with 350 mg/kg bw/day E341 (TCP Group 2) everyday early in the morning by gavage during pregnancy period. We determined the concentration of E341 (TCP) as 2.5-fold (175 mg/kg bw/day) and 5-fold (350 mg/kg bw/day) higher dose than the ADI dose of humans to examine maternal toxicity. The study has conducted in full conformity with the National Research Council guidelines for animal experimentation [9].

Maternal observations

The weight of each dam was recorded on each gestation day. On day 20th of pregnancy, pregnant rats were sacrificed by CO_2 inhalation. The entire uterus was incisized to examine the contents. Empty uterus was stained with 10% ammonium sulfide in order to detect full resorptions at an early stage. The initial and final maternal weights of the control as well as the treatment groups were presented as mean and standard error. Body weight gains were calculated as grams (g) and percentages (%). Absolute and relative weights (organ weight/body weight) of maternal liver, kidney, heart and brain were recorded at the time of cesarean section on the 20th day of gestation. Placenta and all other tissue samples were fixed in Bouin's fixative for further histopathological examinations. Leica RM 2125 microtome was adjusted at 5 μm. The sections were placed on slides. Then the slides were stained with hematoxylin eosin (H&E) and examined blind independently by three observers by Olympus BX51 system light microscope. The photographs were captured with Bs200prop software. Maternal food and water consumptions were recorded everyday during pregnancy.

Fetal observations

The uterine horns were exteriorized through a midline abdominal incision and observed for implantations and resorptions. Living fetuses were counted and examined for externally-visible anomalies. In addition, fetal liver, kidney, heart and brain were removed, weighed and fixed in Bouin's fixative for further histopathological examinations under light microscope.

Maternal haematological and biochemical analysis

Dams were sacrificed and trunk blood was collected into vacutainer tubes in order to make complete blood count with Shimadzu Blood Counter. The parameters were: white blood cells, red blood cells, and thrombocytes/mm^3; percentage of lymphocyte, monocyte, neutrophil, eosinophil, basophile; MCV (Mean Corpuscular Volume), HCT (Hematocrit), MCH (Mean Corpuscular Hemoglobin), MCHC

Table 2. The microbiological and typical analysis of TCP

Microbiological Analysis	
Total live bacteria	< 50.000 / g
Coliform	< 30 / g
E.coli	0 / g
Salmonella	0 / g
Mold	< 1000 / g
Typical Analysis	
Arsenic (ppm)	< 3
Lead (ppm)	< 5
Cadmium (ppm)	< 1
Mercury (ppm)	< 1
Total heavy metals (ppm)	< 30
Copper (ppm)	<20

(Mean Corpuscular Hemoglobin Concentration), Hb (Hemoglobin), RDW-SD (Red Cell Distribution Width–Standard Deviation, RDW-CV (Red Cell Distribution Width–Coefficient Variation), MPV (Mean Platelet Volume) and PDW (Index of Thrombocytes Heterogeneity). From the rest of blood samples, serum was separated after centrifugation at 3000 rpm for 15 min. Serum samples were analyzed in order to determine the concentrations of urea and creatinine to measure enzyme activities of aspartate aminotransferase (AST) alanine aminotransferase (ALT) and alkaline phosphatase (ALP) with CL-770 Shimadzu clinical spectrophotometer.

Statistical analysis

Statistical analysis was performed using SPSS software for Windows. Statistical significance was assigned at the $P \leq 0.05$ level. The homogeneity of variance and normal distribution between groups were evaluated by General Linear Model procedure and Kolmogorov-Smirnov ***nonparametric*** test. Serum parameters were analyzed by one-way ANOVA. In order to identify the sources of main significant effects, ***post hoc*** comparisons (Games-Howell, Tukey) were used. Body weights, absolute and relative organ weights were examined by one-way ANOVA and Games-Howell *post hoc* test [10].

RESULTS

Maternal observations

There were no significant differences between parameters of Control and Oil Control Group; therefore the values of Control Group were not considered. All female rats in all groups became pregnant. There was no female mortality in any group during the course of the study. There were statistically significant decreases in absolute maternal liver, kidney, heart and relative weights of maternal liver and heart in 350 mg/kg bw/day dose TCP group (Table 3). On the other hand variations in initial and final maternal body weight and gain of weight (g, %) were not statistically significant among control and treatment groups (Table 4). Even though maternal weight gain decreased in group TCP II, the results were not considerable. Besides, food and water consumption decreased significantly in TCP II group. No behavioral changes were observed.

Fetal observations

There were no externally visible gross malformations in fetuses. However, there was a statistically significant ($P \leq 0.05$) increase in absolute fetal liver weight in TCP I and TCP II groups when compared to oil control group (Table 3). The observed resorption numbers were not statistically significant among groups.

Maternal haematological and biochemical analysis

According to the results of haematological analysis, there were differences in the percentages of lymphocytes, neutrophils in TCP I group and in percentage of monocytes in both TCP and TCP II groups (Table 5). Lymphocyte percentage of TCP group was significantly high when compared to oil control group ($P \leq 0.05$). Monocyte percentage of TCP I and TCP II groups statistically decreased when compared to oil control group ($P \leq 0.05$). Additionally, neutrophil percentage of TCP I group showed a significant decrease when compared to oil control group ($P \leq 0.05$). In haematological analysis, no statistically significant differences between other types of leukocytes, erythrocytes and thrombocytes related parameters were observed.

The toxicity of TCP on liver and kidney tissues were determined by the analysis of urea, creatinine and the estimation of enzyme activities of AST and ALT (Table 6). The levels of ALP in TCP I group were increased significantly; but no other statistically significant changes were observed in amounts and the levels of these parameters among control, TCP I and TCP II groups ($P \leq 0.05$).

Histopathological examinations

In the present study, maternal liver, kidney, heart, brain tissues and fetal liver, brain and kidney tissues belonging to control and two dose group of TCP were examined and compared. Maternal and fetal histopathological results are shown in Figure 1 and Figure 2. The observed histopathological changes were present in all animals of the mentioned group.

Maternal histopathology

Maternal liver, kidney, heart and brain tissues of oil control group were compared with treatment groups. The histopathological alterations were not dose dependent but observed in each slide examined. In liver tissues of hepatocytes and all sinusoidal areas were normal (Figure 1A). However, an increase in apoptotic cells and different levels of granular degenerations were observed in livers of TCP administered rats (Figure 1B). Additionally, mononuclear cell infiltration was increased locally in livers of TCP I and TCP II groups. In kidney tissues of oil control group, both cortex and medullar regions were in regular appearance (1E). However, in TCP treatment groups, increased periglomerular space and tubular degenerations in proximal and distal tubules were observed (Figure 1F). In heart tissues of oil control group, myofibrils and myofibrillar areas were normal (Figure 2A). On the other hand, local enlargements and adipose tissue accumulations in intermyofibrillar areas of heart tissues of TCP treated rats were detected (Figure 2B). In maternal brain tissues of TCP treatment groups, enlargements in perivascular areas and vacuolization were observed (Figure 2D). In addition to these findings, local glial fibrillar reaction areas (Figure 2E) and hyperchromatic cells (Figure 2F) were detected in the cortex area.

There were no dose dependent histopathological changes in placental sections among TCP groups. When compared with control (Figure 3A), in the placental sections of TCP group; irregular vessel formation in the labyrinth, (Figure 3B); degeneration in the trophoblastic giant cells, increased phagocytic activity in the basal zone (Figure 3C) and hemorrhage (Figure 3D) were observed.

Fetal histopathology

Fetal liver (Figure 1C) and kidney (Figure 1G) tissues of oil control group showed regular morphology. On the other hand, local lysis in liver parenchyma (Figure 1D), increased number of megakaryocytes were observed in TCP treatment groups. In kidney tissues of TCP treatment groups, increased periglomerular spaces were observed (Figure 1H). These observed changes were not dose-dependent. Besides, there were no histopathological changes in fetal heart and brain tissues in any group.

DISCUSSION

Toxicological studies dealing with widely used food additive E341 (TCP) are rare. Besides, maternal and developmental toxicological data are scarce. In the only acute oral study, Stauffer [5] had pronounced that LD_{50} (mg/kg) of TCP is >5000 for rat. Using the available toxicological data, the Joint FAO/WHO Expert Committee on Food Additives [8] had established an acceptable daily intake (ADI) of TCP for man as 70 mg/kg

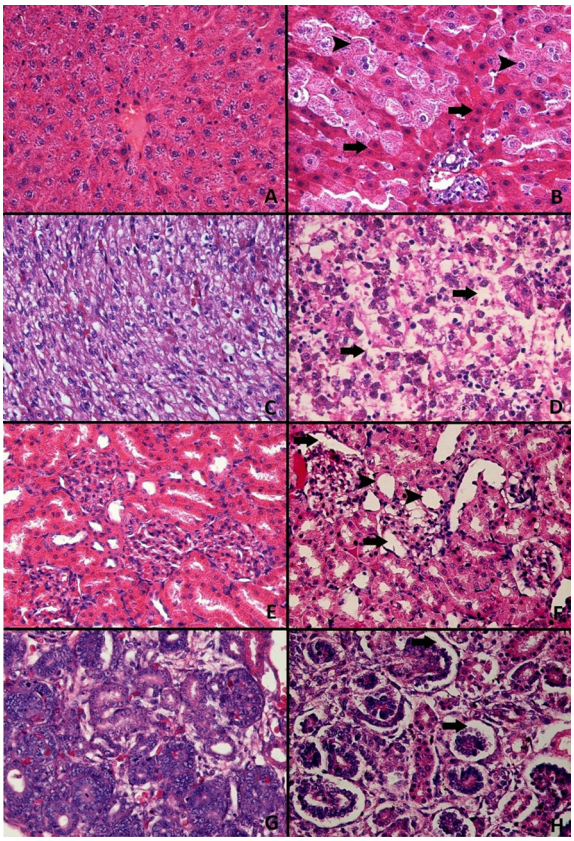

Figure 1. Photomicrographs of maternal and fetal liver and kidney tissues of rats stained with H&E. Liver tissues of control A. maternal, C. fetal. Kidney tissues of control E. maternal, G. fetal. Liver tissues of TCP group B. maternal, D. fetal. Kidney tissues of TCP group F. Maternal, H. fetal. (B) apoptotic cells (arrows) and granular degeneration (arrow heads), (D) lysis in parenchyma (arrows), (F) increased periglomerular space (arrows) and tubular degenerations (arrow heads), (H) wide glomerular dilatations (arrows) (A–H, X 200).

bw/day [1]. In order to test the possible health effects in humans, the most commonly used protocol for assessing developmental toxicity is to test it on laboratory animals. This involves the administration of a test substance to pregnant animals (usually mice, rats, or rabbits) during the period of major organogenesis, evaluation of maternal responses throughout pregnancy, and examination of the dam and the uterine contents just prior to term [11].

In the only developmental study conducted by Güngörmüş et al. [4], it was shown that after treatment of Wistar rats with E341 at 175 mg/kg dose during gestation, there were alterations in weight of placentas, trans-umbilical diameters and morphometric measurements in fetal ulna, femur, and skull. These developmental effects led us to

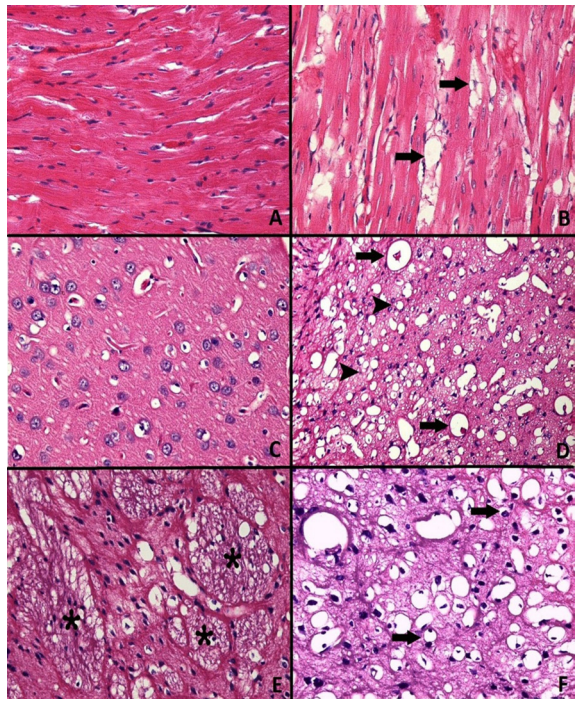

Figure 2. Photomicrographs of maternal heart and brain tissues of rats stained with H&E. A, C maternal oil control groups; B, D, E and F maternal TCP treatment groups. In maternal heart, (B) local enlargements and adipose tissue accumulations in intermyofibrillar areas (arrows) are shown. In maternal brain, (D) enlargements in perivascular areas (arrows), and vacuolization (arrow head), (E) large glial fibrillar reaction areas (stars) and (F) widespread hyperchromatic cells in cortex (arrows) are shown. (D. X 100, A, B, C, E, F. X 200).

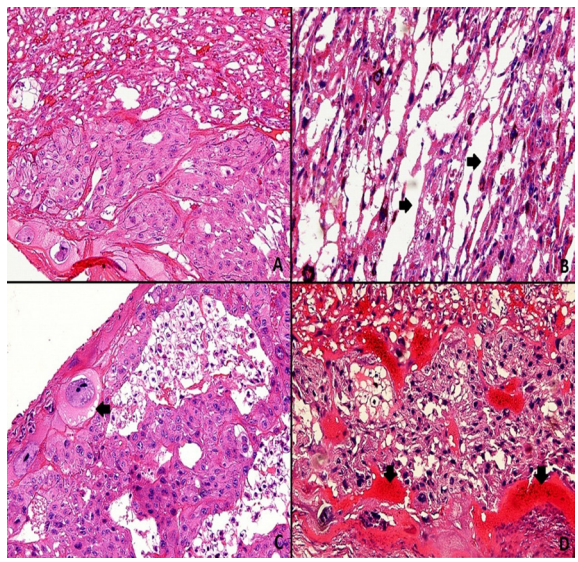

Figure 3. Photomicrographs of placenta tissues of rats stained with H&E. A. Oil control group; B, C and D TCP treatment groups. B. irregular vessel formation in the labyrinth (arrows), C. degenerations in trophoblastic giant cell and an increase in phagocytic activity (arrows). D. hemorrhage in basal zone (arrows). (X 100).

Table 3. Maternal and fetal organ weights(g) in control and treatment groups

			TCP	
Maternal		Oil Control	175 mg/kg bw	350 mg/kg bw
Liver	Absolute	9.9 ± 0.61	9.47 ± 0.36	5.83 ± 0.31[a]
	Relative (x10^{-2})	4.26 ± 0.15	4.39 ± 0.07	3.53 ± 0.23[a,b]
Kidney	Absolute	0.7 ± 0.02	0.67 ± 0.02	0.51 ± 0.02[a,b]
	Relative (x10^{-2})	0.30 ± 0.02	0.30 ± 0.02	0.26 ± 0.03
Heart	Absolute	0.81 ± 0.10	0.62 ± 0.03	0.46 ± 0.08[b]
	Relative (x10^{-2})	0.35 ± 0.05	0.23 ± 0.02	0.20 ± 0.04[a]
Brain	Absolute	1.25 ± 0.36	1.26 ± 0.24	1.32 ± 0.42
	Relative (x10^{-2})	0.69 ± 0.09	0.74 ± 0.06	0.57 ± 0.03
Foetal		Oil Control	175 mg/kg bw	350 mg/kg bw
Liver	Absolute	0.15 ± 0.01	0.24 ± 0.008[a]	0.26 ± 0.045[a]
	Relative (x10^{-2})	0.08 ± 0.01	0.06 ± 0.01	0.05 ± 0.01
Heart	Absolute	0.023 ± 0.002	0.025 ± 0.002	0.020 ± 0.001
	Relative (x10^{-2})	0.02 ± 0.05	0.01 ± 0.03	0.01 ± 0.01
Brain	Absolute	0.29 ± 0.02	0.26 ± 0.01	0.21 ± 0.04
	Relative (x10^{-2})	0.09 ± 0.01	0.8 ± 0.07	0.08 ± 0.02

[a] : is statistically significant from oil control group ($P \leq 0.05$)
[b] : is statistically significant from 175 mg/kg bw group ($P \leq 0.05$)

investigate the potential maternal and fetal toxicity of TCP, by administering Wistar rats at 175 mg/kg and 350 mg/kg doses, which are higher doses than the ADI dose which may be expected to cause maternal toxicity. In the present study, Wistar rats were chosen because of high fertility rate, short gestation period, low malformation rate and genetic stability.

The results of the study demonstrated that there were no statistically significant changes in maternal body weight and organ weights, except the decrease in maternal liver, kidney and heart liver weights in 350 mg/kg bw/day dose TCP group and increase in fetal liver weights in both treatment groups. Additionally water and food consumption decreased in TCP II group. Besides, maternal monocyte and neutrophil % are decreased significantly; lymphocyte %

Table 4. Maternal food, water consumption, body weight gain (gr, %)

		TCP	
	Oil Control	175 mg/kg bw	350 mg/kg bw
Food consumption (g / day)	18.23 ± 0.62	16.80 ± 0.24	14.04 ± 0.38[a]
Water consumption (ml / day)	31.71 ± 1.76	30.76 ± 1.65	23.56 ± 1.09[a]
Initial body weight	148 ± 18.33	140.33 ± 13.01	167.0 ± 9.22
Final body weight	232.66 ± 27.30	219.22 ± 28.23	231.3 ± 15.12
Gain of weight (g)	84.66 ± 13.32	75.66 ± 20.60	64.56 ± 16.86
Gain of weight %	57.32 ± 9.03	54.31 ± 11.11	38.32 ± 10.43

[a] : is statistically significant from oil control group ($P \leq 0.05$)

Table 5. Results of haematological analysis (Mean ± S.E)

		TCP	
Parameters	Oil Control	175 mg/kg bw	350 mg/kg bw
Leukocytes			
White Blood Cell /mm^3	9.81 ± 1.68	6.10 ± 1.17	6.52 ± 1.89
Lymphocyte %	54.75 ± 2.05	69.15 ± 2.28[a]	60.2 ± 3,21
Monocyte %	15.65 ± 0.38	10.05 ± 0.66[a]	10.8 ± 0,24[a]
Neutrophil %	25.4 ± 1.73	19.88 ± 2.33[a]	20.8 ± 1.33
Eosinophil %	0.72 ± 0.45	0.79 ± 0.06	0.9 ± 0.03
Basophil %	1.9 ± 0.17	1.67 ± 0.08	1.0 ± 0.02
Erythrocytes			
Red Blood Cell /mm3	6.03 ± 0.36	6.51 ± 0.56	6.42 ± 0.43
MCV (Mean Corpuscular Volume)	50.77 ± 2.67	55.4 ± 2.77	49.8 ± 1.99
HCT (Haematocrit)	30.8 ± 3.41	36.28 ± 4.34	32.0 ± 2.37
MCH (Mean Corpuscular Hemoglobin)	19.77 ± 0.48	20.68 ± 0.94	20.4 ± 0.71
MCHC (Mean Corpuscular Hemoglobin Concentration)	39.05 ± 1.09	37.4 ± 1.38	40.9 ± 1.22
Hb (Hemoglobin)	11.93 ± 0.98	13.43 ± 1.24	13.10 ± 0.78
RDW-SD (Red Cell Distribution Width- Standard Deviation)	37.35 ± 0.83	38.45 ± 0.78	37.1 ± 0.56
RDW-CV(Red Cell Distribution Width- Coefficient Variation)	20.05 ± 0.83	18.93 ± 0.65	20.20 ± 0.79
Thrombocytes			
Thrombocyte /mm3	1014.67 ± 116.36	698.25 ± 168.30	701.0 ± 135.98
MPV (Mean Platelet Volume)	7.02 ± 0.94	7.275 ± 0.914	5.82 ± 1.06
PDW (Index of Thrombocytes Heterogeneity)	10.4 ± 1.07	9.83 ± 0.45	9.5 ± 0.67

a : is statistically significant from oil control group ($P \leq 0.05$)

is increased in TCP treated groups. Results of biochemical analysis showed that enzyme activities of AST, ALT and levels of urea and creatinine did not change among groups. Some histopathological changes were observed in maternal liver, kidney, brain, heart, placenta and fetal liver, kidney.

According to Economic Research Service, in most of developing countries, food budget shares for bread and cereals are considerably higher as compared to developed countries, because of the higher consumption levels and economical conditions in those countries [12]. Accordingly, bakery products and their ingredients need more consideration. The dose used in this present study, was higher than ADI dose of E341 which cause maternal toxicity.

In a developmental toxicity study, annatto which is used as a food additive in butter and cheese was given by gavage to Wistar rats on days 6–15 of pregnancy. The weight gain of annatto-treated mothers during the whole pregnancy (days 0–21) did not differ statistically from that of the control group. These findings are correlated with our maternal weight gain results. On the other hand, no gross pathological alterations in the maternal organs of any group were found [13]. In contrary, there were histopathological changes in liver, kidney, heart and brain tissues according to our maternal examinations.

According to histopathological examinations, there were changes in maternal and fetal liver and kidney tissues independently from the doses of TCP. The maternal liver and kidney were severely affected

while fetal tissues showed mild degenerative changes. The hepato- and nephrotoxic effects of TCP have not been documented but these results suggested that placental transmission of TCP to fetuses causing pathological changes in the vital organs of developing fetuses and this might be possible due to its lipid solubility and its chemical constituent. But, maternal biochemical results on levels of AST, ALT and concentrations of urea, creatinine which are indicators of the severe failure in liver-kidney functions did not change among groups. Therefore, these alterations could be reversible in maternal tissues. Further, we suggest that the mechanism of toxicity is similar in both dams and their fetuses with respect to pathological changes in liver and kidneys. The weight gain of fetal liver tissue in our treatment group also supports these suggestions.

Nelson et al. [14] reported the results of a developmental toxicity study in which Sprague-dawley rats were exposed to 1-butanol, which is used as a flavoring agent, by inhalation for 7 hr/day on days 1–19 of pregnancy at 3 doses.

They observed maternal deaths at highest dose, decreases in maternal food consumption and fetal weight at two highest dose groups, and no maternal toxicity at any dose of 1-butanol.

Interestingly, they found significant increase in the incidence of fetuses with dilation of the subarachnoid space and dilation of the lateral ventricle and/or third ventricle of the brain even at the lowest dose. They have concluded that 1-butanol was a developmental toxicant and produced anomalies in the skeleton and central nervous system. In our study, enlargements in perivascular areas, local glial fibrillar reaction areas and widespread hyperchromatic cells were detected in the cortex of maternal brain tissues which might be reflecting TCP can pass blood-brain barrier and induce inflammatory reactions. But, there were no histopathological changes observed in fetal brain tissues. One of the most important indicators of immune response is the increase in lymphocytes. In the present study, lymphocyte percentage increased in blood, possibly due to the increase in inflammatory reaction in tissues including mononuclear cell infiltration in liver and kidney. Monocytes are established circulating precursors for tissue macrophages and dendritic cells [15]. The reason of decrease in blood monocyte percentage in TCP group might be the migration of monocytes into tissues after inflammation signal starts.

E341 as a food additive is not only composed of TCP but it also includes heavy metals in ppm levels such as arsenic, lead, cadmium, mercury, copper [4]. These heavy metals may be the reason of maternal and fetal toxicity. In Wang et al. [16], it has been investigated the toxicity of lead exposure on the placenta at different dosages in Wistar rats. They suggested that injury in the placenta may interfere with the nutrition and oxygen exchange between mother and fetus, which may contribute to abnormal pregnancy outcomes. In this study, the heavy metals in the composition of TCP could be reason of the alterations in liver, kidney and brain histology. Kidney is a target organ for cadmium toxicity [17] and even though cadmium can hardly get into brain parenchyma because of brain barrier,

Table 6. Results of biochemical analysis of control and treatment groups (Mean±S.E)

		TCP	
Parameter	Oil Control	175 mg/kg bw	350 mg/kg bw
AST (U/L)	159.6 ± 22.00	174.33 ± 33.80	219.8 ± 20.10
ALT (U/L)	65.63 ± 10.55	81.33 ± 7.23	68.3 ± 8.98
ALP (U/L)	229.3 ± 27.17	323.80 ± 77.32[a]	218.40 ± 45.67
Creatinine (mg/dL)	0.50 ± 0.15	0.43 ± 0.05	0.50 ± 0.02
Urea (mg/dL)	68.73 ± 5.50	59.45 ± 8.51	88.7 ± 6.78

[a] : is statistically significant from oil control group ($P \leq 0.05$)

central nervous system disorders have been reported after chronic exposures or exposure during pregnancy [18,19]. Combination of lead [20,21], arsenic and mercury might be the reason of glial fibrillar reaction and also increase in hyper chromatic cells in TCP treated maternal brain cortex. However, there were no changes observed in fetal brain.

Histopathological changes such as enlargements in myofibrillar area and lipid accumulation in maternal heart might be due to heavy metal content of E341, especially arsenic and cadmium. As Yáñez et al. [22] suggested, heart tissue can be affected not only by the mixture of arsenic and cadmium, but also by either metal alone in rats. However, no change in fetal heart was observed.

It seems likely that the irregular vessels formation in the labyrinth was caused by the reduced functional capacity of trophoblastic barrier because of TCP treatment. Güngörmüş et al. [4] have reported that E341 decreases the weight of placenta in rats. Our histopathologic results in placental sections are consistent with their results. TCP treatment during pregnancy had some histopathologic changes in placenta such as congestion in basal zone. This change may be interpreted as a compensatory mechanism via diluting the toxic molecules [23].

In the light of the above findings, it has been concluded that the treatment at higher E341 dose of ADI during pregnancy in Wistar rats may cause maternal toxicity including liver, kidney, heart, brain, placenta and changes in haematological parameters. On the other hand, the observed histopathological effects in fetal liver and kidney tissues are supportting the alterations in skeletal development and morphometric measurements relating to placental data reported before

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