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The role of chrysin against harmful effects of formaldehyde exposure on the morphology of rat fetus liver and kidney development

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Abstract

This study was aimed to investigate possible harmful effects of formaldehyde (FA) exposure on the morphology of fetus liver and kidney development during pregnancy and also to determinate possible protective role of chrysin (CH) against these harmful effects. For this aim, after pregnancy was induced, 58 female rats were divided into 6 groups. Serum physiologic (SF) was injected to the Group I rats intraperitoneally (i.p.). 20 mg/kg CH was given to the Group II via gavage. 0.1 mg/kg FA was applied to the Group III (i.p.), 1 mg/kg FA was injected to Group IV (i.p.) 0.1 mg/kg FA was given to Group V i.p., and 20 mg/kg CH was given to the same group via gavage. 1 mg/kg FA was applied to Group VI i.p., and 20 mg/kg CH was given to the same group via gavage. Fetuses were taken from each pregnant rat with cesarean section on the 20th day of the pregnancy. The morphological analyses of the fetuses, liver and kidney; biochemical and histological analyses of the liver and kidney were performed. The fetal body, liver and kidney weight of the FA groups demonstrated a statistically significant decrease the compared to control group. Also the FA-1 group were observed histopathological changes on the fetus liver and kidneys. FA exposure causes harmful effects on fetus the liver and kidneys. CH reduces the negative effect on morphological variables statistically. Although CH is insufficient to fix the histopathological changes that occur in the liver, damaging effects that occur in the kidney decreased statistically.

Keywords: Formaldehyde, chrysin, rat fetuses, liver, kidney

Introduction

Fetal growth is the change in the anatomic sizes of fetus in the course of time. In the embryonic period, growth is mostly determined by fetal genes. As the size of the fetus increases, it is affected by environmental and epigenetic factors. If these effects are negative, undesirable results emerge during the fetal and subsequent developmental periods. In the pregnancy period, the effects of harmful factors may vary by the period of duration and the dosage of exposure to these factors [1-3]. The most sensitive period for teratogenic substance is the 3rd to 8th weeks of pregnancy in human fetuses, and is the 9th to 10th days of pregnancy in rat fetuses [4,5]. This period corresponds to the organogenesis period in rats. Organogenesis starts to occur during the above mentioned period and is shaped until the 16th day (included) of pregnancy [1,5,6].

There are a lot of harmful chemicals that we are exposed to in our daily lives. One of these is formaldehyde (FA), which swiftly turns into gas at ambient temperatures, dissolves in water very well, and is inflammable, colorless, pungent, and irritant [7,8].FA is used as an antimicrobial agent in many products such as shampoo, soap, lotion, deodorant, and cosmetic products. It is also used as a disinfectant in hospitals [9]. FA is used in preparation of tissues in pathology and histology laboratories and in determination and long term storage of cadavers in anatomy [10]. FA penetrates into body through dermal absorption, digestion, and respiration and creates toxic and carcinogenic effects. It has toxic effects on reproductive system and during pregnancy, as well [11,12].

Epidemiologic studies reported that risks of spontaneous abortion, premature birth, and giving birth to a low weight baby increased in pregnant individuals who were exposed to FA in their work environments [13-16]. It was found that a rise in congenital heart malformation occurred in settings where FA level was over 2.4 mg/m³[17]. Experimental studies, on the other hand, reported a considerable fall in fetus body weight and an increase in fetal deaths as a result [18-20]. In addition, anomalies such as umbilical hernia, bobtail, oligodactyly, encephalocele, cryptorchidism, and meromelia were found [21,22].

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Chrysin (CH), which is known as a strong antioxidant, has 5,7- dihydroxyflavone molecule structure. It is naturally found in strawberry, broccoli, celery, fruit coats, cornelian cherry, grape, lettuce, olive, onion, parsley, honey, andpropolis in high quantities [23-26]. In addition, it was reported that CH has anti-inflammatory, anti-allergic, anxiolytic, antihypertensive, and anti-cancer effects [27-35].

To our best knowledge, there was no study that demonstrated the effects of CH against the possible harmful effects of FA exposure during pregnancy. Therefore, this study aims to explore the possible protective role of CH against the possible harmful effects of exposure to FA during pregnancy on the morphological characteristics and liver and kidney development of the fetus.

Materials and Methods

This study was conducted upon the approval of Inönü University Faculty of Medicine Experimental Animals Ethics Committee (2012/A-58).

Chemicals

37% FA solution (formalin) procured from Deisenhofen-Germany, Sigma-Aldrich and CH with a purity of 97% acquired from Sigma-Aldrich were used. CH was dissolved in maize oil with a magnetic mixer and applied with the gavage method.

Experimental Animals

58 mature female Wistar Albino rats with an average weight of 200 to 250 g. were used. The rats were kept in rooms with a temperature of 19 to 21º C that were illuminated for 12 hours (07:00 - 19:00) and dark for 12 hours (19:00- 07:00) and were automatically ventilated via an air-conditioner. The rats were fed ad libitum throughout the experiment. The rats were separated into cages in the evening as one male and one female rat each. Vaginal smear samples were taken from female rats in the following morning and scrutinized under light microscope. The rats with sperm observed in their smears were accepted as pregnant for half a day. 58 female rats were divided into 6 groups, and an each were attempted to be impregnated. 10 rats that did not become pregnant were excluded from the experiment.

Experimental Groups and Drug Applications

Serum physiologic (SF) was injected in Group I (Control, n:8) i.p..20 mg/kg CH was given to the Group II (CH group, n:8) via gavage. 0.1 mg/kg FA was applied to the Group III (FA-0.1 group, n:8) i.p. 1 mg/kg FA was injected in the Group IV (FA-1 group, n:8) i.p. 0.1 mg/kg FA was given to Group V (FA-0.1+CH group, n:8) i.p., and 20 mg/kg CH was given to the same group via gavage. 1 mg/kg FA was applied to Group VI (FA-1+CH group, n:8) i.p., and 20 mg/kg CH was applied to the same group via gavage. These applications were performed every other day between the 7th and the 20th days of pregnancy. Fetuses were taken from each pregnant rat via cesarean section on the 20th day of the pregnancy. In CH + FA groups, the first CH application was performed one day before the first FA application. Other CH applications were performed just before FA applications on FA application days.1 cc/kg SF was injected i.p. based on the weight of the pregnant rat.

Fetus preparation

All rats were anesthetized on the 20th day of the pregnancy with Ketamine (80 mg/kg)-Xylazine (5 mg/kg) i.p..The fetuses and placentas were removed with cesarean section. All the fetuses were weighed with a precision scale and measured with a precision caliper. Then the fetuses were evaluated for macroscopic anomalies. The abdomens of the fetuses were opened with a median laparotmy, and their liver and kidneys were removed. The livers and the kidneys were weighed with a precision scale. After that, the fetuses were terminated with a cervical dislocation. All surgical procedures were carried out by the same person.

Biochemical Analyses

The tissues were kept at -30 ºC until biochemical analyses were conducted. 10 tissues were randomly chosen from the liver tissues of the fetuses taken from each rat in a group. The tissues were homogenized in different solutions for different parameters. For 20 minutes, they were centrifuged at 5000 rpm in 0.15 M potassium chloride solution with a homogenizer. Glutathione (GSH), glutathione peroxidase (GSH-Px), catalase (CAT), superoxide dismutase (SOD), and malondialdehyde (MDA) levels were measured in the supernatants obtained. Biochemical studies could not be conducted due to small kidney tissue sizes.

Determination of GSH: GSH analysis was conducted with the method described by Ellman [36]. According to the GSH measurement principle, glutathione in the analysis tube reacts with 5, 5' dithiobis 2-nitrobenzoic acid and produces a yellow-greenish color. Reduced glutathione amount was determined by reading the light intensity of this color at 410 nm wavelength via a spectrophotometer. Tissue GSH levels were expressed as nmol/gr wet tissue.

Determination of GSH-Px: GSH-Px analysis was conducted with Paglia et al. [37] method. GSH-Px activity was calculated by reading the decrease in absorbance during oxidation of NADPH to NADP+ at 340 nm and was expressed as unit/gram (U/g) tissue protein.

Determination of MDA: MDA analysis, which is the most important indicator of lipid peroxidation, was conducted based on the measurement method determined by Ohkawa et al. [38]. Fifteen minutes later, the absorbance of the rapidly cooled samples was spectrophotometrically read at 532 nm. The tissue MDA levels were expressed as nmol/gr wet tissue.

Determination of SOD: Superoxide dismutase enzyme analysis was conducted based on the method modified by Sun et al.[39]. This method is based on the principle that nitrobluetetrazolium is reduced by xanthine-xanthine oxidase system, which is a superoxide producer. SOD activity was measured based on the absorbance provided by the formazan emerging during such reduction at 560 nm. SOD activity was expressed asunit/gram (U/g) tissue protein.

Determination of CAT: CAT enzyme analysis was conducted based on the method developed by Aebi [40]. This method determines the change in speed of the decomposition of H_2O_2 by CAT at 240 nm. The change in the speed of the enzyme was calculated via measurement of absorbance changes per a minute basis. The activities were expressed as katal/gram (K/g) tissue protein.

Histological Applications

Fetus liver and kidney tissues obtained from all rats were fixed with a 10% FA solution. Sections with a thickness of 4μm were obtained from paraffin blocks. Hematoxylin-Eozin (H-E) and Periodic Acid Schiff (PAS) histological stains were applied to tissue sections.

Statistical Analysis

Morphological and biochemical datas were analyzed by "SPSS 20.0 for Windows" statistical software package. The fitness of the data for normal distribution was explored with "*Kolmogorov-Smirnov*" test. The groups were compared via "*Kruskal-Wallis H*"test. When a significant difference was identified, multiple comparisons were conducted with "*Conover*" test. p<0.05 value was accepted as the level significance. The data were summarized as median (minimum-maximum) values. Histopathological data for liver and kidney tissues were analyzed via SPSS (SPSS for Windows version 17) and MedCalc (2007, Belgium) statistical software packages. The general comparison of the groups was conducted with Kruskal Wallis variance analysis. All results were expressed as arithmetic average ± standard error (x ± SE) values. Inter-group comparisons were made via Mann-Whitney U test. p<0.05 was accepted as the level of significance.

Results

The number of fetuses, CRL, and fetal body weights

372 fetuses were obtained via the cesarean sections. No significant difference was found between the groups in terms of the number of fetuses and crown-rump length (CRL). However, the number of fetuses in the groups exposed to only FA was smaller than the control group. The fetus weights of the groups exposed to only FA were significantly lower compared to those in the control group. The fetus weights in the groups exposed to CH together with FA were significantly higher than those of the groups exposed to only FA (p<0.05) (Table 1.).

Table 1. The number of fetuses, CRL, and body weight.

Group	The number of fetuses	CRL (cm)	Fetal weight (g)
Control	9.5 (8-12)	3.3 (3-4.2)	4.3 (2.6-6.3)
CH	11 (9-13)	4.0 (3-4.3)	5.1 (3.1-6.1)
FA-0.1	9 (7-12)	3.6 (2.1-4.2)	3.4 (1.3-6.3)[a]
FA-1	8 (3-11)	3.4 (2.4-4.1)	3.2 (0.9 -4.3)[a]
FA-0.1+CH	10.5 (7-14)	3.9 (2.5-4.2)	4.6 (1.8-5.7)[b]
FA-1+CH	10 (7-11)	3.9 (3-4.2)	4.7 (2.6-5.8)[c]

[a] Significant decrease compared to the control group (p<0.05).
[b] Significant increase compared to the FA-0.1 group (p<0.05).
[c] Significant increase compared to the FA-1 group (p<0.05).

Fetal liver and the kidney weights

The liver and the kidney weights of the fetuses in the FA-1 group significantly decreased compare to the control group. In addition, the liver and the kidney weights of the fetuses in the FA-1+CH group significantly increased compared to the FA-1 group (p<0.05) (Table 2).

Table 2. The liver and the kidney weights of the fetuses.

Group	Liver weight (g)	Kidney weight (g)
Control	0.24 (0.11-0.35)	0.03 (0.02-0.05)
CH	0.26 (0.1-0.43)	0.04 (0.02-0.07)
FA-0.1	0.23 (0.06-0.39)	0.03 (0.003-0.06)
FA-1	0.21 (0.03-0.67)[a]	0.02 (0.003-0.07)[a]
FA-0.1+CH	0.26 (0.11-0.43)	0.04 (0.01-0.4)
FA-1+CH	0.26 (0.11-0.42)[b]	0.04 (0.01-0.2)[b]

[a]Significant decrease compared to the control group (p<0.05).
[b]Significant increase compared to the FA-1 group (p<0.05).

The biochemical variables of the liver tissue of the fetuses

GSH level of the fetuses in the FA-1 group significantly decreased compared to the control group (p<0.05).Moreover, GSH level significantly increased in the FA-1+CH group in compare to the FA-1 group (p<0.05). Differences were found between the FA-1 group and the control group in terms of GSH-Px, SOD, CAT, and MDA levels and between the FA-0.1 group and the control group in terms of all biochemical variables, but these differences were not statistically significant (Table 3).

Table 3. The biochemical parameters of the liver tissue of the fetuses.

Group	GSH(nmol/gr)	GSH-Px (U/g)	MDA (nmol/gr)	CAT (K/g)	SOD (U/g)
Control	923 (577-2077)	312(228-338)	631 (335-941)	82 (46.9-109.5)	129 (58-190)
CH	1106 (583-1333)	354(288-384)	844 (472-1039)	100 (58.1-121.6)	125 (78-156)
FA-0.1	980 (782-1474)	331(273-443)	906 (680-1051)	85 (56.5-109.4)	153 (109-199)
FA-1	554 (423-1045) [a]	327(288-459)	723 (546-1028)	95(65.5-117.2)	119 (105-145)
FA-0.1+CH	1214 (647-1365)	349(297-677)	541 (409-1162)	111 (70.7-146.1)	131 (113-254)
FA-1+CH	1189 (814-2051) [b]	626(509-881)	631 (505-954)	106 (52.9-134.3)	143 (114-206)

[a]Significant decrease compared to the control group (p<0.05).
[b]Significant increase compared to the FA-1 group (p<0.05).

Histopathological Findings
Liver tissue

Classic liver lobule structure was not completely observed in the control and CH groups. Structures of portal triads other than bile ducts were not distinguished. Multinucleate, acidophilcytoplasm megakaryocytes were observed among hepatocytes (Figure 1-A). The number of megakaryocytes in the FA-0.1 group decreased compared to the control group (p<0.05) (Figure 1-B). The number of megakaryocytes in the FA-0.1+CH group increased compared to the FA-0.1 group. However, the increase was statistically nonsignificant. In the FA-0.1+CH group, megakaryocytes and hepatocyte nuclei appeared to be normal (Figure 1-C). Degenerated megakaryocytes stood out in the FA-1 group (Figure 1-D). The number of megakaryocytes decreased compared to the control group (p<0.001). In addition, there were not hepatocytes and hematopoietic cells in some areas (Figure 1-E). The number of megakaryocytes in the FA-1+CH group increased compared to the FA-1 group. However, the increase was statistically nonsignificant. FA-1+CH group compared to FA-1 group, a decrease was observed in the area without hepatocytes and hematopoietic cells Also, in FA-1+CH group, hepatocyte nuclei had smoother contours and megakaryocytes contained structures similar to the control group (Figure 1-F). Table 4 indicates the numbers of megakaryocytes within the groups.

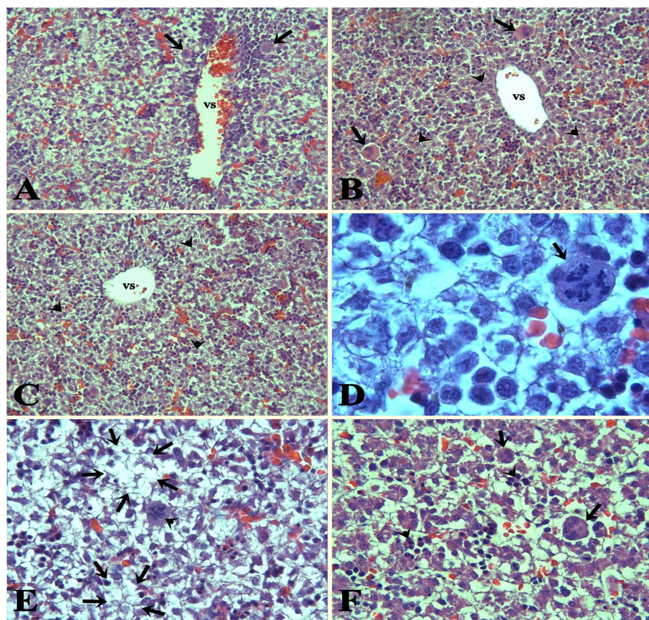

Figure 1-A. Hepatocytes and megakaryocytes (arrows) observed around a large vena centralis (VC) in the control group. H-EX20. **B.** Megakaryocytes (arrows) observed between hepatocytes (arrow heads) around vena centralis (VC) in the FA-0.1 group, H-EX20. **C.** The view of vena centralis (VC) and hepatocytes (arrow heads) in the FA-0.1+CH group, H-EX20. **D.** Degenerated megakaryocyte in the FA-1 group (arrow), H-EX100. **E.** The areas in theFA-1 group that did not involve hepatocytes and hematopoietic cells (arrows) and the degenerated megakaryocyte (arrow head), H-EX40. **F.** The view of hematopoietic cells (arrow heads) and megakaryocytes (arrows) in the FA-1+CH group, H-EX40.

Table 4. The numbers of megakaryocytes in the groups.

Group	The numbers of megakaryocytes
Control	27.7 ± 2.9
CH	19.4 ± 2.1
FA-0.1	16.6 ± 2.4[a]
FA-1	14 ± 2.2[a]
FA-0.1+CH	17.2 ± 1.3[a]
FA-1+CH	15.9 ±1.8[a]

[a]Significant decrease compared to the control group (p<0.001).

Kidney Tissue

In the sections examined in control and CH groups, glomeruli that had not complete their development were observed just below the capsule while there were mature glomeruli in the parts of the renal cortex close to medulla (Figure 2-A, 2-B). There was a statistically significant difference between the FA-1 group and the control group in terms of the number of immature glomeruli (p<0.05), but the difference between the FA-0.1 group and the control group was not statistically significant (Table 5.). The number of mature glomeruli decreased statistically in FA groups compared to the control group (p<0.05). In addition, the number of mature glomeruli in the FA-1 group significantly decreased compared to the FA-0.1 group (p=0.001). The number of mature glomeruli in the FA groups treated to CH statistically increased compared to the groups exposed to only FA (p<0.05) (Figure 2-C, 2-E). Also, total number of glomeruli in the FA-1 group significantly decreased compared to the control group (p=0.01) and the total number of glomeruli in the FA-1+CH group significantly increased compared to the FA-1 group (p=0.02) (Figure 2-D, 2-F) (Table 5).

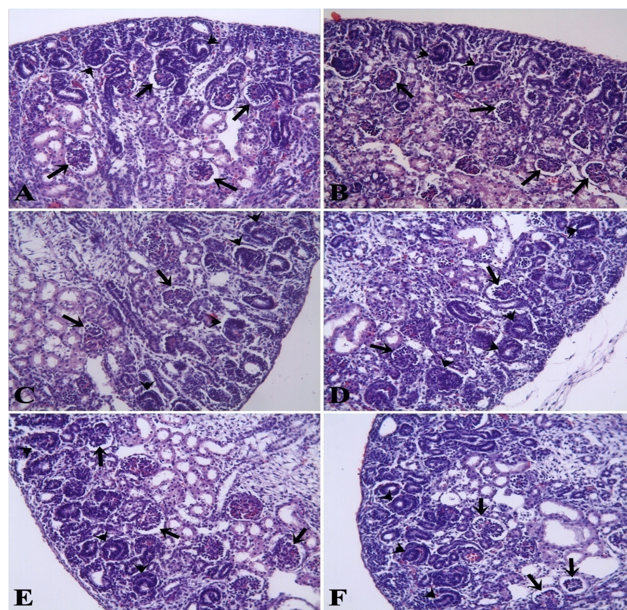

Figure 2-A. Control **B**; CH groups have not completed their development in the outer cortex of immature glomeruli (arrowheads), mature glomeruli in the cortex, medulla close sections (arrows) are monitored HEX20. **C**; FA-0.1, **D**; FA-1 in immature group (arrowheads) and mature glomeruli (arrows). Immature glomeruli of the FA-1 group draws attention is more pronounced. **E**; FA-0.1 + CH, **F**; FA-1 + CH groups are monitored on an increase in mature glomeruli. H-EX20.

Table 5. The number of glomeruli of groups.

	Control	CH	FA-0,1	FA-1	FA-0,1 + CH	FA-1 + CH
Immature	4.4 ± 1.5	4.7 ± 1.9	4.9 ± 1.4	5 ± 1.8 [a]	4.7 ± 1.5	5.1 ± 1.7
Mature	3.3 ± 1.3	3.8 ± 1.5	2.8 ± 1.5 [b]	2 ± 1.3 [b]	3.4 ± 1.5 [c]	2.6 ± 1.3 [d]
Total	7.7 ± 1.9	8.6 ± 2.1	7.7 ± 2.1	7.1 ± 2.1 [e]	8.1 ± 1.6	7.7 ± 2.1 [f]

[a] Significant increase compared to the control group. ($p<0.05$).
[b] Significant decrease compared to the control group. ($p<0.05$).
[c] Significant increase compared to the FA-0.1 group. ($p<0.05$).
[d] Significant increase compared to the FA-1 group. ($p<0.05$).
[e] Significant decrease compared to the control group. ($p<0.05$).
[f] Significant increase compared to the FA-1 group. ($p<0.05$).

Discussion

Pregnancy is a physiological process in which there is an increased need for oxygen in order to provide the growing fetus with energy. Thus, free oxygen radicals increase during the pregnancy. Oxidative balance may be disrupted if antioxidant defense system fails during this period. Disruption of oxidative balance may lead to a decrease in fetal growth and complications such as preeclampsia [41]. The results of the effects of harmful factors during pregnancy might vary by the period when such effects manifest themselves and based on the duration and dosage of exposure to this factors [1-3].

Among the studies involving FA, a study conducted with hamsters found that when FA was applied percutaneously on the 8th, 9th, 10th, and11th days of the pregnancy, fetal deaths increased, but fetal weight and height were not affected, and no anomaly was detected. It was stated that the reason for the increase in fetal deaths was stress [18]. Saillenfait et al. [19] exposed rats to FA in doses of 5 ppm, 10 ppm, 20 ppm, and 40 ppm for 6 hours per day from the 6th day to the 20th day of the pregnancy. A considerable decrease occurred in the fetal weights of those who were exposed to FA in doses of 20 ppm and 40 ppm. Another study conducted with rats determined that exposure to FA in doses of 2 ppm, 5 ppm, and 10 ppm through inhalation did not have any effects on fetal weights from the 6th day to the 15th day of the pregnancy [20]. In a study conducted by Thrasher et al. [21], rats were exposed to FA in doses of 0.012 mg/m³ (0.00928 ppm) and 1.0 mg/m³ (0.773 ppm) through inhalation during pregnancy. They concluded that the number of fetuses decreased in FA groups compared to the control group. In addition, a decrease was detected in liver and kidney weights of fetuses while increases were detected in their body, heart, liver, and thymus weights. It was considered that the reason was an increase in RNA concentration and a decrease in DNA concentration in fetal organs. In another study, rabbits were exposed to FA through inhalation in a dose of 12 ppm during pregnancy, and it was determined that 35.6% of the offspring had low birth weight while 64.4% had very low birth weight [22].

The above-mentioned studies involved exposure through inhalation and at high doses. In the present study, however, FA exposure was i.p. and at low doses in order to create systemic effect. A significant decrease occurred in the weights of rats exposed to FA in a dose of 0.1 mg/kg and of rats exposed to FA in a dose of 1 mg/kg. Also, both liver and kidney weights of the fetuses obtained from the group exposed to FA in a dose of 1 mg/kg fell. Although FA was applied at lower doses in the present study compared to doses utilized in the literature, the body, liver, and kidney weights of the fetuses decreased. That was attributed to the fact that systemic effect was created through exposure to FA i.p.. FA that had a systemic effect may have reduced fetal weights by inhibiting protein synthesis. CH application significantly increased fetal weight and liver and kidney weights in the groups exposed to FA. It was considered that these increases were due to the fact that CH displayed an antioxidant feature with its free radical scavenging mechanism [25,42,43].

Free oxygen radicals are continuously formed in high-structure living being cells. The level of these radicals is controlled by the enzymatic and non-enzymatic antioxidants in the cells via detoxification. Cellular enzymatic antioxidants are SOD, CAT, and GSH-Px. Non-enzymatic antioxidants include GSH, melatonin, vitamins A, E, and C, selenium, and other organic and inorganic antioxidant molecules [43,44]. In pathological situations, the balance is disrupted in favor of oxidants as a result of an excessive free radical production or a decrease in the amount of antioxidants. As a result, oxidative stress that leads to the beginning of events that cause cellular damage. Oxidative stress causes damage in building blocks of cells such as DNA, RNA, lipid, protein, and carbohydrates and cells to fade away [45,48]. The superoxide radical that emerges first during oxidative stress is quite a toxic substance. SOD converts superoxide radical into H_2O_2, which is a radical having a much lower toxic effect. Thus, SOD detoxifies the superoxide radical and protects tissues against oxidative damage [43,49,50]. In the present study, as a response to FA toxicity in the FA-0.1 group, fetal liver cells increased SOD activity levels considerably compared to the control group and thus developed a compensation (cellular compensation) mechanism. However, in FA-1 group fetuses, SOD activity decreased compared to the control group. In other words, a cellular compensation mechanism did not develop. Most probably, in the FA-1 group, the applied FA suppressed SOD activity in liver cells (in transcriptional and/or transnational level) [51,53]. In the fetuses exposed to CH in addition to FA as a protector, an increase in SOD activity in the fetuses in the

FA-0.1 group (cellular compensation) were observed. Especially, the FA-1+CH group was observed that were normalized to of the decrease in SOD activity in the FA-1 group. It was considered that the antioxidant effects of CH. However, histopathological results did not confirm this normalization at SOD level, since the number of megakaryocytes considerably decreased in fetal liver cells in the FA-0.1 and FA-1 groups. CH application failed to compensate for this decrease completely.

CAT and GSH-Px are intense in the liver tissue. In the pathologies where oxidative stress becomes chronic, the large part of H_2O_2 emerging in the cells is decomposed by GSH-Px. The protective effect of CAT is much weaker in chronic pathologies [44,50,53,55]. No considerable change was observed in the CAT levels of the fetuses in the FA-0.1 group, but CAT levels in the FA-1 group considerably increased compared to the control group. A slight increase took place in the GSH-Px activity of the fetuses in the FA-0.1 group compared to the control group. Although FA dosage increased 10 times in the FA-1 group, no significant increase occurred in GSH-Px activity. It was considered that this was because the liver GSH level of this group was considerably lower compared to the control group and thus GSH-Px activity decreased in the course of time, since GSH is the coenzyme of GSH-Px. When cells do not contain adequate GSH, GSH-Px activity would decrease substantially. As a result, substantial oxidative damages would occur in macro building blocks of cells such as protein, lipid, and carbohydrate due to oxidation. The liver MDA levels and the histopathological results of the fetuses in the FA-0.1 and FA-1 groups confirmed that. Since fetal MDA levels, which are the most important indicator of lipid peroxidation, in FA-0.1 and FA-1 groups increased, while the number of megakaryocytes in liver cells decreased considerably. The fact that the liver MDA levels of the fetuses in the groups exposed to CH in addition to FA did not increase considerably compared to the control group was attributed to the protective effects of CH against the harmful effects of FA [25,42,43].

GSH is a very important non-enzymatic antioxidant that is common in all body cells and protects cells against oxidative damage [56-58]. Approximately 40% of total GSH levels in the body exist in liver tissues. GSH displays an antioxidant effect by scavenging the free oxygen radicals emerging in the cells. H_2O_2, formed in cells due to oxidative stress, is eliminated through decomposition into water through GSH-Px activity when sufficient amount of GSH is available. GSH is the coenzyme of this enzyme [59]. If there is not enough GSH in the environment, GSH-Px activity takes place at a lower level. Consequently, H_2O_2 levels could not be controlled, and oxidant/antioxidant balance is disrupted in favor of the oxidants. As a result, oxidative damages take place in tissues. Secondly, GSH converts FA into S-hidroximetil-GSH by directly interacting with FA in the liver and helps FA turn into formic acid and be discharged from the body [60]. In the present study, no significant changes were observed in fetal GSH levels in the FA-0.1 group, but a significant decrease was found in fetal GSH levels in the FA-1 group compared to the control group. Such considerable fall substantially limited the increase in GSH-Px activity. Moreover, a considerable decrease in GSH levels in the liver would limit the conjugation of FA with GSH in liver, its decomposition into formic acid, and discharge from body. The fact that no decrease took place in fetal GSH levels compared to the control group when CH was applied with FA was attributed to the fact that CH displayed an antioxidant effect against the toxic effects of FA [25,42,43].

The histological damages were observed on the fetus liver and kidney of only FA exposed groups. These harmful effects were more significantly in the FA-1 group. It was considered that FA caused oxidative stress and lead to these histopathologic changes. In a study, there were histological damages in the liver and kidney tissues of rat fetuses exposed to 1.0 mg/m³ FA by inhalation during pregnancy [21]. In an another study, considerable irregular glomeruli and quite prominent dilatation of the distal tubules were observed in the rats exposed to FA (10 mg/m³,i.p.) [61].In a study conducted byBakar et al. [62] reported that FA (10mg/m³,i.p.) caused degeneration and vacuolization in the hepatocytes.

CH treatment with FA increased (non-significant) the number of megakaryocytes of the liver tissues compared to the only FA groups. It was believed that CH failed to protect the fetal liver against oxidative damage most probably because the application dosage was low and/or duration of the application was short, and transition from mother's blood to the fetus was low. In addition application of CH with FA improves the histological damage of the kidney tissues. This can be attributed to its antioxidant capacity [25,43,44]. But as a limitation of our study we couldn't analyse biochemical parameters.

Conclusions

The present study demonstrates that exposed to FA during pregnancy caused liver and kidney damage in their fetuses. And also, treatment of CH reduces these oxidative damages. These therapeutic effects can be attributed to its action on oxidant-antioxidant systems. Although, further experimental and clinical studies are required to confirm these findings. In the light of the above discussion, several protective measures should be taken for employees, especially the pregnant, who work in environments where FA is present.

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Conflict of interest
All authors declare that they have no conflicts of interest.

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