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Gingerol fractions bioactivity against butanone cytotoxicity induced in newborns of mice

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Abstract. – OBJECTIVE: Accumulating studies have demonstrated the potential activity of ginger in treating and manage several diseases whoever little is known about its protective effects against teratogenicity of chemical toxins. Thus, in this study, we have evaluated the protective effect of gingerol fraction (GF) against methyl ethyl ketone (MEK) induced teratogenic effects in newborn of mice.

MATERIALS AND METHODS: A total of 30 mature females and fifteen male mice (Mus musculus) weighing 25-30 g were included in this study. The pregnant mice were divided into three groups (10 mice each); control group (GI, mice received normal drinking water; NDW), methyl ethyl ketone (MEK) treated group (GII, received MEK at a dose of 350 mg/kg body weight in NDW), and GF treated group (GIII; mice received GF at a dose of 25 mg/kg in NDR). Histological analysis, cellular oxidative, and antioxidant enzymes, fibrosis, and apoptosis of brain, liver, and kidney tissues were estimated by histological and immunoassay techniques.

RESULTS: In this study, the treatment of pregnant female mice with gingerol fractions (GF) at a dose of 25 mg/kg significantly protected all tissues organs of mothers and their offspring against the teratogenic effects induced by MEK at a dose of 350 mg/kg. A significant improvement in cellular antioxidant enzymes GSH, SOD, and peroxidase activities along with a reduction in the initiation of cellular oxidative free radicals (TBARS) was reported in GF treated mice compared to mice intoxicated with MEK (350 mg/kg). In addition, a significant reduction in cellular fibrosis and apoptosis was reported in all tissues of mothers and their offspring's following treatment with GF. HPLC analysis of ginger extracts estimated a set of polyphenolic compounds such [6]-gingerol, [8]-gingerol, [10]-gingerol, and [6]-shogaol which are responsible for the antioxidant, anti-fibrotic, and anti-apoptotic protective effects against teratogenic effects of MEK.

CONCLUSIONS: Gingerol fractions (GF) at a dose of 25 mg/kg significantly predicted all tissues organs of mothers and their offspring against the teratogenic effects induced by MEK at a dose of 350 mg/kg. The beneficial effects of ginger phenolic compounds; [6]-gingerol, [8]-gingerol, [10]-gingerol, and [6]-shogaol against teratogenic effects of MEK proceeded through their antioxidant, anti-fibrotic, and anti-apoptotic properties.

Key Words:
Methyl ethyl ketone (MEK), Gingerol fractions, Teratogenicity, Cytotoxicity, Oxidative stress, Fibrosis, Apoptosis.

Introduction

Methyl ethyl ketone (MEK) is a volatile solvent with a sweet and sharpacetone-like odor. MEK is widely used as solvent in common household products, such as inks, paints, cleaning fluids, varnishes, and glues[1-3]. In most industrial applications, it is used as a component of a mixture of organic solvents. It has also been detected in a wide variety of natural products and may be a minor product of normal mammalian metabolism[1-4]. The relative toxicity of ketones is low[2], and no studies were located regarding deaths of humans following inhalation, oral, or dermal exposure to MEK[4,5]. Several studies showed that no cellular toxicological effects appeared on humans inhaled MEK at low doses 200-400 PPM for 2-4 hours/day[2-5]. Only recommended little irritations, psychological, as well as neurological disturbances were present among humans with dermal contact along with inhalation to MEK[2-5].

No information was found specific to genotoxicity, the developmental and reproductive toxicity of MEK in humans. Two retrospective epidemiology studies of workers chronically exposed to

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MEK at petroleum refining plants reported that deaths due to cancers were fewer than expected[6-10]. Data from experimental studies with animals showed that exposing rats, mice or guinea pigs to higher doses of MEK at longer periods significantly produce cellular toxicity in all organelles and increase the rate of mortality[11-15]. A single 8-hour exposure of rats to a 2-butanone concentration of 10000 ml/m3 led to the death of the animals; guinea pigs, however, survived a 4-hour exposure to the same concentration[4,5]. Also, other studies showed that acute inhalation exposure to ≥8,000 ppm 2-butanone resulted in death in rats, mice, and guinea pigs within a few hours[15,16]. In most studies, the cause of death for all rats exposed to 2-butanone at higher doses with longer periods might be due to severe bronchopneumonia confirmed pathologically and histologically[16-18].

Previous research studies[19-23] showed that exposure to MEK at higher doses by inhalation routes, significantly produces a slight to moderate increase in teratogenic effect and the incidence of malformations in rats during the developmental of newborns. Acaudia, imperforate anus, sternebral anomalies, and brachygnathia were found in new offspring's or fetuses[19-21]. Also, significant changes in body weight gain and increased water consumption in the pregnant rats at higher doses of 3,000 ppm 2-butanone indicated that some maternal and fetal toxicity may have occurred at this exposure level[19-24]. Indeed, developmental studies on different animal strains have immensely helped in determining several factors affecting disease progression[25]. The altered expression of reflexes may be explained in terms of impaired neurotransmitters as a result of oxidative stress-induced nervous tissue damage[26]. Declined glutathione and elevated lipid peroxidation are used as indicators of tissue damage[27]. The increased activity of oxidative stress enzymes such as super oxide dismutase (SOD), peroxidase, and catalase in the nervous system tissues indicate the presence of reactive oxygen species, which is a sign of toxicity and causes cellular damage and DNA mutations[26,27].

The pivotal biological toxicity of several organic solvents widely used in industry particularly methyl ethyl ketone (MEK) significantly underlines the urgent need for further clinical and experimental research on herbal medicine as protective remedy to overcome this problem. Previous studies reported that ginger (Zingiber officinale Roscoe) has been commonly consumed as a spice and an herbal medicine for a long time[28,29]. Ginger showed to treat and attenuate the severity of many common diseases such as headaches, colds, nausea, and emesis[29,30]. The protective and treating activities of ginger are relating to the presence of many bioactive compounds such as phenolic and terpene compounds[31,32]. The phenolic compounds are mainly gingerols, shogaols, and paradols, which account for the various bioactivities of ginger[31-33]. In recent years, ginger has been found to possess biological activities, such as antioxidant, anti-inflammatory, antimicrobial, and anticancer activities[28-34]. In addition, accumulating studies have demonstrated the potential activity of ginger in treating and manage several diseases[34-40].

However, little is known about GF activity against cellular toxicity induced by chemical toxins. Thus, in this study, we have evaluated the protective effect of gingerol fraction (GF) against methyl ethyl ketone (MEK) induced teratogenic effects in newborn of mice. We expect that gingerol fractions have a protective role against the oxidative stress induced within the main organs such as the brain regions, liver, and kidney of the newborns following MEK toxicity.

Materials and Methods

Chemicals

All chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA) and other standard commercial sources.

Estimation of Gingerol Fractions (GF) by HPLC Analysis

In this study, column chromatography (18.0 cm by 4.2-cm internal diameter [i.d.]) on silica gel (70-230 mesh) with gradients of n-hexane and ethyl acetate was used to fractionate a total of 10 gr of fresh ginger rhizomes extract into 14 fractions (F1 to F14)[40]. The gingerols were identified in F3 (gingerol fraction [GF]) following high-pressure liquid chromatography (HPLC) analysis as previously reported[40].

Animals and MEK Dosing Schedule

The experiment and the procedures were performed in the labs of the experimental animals at King Saud University in the period of 2017-2018 and significantly approved according to the Ethics Committee of the Experimental Animal Care Society at King Saud University (Permit Number:

PT 1204). Thirty mature female and fifteen male mice (Musmusculus) weighing 25-30 g, obtained from the animal house at the College of Pharmacy, King Saud University were used in the present investigation. The animals were marked, housed (one/cage) and provided a standard diet of known composition (Saudi Company for animal foods) and water ad libitum. To obtain healthy environments, the animals were distributed in special cages equipped with drinking water in their ventilated rooms subject to the appropriate natural factors of moisture, light and temperature ranging from 25°C to 35°C. Mating was accomplished by placing pro-estrous females and male mice overnight. The vaginal plug was detected based on the presence of sperm in vaginal smear and considered as the first day of gestation. Pregnant mice were randomly divided into 3 groups each comprising 10 animals as follows:

Control group (GI): the pregnant mothers received only drinking water by oral intubation.

MEK treated Group (GII): the pregnant mothers received a daily oral dose (350 mg/kg body weight) of MEK dissolved in drinking water for 7 days.

GF treated group (GIII): the pregnant mothers received a daily oral dose (350 mg/kg body weight) of MEK dissolved in drinking water and GF at a dose of 25 mg/kg for 7 days.

Feeding

The animals were given the appropriate food, which is the animal feed experiment No. (P 648) obtained from the General Organization of grain silos and flour mills in Riyadh and it consisted of: raw protein 20%, phosphorus 6.0%, raw fat 5.3% vitamin A 20% international unit /g, ash 6% vitamin D 2.2% international unit/gm, calcium 1% vitamin e 70% international unit/g, salt 5.0%. In addition to the rare mineral elements: cobalt, copper, iodine, iron, manganese and zinc, water is left as needed. The experimental animals were selected at an average age of 12-15 weeks and the average body weight was 60 g.

Postnatal Investigations

In all groups, newborns of each mother were fixed to be 8 pups postnatal day 1 (D1) by transferring newborns from the mother to the other within the same group if the number of pups was less or more than 8. The first-generation pups were observed daily by the experimenter, and the body weights of 10 newborns/group were recorded daily[41-43].

Biochemical Assays

At D7, D14 and D21, newborns from all groups were decapitated to harvest kidney, liver and brain (cerebrum, cerebellum and medulla oblongata). The tissues were homogenized in chilled saline and used for the determination of lipid peroxidation, reduced glutathione, peroxidase, and superoxide dismutase (SOD) as previously reported[44-46]. Liver and kidney function tests were performed according to routine lab methodology previously reported in the literature[47-49].

Histological Preparations

After decapitation at D7, D14 and D21, samples from the kidney, liver, brain (cerebrum, cerebellum and medulla oblongata) and brachial region of the spinal cord were fixed in 205 formalin and processed for hematoxylin and eosin, periodic acid-Schiff's stain (PAS) staining[49-51]. The METAVIR system was applied to measure the score and the degree of fibrosis in liver and kidney tissues and no fibrosis was defined as F0, mild fibrosis as F1, moderate fibrosis as F2, severe fibrosis as F3, and cirrhosis as F4. Significant fibrosis was also defined as F2-4. Hepatic inflammatory activity and apoptotic index were also scored as before[49-51].

Statistical Analysis

Statistical analysis was carried out with SPSS (Statistical Package for Social Science) program version 10 for Windows (SPSS Inc, Chicago, IL, USA). All data tabulated as mean ± SD. The statistical differences were performed by using one-way analysis of variance (ANOVA) and Student's *t*-test. p-value < 0.05 was considered statistically significant.

Results

Estimation of Gingerol Fractions (GF)

In this study gingerol fractions were estimated in fresh ginger rhizomes extract using HPLC analysis. The data showed [6]-gingerol present in 47.9%, followed by 5.6% of [8]-gingerol, 3.8% [10]-gingerol, 1.2% [6]-shogaol, and 41.81%.

Assessments of Liver and Kidney Function

Liver and kidney function were estimated in all mice groups; control (GI), MEK (GII), and GF (GIII) treated mice (Table I). In MEK treated groups, significant change (p-value < 0.001) in the

concentrations of both kidney and liver biochemical functions was estimated in pregnant mothers and their offspring compared to those obtained in normal control group (Table I). The data showed significant increase (p-value < 0.001) in the levels of creatinine, blood urea, SGPT, SGOT, and lower concentrations of albumin among pregnant mothers and their offspring's respectively as in Table I. In addition, total body weight (TBW) was decreased and both relative liver weight, and relative kidney weights were significantly increased in pregnant mothers and their offspring's of MEK treated groups compared to normal control ones (Table I).

When MEK intoxicated pregnant mothers and their offspring's treated with gingerol fractions (GF) at a dose of 25 mg/kg significantly (p-value =0.001) improved both kidney and liver biochemical functions as well as enhancing (p-value =0.001) total body weight (TBW) and relative weights of kidney and liver organs in comparison with mice treated with MEK at a dose of 350 mg/kg as shown in Table I. Significant decrease in the levels of creatinine, blood urea, SGPT, SGOT, and an increase in the concentrations of albumin among pregnant mothers and their offspring's following GF treatment at a dose of 25 mg/kg respectively for seven days (Table I). The results reported that treatment with GF significantly protect pregnant mothers and their offspring from teratogenic effects produced from MEK.

GF Activity Against on Cellular Apoptosis and Fibrosis

Kidney and liver tissues of mothers and their offspring were subjected for evaluating cellular fibrosis and apoptosis by histological examination (Table I). The data showed that MEK at a dose of 350 mg/kg significantly (p-value =0.01) causes the synthesis of cellular fibrosis and expression of apoptosis within kidney and liver tissues of pregnant mothers and their offspring respectively. The data showed that MEK produces cellular toxicity via initiation of cellular fibrosis and apoptosis. However, when MEK intoxicated mice treated with GF at dose of 25 mg/kg significantly (p-value =0.001) decrease fibrosis index and reduce the expression rates of apoptosis compared to MEK treated mice (Table I).

Effect of GF on Cellular Oxidative Stress Induced by MEK in Newborn Mice

Oxidative stress and antioxidant parameters; TBARS, GSH, peroxidase, and SOD were estimated in kidney, liver, cerebrum, and medulla

Table I. Changes in body weight, kidney and liver weights, and the levels of liver and kidney function biomarkers in MEK and GF treated experimental mice.

Biological parameters	Groups					
	Control (GI)		MEK treated (GII)		GF treated (GIII)	
	Mother	offspring	Mother	offspring	Mother	offspring
Final weight (g) TBW LW KW	172 ± 1.9 0.52 ± 0.14 0.62 ± 0.21	131 ± 2.7 0.46 ± 0.23 0.42 ± 0.21	152 ± 2.3[a] 0.85 ± 0.24[a] 0.89 ± 0.62[a]	105 ± 38 [a] 0.68 ± 0.23[a] 0.7 1± 0.28 [a]	168 ± 6.8[b] 0.62 ± 0.15[b] 0.49 ± 0.32[b]	128 ± 48 [b] 0.52 ± 0.17[b] 0.38 ± 0.18 [b]
Kidney function Creatinine (mg/dl) Urea (mg/dl)	0.6 ± 0.32 26.5 ± 3.6	0.52 ± 0.11 16.8 ± 2.3	3.6 ± 2.3 [a] 91.6 ± 12.2 [a]	3.9 ± 0.21 [a] 35.8 ± 3.7 [a]	1.8 ± 1.2 [b] 45.3 ±11.1 [b]	2.1 ± 0.15 [b] 28.3 ± 2.6 [b]
Liver function SGPT (U/L) SGOT (U/L) Albumin (mg/dL)	18.7 ± 2.8 22.6 ± 1.7 4.1 ± 2.1	16.8 ± 4.2 19.4 ± 4.3 3.9 ± 2.3	62.4 ± 3.4 [a] 48.6 ± 3.1[a] 2.9 ± 0.26[a]	36.4 ± 6.8[a] 45.1 ± 3.4[a] 3.6 ± 2.4[a]	32.8 ± 4.2 [b] 29.5 ± 5.1[b] 4.2 ± 0.46[b]	26.2 ± 9.1[b] 31.1 ± 7.5[b] 3.9 ± 3.2[b]
Fibrosis score: (N, %) No fibrosis (0-1) Fibrosis (2-3)	- -	- -	1 (10%) 9 (90 %) [a]	2 (20 %)[a] 8 (80%)[a]	6 (60 %)[b] 4 (40 %) [b]	7 (70 %)[b] 3 (30%)[b]
Apoptotic index	0.56 ± 0.08	0.18 ± 0.13	4.8 ± 2.6[a]	12.9 ± 0.89 [a]	2.9 ± 1.8[b]	4.6 ± 1.96 [b]

All values represent mean ± SD. [a]p-value < 0.01 (MEK *vs*. Control). [b]p-value < 0.001 (GF *vs*. MEK). Student's t-test. GOT: Glutamic oxaloacetic transaminase, GPT: Glutamic pyruvic transaminase, TBW: total body weight, LW: liver weight, KW: kidney weight.

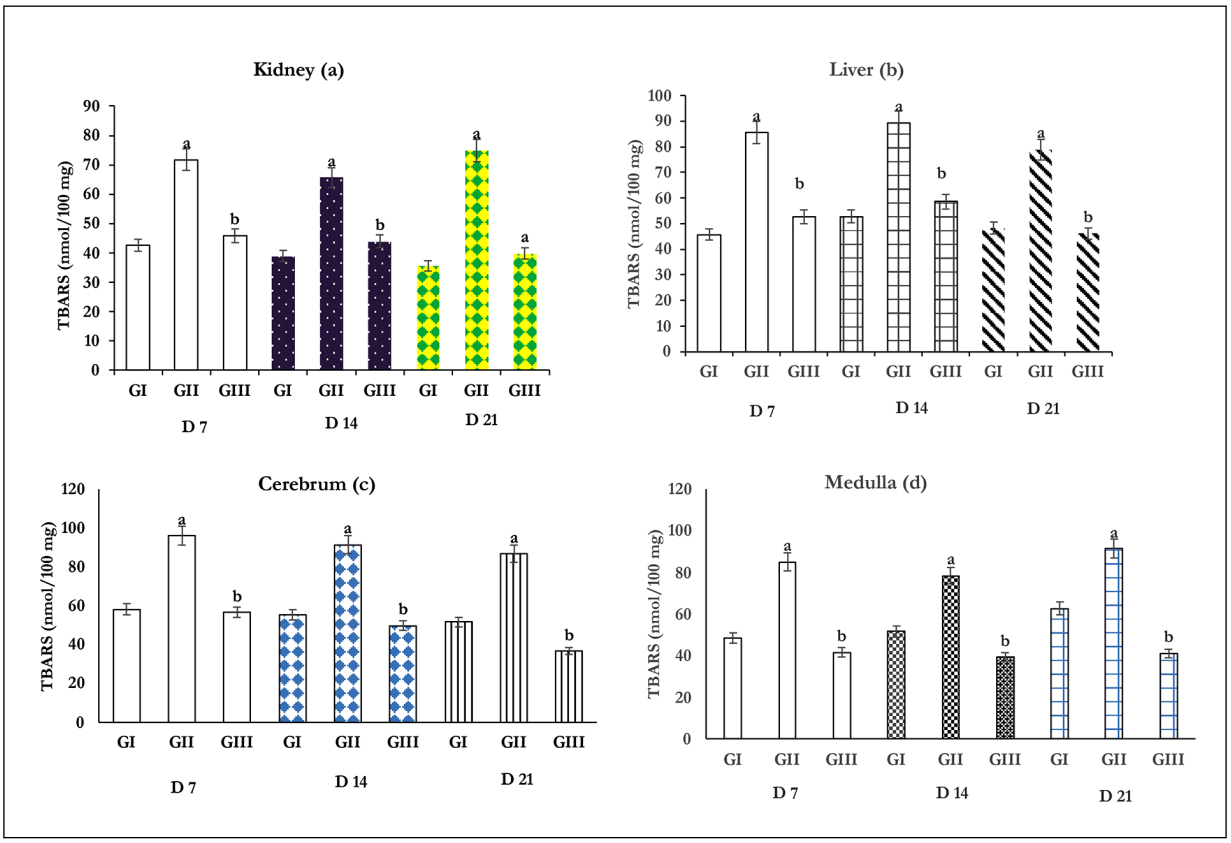

Figure 1. TBARS in kidney **(a)**, liver **(b)**, cerebrum **(c)**, and medulla oblongata **(d)** of the pups of normal control group (GI) and MEK-treated group (GII), and GF treated mice (GIII) at postnatal day 7, 14 and 21. Data are expressed as mean ± SE (N = 10; a *p*-value <0.01 and b *p*-value <0.001, significantly different between the groups). MEK: methyl ethyl ketone; GF: gingerol fractions.

of all mice groups as shown in Figures 1-4. In mice treated with MEK at a dose of 350 mg/kg, a marked increase (*p*-value=0.01) was found in the expression of TBARS in all tissue organs kidney (Figure 1a), liver (Figure 1b), cerebrum (Figure 1c), and medulla (Figure 1d) at D7, D14, and D21, respectively. In addition, when the mice treated with GF at a dose of 25 mg/kg significantly reduced the expression of the cellular TBARS in all studied organs, cellular functions significantly improved as shown in Figure 1 (a-d). GF treated mice exhibited marked decrease in TBARS level in all tested brain regions (Figures 1c-d), liver (Figure 1b) and kidney (Figure 1a) at D7, D14, and D21, respectively.

Similarly, cellular GSH and antioxidant enzymes; peroxidase and SOD activity were significantly improved toward normal levels in all tissue organs following treatment with GF at a dose of 25 mg/kg (Figure 2 and Figure 3). Compared to MEK treated mice, a significant (*p*-value <0.001) increase in GSH levels in kidney (Figure 2a), liver (Figure 2b), the cerebrum (Figure 2c), and medulla oblongata (Figure 2d) were observed in all mice tissues at D7, D14, and D21, respectively, following treatment with GF (25 mg/kg). In the same manner, both peroxidase and SOD activities were significantly increased in all tissue organs following treatment with GF at a dose of 25 mg/kg (Figure 3 and Figure 4). The data obtained were significantly compared to that obtained from MEK treated mice, whereas a significant decrease (*p*-value =0.01) in peroxidase and SOD activity in kidney (Figure 3e and Figure 4e), liver (Figure 3b and Figure 4b) and brain tissues cerebrum (Figures 3c and 4c) and medulla oblongata (Figures 3c and 4c) respectively at D7, D14, and D21. The data confirmed that GF obtained from ginger extracts significantly protect all tissue organs from MEK cellular toxicity via antioxidant pathways.

Table II. Association between oxidative stress parameters with apoptosis and fibrosis in kidney and liver tissues of newborn mice intoxicated with MEK (350 mg/kg) and treated with GF at dose of 25 mg/kg.

Oxidative stress parameters	Fibrosis											
	MEK treated (GII)						GF treated (GIII)					
	At D7		At D14		At D21		At D7		At D14		At D21	
	Liver	Kidney	Liver	Kidney	Liver	Kidney	Liver	Kidney	Liver	Kidney	Liver	Kidney
GSH (nmol/gm)[b]	-0.18	-0.38	-0.39	-0.58	-0.35	-0.86	0.45	0.68	0.45	0.75	0.74	0.96
SOD (U/gm)[b]	-0.36	-0.254	-0.518	-0.612	-0.16	-0.19	0.51	0.312	0637	0.458	0.247	0.238
TBARS nmol/100gm)[c]	0.368	0.125	0.458	0.314	0.234	0.825	-0.59	-0.164	-0.49	-0.529	-0.29	-0.831
Oxidative stress parameters	**Apoptosis**											
	MEK treated (GII)						GF treated (GIII)					
	At D7		At D14		At D21		At D7		At D14		At D21	
	Liver	Kidney	Liver	Kidney	Liver	Kidney	Liver	Kidney	Liver	Kidney	Liver	Kidney
GSH (nmol/gm)[c]	-0.256	-0.115	-0.38	-0.235	-0.29	-0.28	0.125	0.247	0.347	0.128	0.234	0.154
SOD (U/gm)[c]	-0.315	-0.231	-0.18	-0.361	-0.19	-0.128	0.342	0.275	0.124	0.348	0.134	0.185
TBARS (nmol/100 gm)[c]	0.232	0.235	0.358	0.261	0.345	0.167	-0.28	-0.291	-0.38	-0.224	-0.39	-0.123

Correlation coefficients (R); All values represent mean ± SD. [b]$p < 0.01$.[c] $p < 0.001$. Student's *t*-test.

In this study, the data showed that MEK toxicity to the newborn mice proceeds via cellular damage, fibrosis, and increasing cellular apoptosis, which occurs via free radical oxidative stress. The data showed that both fibrosis and apoptosis in liver and kidney cells significantly correlated positively with higher TBARS expression levels, and negatively associated with the decline in the levels of GSH and SOD, respectively, as shown in Table II. Also, the improvement in cellular antioxidant enzymes, reduction in the initiation of cellular free radical (TBARS), fibrosis, and apoptosis were significantly correlated in all tissues of GF treated mice. The improved fibrosis and apoptosis in kidney and liver tissues positively correlated with GSH, SOD, peroxidase, and negatively with cellular free radical (TBARS) (Table II).

Discussion

In this study, the treatment of pregnant female mice with gingerol fractions (GF) at a dose of 25 mg/kg significantly protected all tissues organs of mothers and their offspring against the teratogenic effects induced by MEK at a dose of 350 mg/kg.

Ginger showed to treat and attenuate the severity of many common diseases such as headaches, colds, nausea, and emesis[29,30]. The protective and treating activities of ginger are relating to the presence of many bioactive compounds such as phenolic and terpene compounds[31,32]. The phenolic compounds are mainly gingerols, shogaols, and paradols, which account for the various bioactivities of ginger[31-33].

In this study, MEK at a dose of 350 mg/kg significantly produces severe weight loss, cytotoxicity, increased oxidative stress, and teratogenicity in all brain parts, as well as fibrosis, and apoptosis in the liver and kidney tissues of the postnatal mice. Similarly, several studies[26,27] reported that the altered expression of reflexes may be explained in terms of impaired neurotransmitters, as a result of oxidative stress-induced nervous tissue damage[26]. Declined glutathione and elevated lipid peroxidation are used as indicators of tissue damage[27]. Also, the increased activity of oxidative stress enzymes such as super oxide

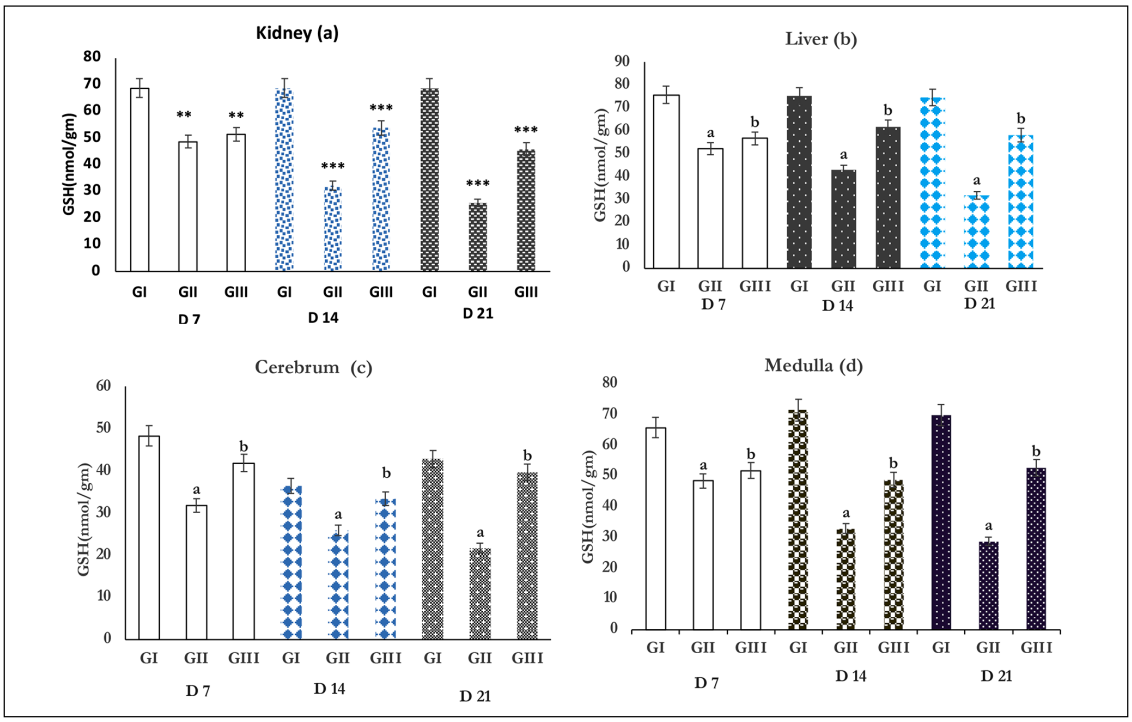

Figure 2. Shows GSH in kidney **(a)**, liver **(b)**, cerebrum **(c)**, and medulla oblongata **(d)** of the pups of normal control group (GI) and MEK treated group (GII), and GF treated mice (GIII) at postnatal day 7, 14 and 21. Data are expressed as mean ± SE (N = 10; a *p*-value <0.01 and b *p*-value <0.001, significantly different between the groups). MEK: methyl ethyl ketone ketone; GF: gingerol fractions.

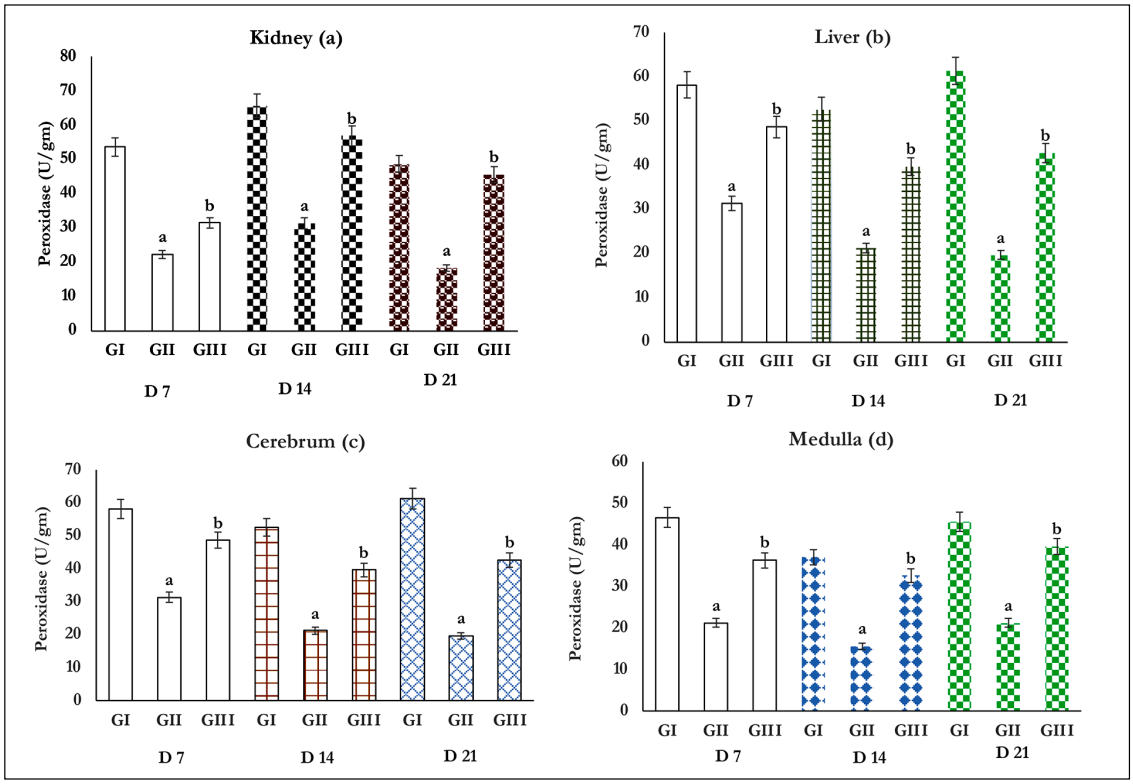

Figure 3. Peroxidase in kidney **(a)**, liver **(b)**, cerebrum **(c)**, and medulla oblongata **(d)** of the pups of normal control group (GI) and MEK-treated group (GII), and GF treated mice (GIII) at postnatal day 7, 14 and 21. Data are expressed as mean ± SE (N = 10; a *p*-value <0.01 and b *p*-value <0.001, significantly different between the groups). MEK: methyl ethyl ketone; GF: gingerol fractions.

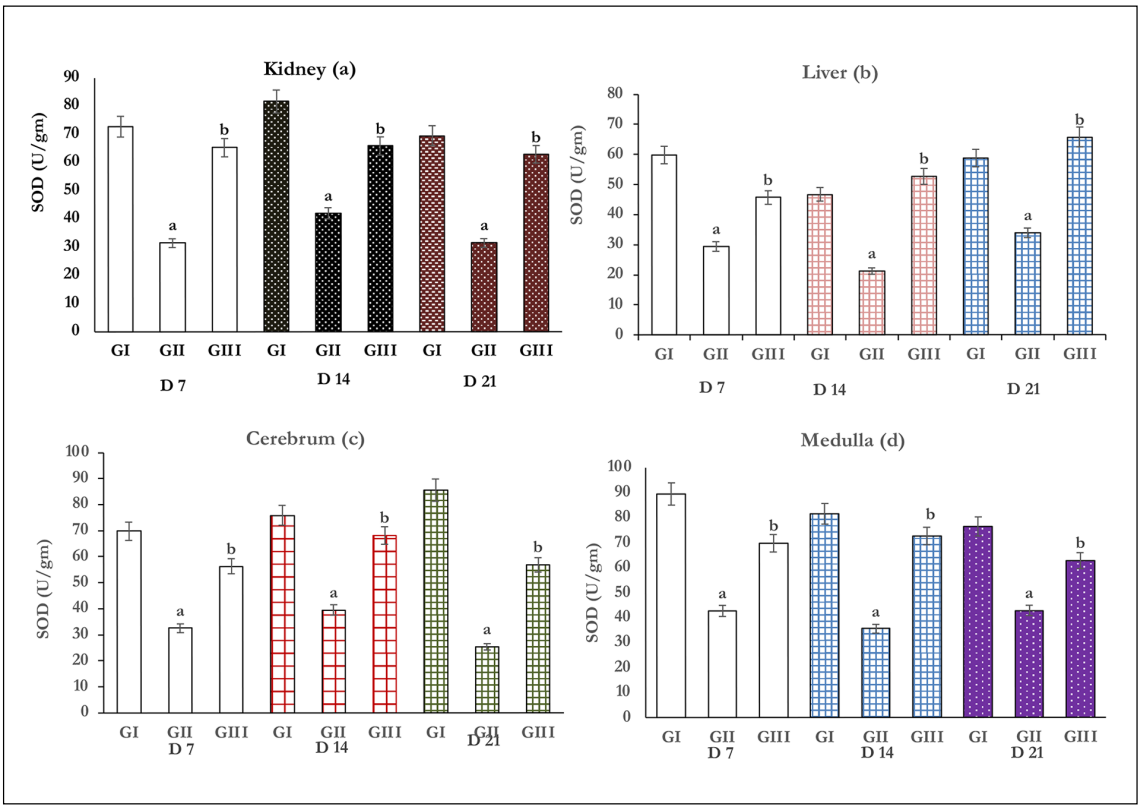

Figure 4. SOD in kidney **(a)**, liver **(b)**, cerebrum **(c)**, and medulla oblongata **(d)** of the pups of normal control group (GI) and MEK-treated group (GII), and GF treated mice (GIII) at postnatal day 7, 14 and 21. Data are expressed as mean ± SE (N = 10; a *p*-value <0.01 and b *p*-value <0.001, significantly different between the groups. MEK: methyl ethyl ketone; GF: gingerol fractions.

dismutase (SOD), peroxidase, and catalase in the nervous system tissues indicates the presence of reactive oxygen species, which is a sign of toxicity which causes cellular damage and DNA mutations[26,27]. Previously, other studies showed that pregnant mothers treated with a certain substance under special conditions are not solely affected, as the offspring could express the clinical features of the disease caused by the mother-treated substance[28]. Postnatal developmental body weights, behavioral reflexes, and even the histological and physiological status of some vital organs in the newborn mice may be the main evident consequences of toxic treatments affecting pregnant mothers[29]. Previous research studies showed that exposure to MEK at higher doses by inhalation routes, significantly produces a slight to moderate increase in teratogenic effect and the incidence of malformations in rats during the developmental of newborns[19-23].

In this current study, when mothers and their offspring treated with GF (25 mg/kg), a significant improvement in cellular antioxidant enzymes GSH, SOD, and peroxidase activities along with a reduction in the initiation of cellular oxidative free radicals (TBARS). In addition, significant reduction in cellular fibrosis and apoptosis was reported in all tissues of mothers and their offspring's following treatment with GF. In recent years, ginger has been found to possess biological activities, such as antioxidant, anti-inflammatory, antimicrobial, and anticancer activities[28-34]. In addition, accumulating studies have demonstrated the potential activity of ginger in treating and manage several diseases[34-39].

Thus, in this study, the protective activity of GF was related to the presence of [6]-gingerol present in 47.9%, followed by 5.6 % of [8]-gingerol, 3.8% [10]-gingerol, and 1.2% [6]-shogaol. These phenolic compounds account for various bioactivities of ginger extracts such as antioxidant, anti-fibrotic, anti-apoptotic, and anti-teratogenic effects against MEK cellular toxicity. Like other various models of cytotoxicity[52-56], our results focus mainly on anti-oxidative, anti-inflammatory, and anti-apoptotic effects of

gingerol-enriched fractions of ginger extract. Also, previous studies showed that ginger extract usually contain polyphenol compounds, such as [6]-gingerol, [8]-gingerol, and [10]-gingerol, which have been cited as the main components responsible for its pharmacological effects[52-54].

Conclusions

In this study, the treatment of pregnant female mice with gingerol fractions (GF) at a dose of 25 mg/kg significantly protected all tissues organs of mothers and their offspring's against the teratogenic effects induced by MEK at a dose of 350 mg/kg. The beneficial effects of ginger phenolic compounds [6]-gingerol, [8]-gingerol, [10]-gingerol, and [6]-shogaol against teratogenic effects of MEK proceeded through their antioxidant, anti-fibrotic, and anti-apoptotic properties.

Conflicts of Interest

The authors declare that they hav no conflicts of interest.

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Authors' Contribution

All authors contributed equally to designing, performing practical, interpreting the results, and contributed in manuscript writing and preparing the final manuscript.

Institutional Review Board Statement

The experimental protocols and investigations comply with the Guide for Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996). The study was approved by the Ethics Committee for Animal Experimentation at PNU.

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