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AMELIORATIVE EFFECTS OF GINGER EXTRACT ON PARABEN-INDUCED LIPID PEROXIDATION IN THE LIVER OF MICE

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Abstract: We have evaluated the ameliorative effect of ginger extract on paraben (*p*-hydroxybenzoic acid)-induced lipid peroxidation in the liver of mice. Adult female albino mice were orally administered with 2.25 or 4.50 mg of paraben in 0.2 mL olive/animal/day (67.5 and 135 mg/kg of body weight) for 30 days. The results revealed significantly higher ($p \leq 0.05$) lipid peroxidation in the liver of paraben-treated mice than that of controls. As compared with the controls, the levels of non-enzymatic antioxidants: glutathione and ascorbic acid, as well as the enzymatic antioxidants: superoxide dismutase, glutathione peroxidase and catalase were significantly ($p \leq 0.05$) lowered in the liver of paraben-treated mice. Oral administration of aqueous extract of *Zinziber officinale* (3 mg/animal/day) along with paraben for 30 days (Groups 6 and 7) caused significant ($p \leq 0.05$) amelioration in paraben-induced lipid peroxidation and increased significantly ($p \leq 0.05$) the activities of enzymatic (superoxide dismutase, glutathione peroxidase, catalase) and contents of non-enzymatic (glutathione and ascorbic acid) antioxidants in the liver of mice, as compared with those given paraben alone (Groups 4, 5). Thus, oral administration of aqueous extract of *Zinziber officinale* along with paraben significantly ($p \leq 0.05$) ameliorates paraben-induced lipid peroxidation in the liver of mice.

Keywords: paraben, lipid peroxidation, liver, ginger extract

Parabens are alkyl esters of *p*-hydroxybenzoic acid. They are commonly used as preservatives since the 1920s, in thousands of products including personal care products, pharmaceuticals, cosmetics and food. The six forms of parabens are used in approximately 13,200 cosmetic products (1). Rastogi et al. found out that parabens were present in 77% of rinse-off cosmetics and in 99% of leave-on products (2). Parabens possess estrogen-like activities and interrupt a normal hormone function (3). Parabens may induce oxidative stress in the skin (4). The determination and toxicological characterization of products of the reaction between *p*-hydroxybenzoic acid esters and reactive oxygen species (ROS) are very important, because of the frequent use of parabens in cosmetics and possible generation of O_2^- in the skin.

It is well known that natural antioxidants including phenolic or thiolic compounds could protect against damages caused by reactive oxidants by various biological mechanisms in living cells (5). In dried ginger powder, shogal, a dehydrated product of gingerol is a predominant pungent constituent (6). The powdered rhizome contains 3-6% fatty oil, 9% protein, 60-70% carbohydrates, 3-8% crude fiber, about 8% ash, 9-12% water and 2-3% volatile oil. Ginger (*Zinziber officinale)* is known to possess antioxidant activity (7). Water and alcoholic extracts of ginger dose dependently inhibit oxidation of fatty acid (8). Ginger extracts also showed selective anti-cancer activity (9).

The present study was undertaken to evaluate the possible ameliorative effect of aqueous ginger extract on paraben induced lipid peroxidation and its antioxidative defence mechanisms in the liver of mice.

EXPERIMENTAL

Materials and methods

All the chemicals used were of analytical grade. All the reactions were run in freshly obtained double distilled water. Paraben (*p*-hydroxybenzoic acid) was purchased from HI Media, Mumbai, India. Inbred young adult Swiss strain female albino mice (*Mus musculus*) weighing approximately 30-35 g were obtained from Vaccine Institute, Gandhinagar, India.

Animals were provided with animal feed and water *ad libitum* and maintained in 12 h light/dark

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cycles at 26 ± 2°C. Guidelines for care and use of animals in scientific research (1991), published by the Indian National Science Academy, New Delhi, India, were followed. Each group consisted of ten animals and was caged separately. Group 1 (untreated control) animals were maintained without any treatment. Animals of Group 2 and 3 received olive oil (0.2 mL/animal/day) or aqueous ginger extract (3 mg/animal/day), respectively, for 30 days and served as pre-treatment controls. Animals of Group 4 and 5 were orally administered with paraben in doses of 2.25 and 4.5 mg/0.2 mL olive oil/animal/day, respectively, for 30 days. Group 6 and 7 animals were orally treated with paraben as mentioned for Group 4 and 5 animals and were given aqueous ginger extract for 30 days.

Olive oil was obtained from Figaro, Madrid. Shade dried ginger (*Zinziber officinale*) was purchased from local market and aqueous extract was prepared according to WHO protocol CG-06 (10). 5 g of finely ground ginger powder and 100 mL of double distilled water was stirred on a magnetic stirrer for 1.5 h. The mixture was filtered twice through Whatman filter paper no.1. The filtrate was collected and allowed to dry. Paraben was dissolved in olive oil; hence it was administered as a vehicle alone in Group 2. The dose of paraben was based on LD_{50} value of paraben (11). Females are comparatively more sensitive than males, therefore, females were used in the present study. All the treatments were given orally for 30 days, using a feeding tube attached to a hypodermic syringe .

On completion of the treatment, the animals were sacrificed by cervical dislocation.

The livers of all animals were quickly isolated, blotted free of blood and used for biochemical analysis. The lipid peroxidation in the liver of animals was measured by quantification of thiobarbituric acid reactive substances (TBARS) determined by the method of Ohkawa et al. (12) using malondialdehyde (MDA) as a substrate. The activity of superoxide dismutase in the liver of controls and treated groups of animals was assayed by the modified spectrophotometric method of Kakkar et al. (13). The activities of glutathione peroxidase and catalase in the liver of controls and all treated groups of mice were assayed by the modified method of Pagila and Valentine (14) and Luck (15), respectively.

The concentration of glutathione and total ascorbic acid were measured in the liver of mice by the method of Grunert and Philips (16) and Roe and Kuether (17), respectively. All the samples were analyzed for protein concentration by the method of Lowry et al. (18).

Phytochemical screening of *Zinziber officinale*

Chemical tests were carried out on the aqueous extract and on the powdered specimens using standard procedures to identify the constituents as described by Sofowara (19), Trease and Evans (20) and Harborne (21).

Tests for tannins

About 0.5 g of the dried powdered sample was suspended in 20 mL of water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added and observed for brownish green or blue black color.

Test for phlobatannins

Deposition of red precipitate when an aqueous extract of each plant sample was boiled with 1% aqueous HCl was taken as an evidence for the presence of phlobatannins.

Test for saponin

2 g of the powdered sample was boiled in 20 mL of distilled water in a water bath and filtered. 10 mL of the filtrate was mixed with 5 mL of distilled water and shaken vigorously. The frothing was mixed with 3 drops of olive oil and shaken vigorously, then observed for formation of emulsion.

Test for flavonoids

5 mL of dilute ammonia solution were added to a portion of the aqueous filtrate of each plant extract, followed by addition of concentrated H_2SO_4. A yellow coloration indicated the presence of flavonoids.

Test for terpenoids (Salkowski test)

5 mL of extract was treated with 2 mL of chloroform and concentrated H_2SO_4 (3 mL) was added. A reddish brown coloration of the interface was formed to show positive result.

Test for alkaloids

The test was performed with Mayer's, Wagner's and Dragendorff's reagents. Observation of white, brown and orange color, respectively, indicated the presence of alkaloids.

After confirmation from qualitative test, these constituents were quantitatively determined. Alkaloids were determined by using Harborne (21) method. Saponins were quantified by Obadoni and Ochuko (22) method and flavanoids were quantitatively estimated acc. to Bohm and Kocipai-Abyazan (23).

The results were expressed as the means ± S.E.M. The data were statistically analyzed using the one way analysis of variance (ANOVA) fol-

Table 1. Effect of aqueous extract of ginger on paraben induced changes in the lipid peroxidation and antioxidative defence mechanism in liver of mice

Parameters	Experimental groups						
	1	2	3	4	5	6	7
Lipid peroxidation (nmoles MDA/mg protein/60min)	4.15 ± 0.06	4.14 ± 0.06	4.11±0.07	5.08 ± 0.01[abcdfg]	7.02 ± 0.01[abcdfg]	4.02 ± 0.01[abcdef]	4.43± 0.05[abcdef]
Total ascorbic acid	4.01 ± 0.05	4.13 ± 0.01	4.05 ± 0.01	2.87± 0.12[abcefg]	1.65 ± 0.18[abcefg]	3.69 ± 0.14[abcdeg]	3.02 ± 0.31[abcdef]
Glutathione peroxidase (nmoles NADPH consumed/mg protein/min)	3.23 ± 0.08	3.84 ± 0.17	3.13 ± 0.26	2.34 ± 0. 90[abcefg]	1.22 ± 0.05[abcdefg]	2.77 ± 0.13[abcdeg]	2.52 ± 0.11[abcdef]
Catalase (μmoles H_2O_2 consumed/mg protein/min)	11.86 ± 0.20	12.11 ± 0.01	12.24 ± 0.02	7.84 ± 0.17[abcefg]	5.86 ± 0.11[abcdeg]	10.49 ± 0.13[abcdeg]	9.25 ± 0.19[abcdef]
Superoxide dismutase (units/mg protein)	2.35 ± 0.15	2.45 ± 0.26	2.42 ± 0.13	1.63 ± 0.17[abcefg]	0.846 ± 0.11[abcdfg]	2.07 ± 0.04[abcdeg]	1.17 ± 0.01[abcdef]
Glutathione (μmoles/100 mg tissue weight)	56.31 ± 0.06	57.16 ± 0.01	54.20 ±0.01	43.36 ± 0.06[abcef]	30.97 ± 0.27[abcdfg]	53.42 ± 0.11[abcdeg]	43.30 ± 0.16[abcef]

[a] as compared to group 1, $p < 0.05$; [b] as compared to group 2, $p \leq 0.05$; [c] as compared to group 3, $p \leq 0.05$; [d] as compared to group 4, $p \leq 0.05$; [e] as compared to group 5, $p \leq 0.05$; [f] as compared to group 6, $p \leq 0.05$; [g] as compared to group 7, $p \leq 0.05$; Values are the arithmetic mean ± S.E.M.; n = 10. Group 1 – untreated control, Group 2 – vehicle control, Group 3 – antidote control, Group 4 – low dose, Group 5 – high dose, Group 6 – low dose + antidote, Group 7 – high dose + antidote.

lowed by Tukey test. The levels of significance was accepted with $p \leq 0.05$.

RESULTS AND DISCUSSION

The photochemical character of *Zinziber officinale* indicated the presence of alkaloids, saponins and flavonoids. Tannins, phlobatannins and terpenoids were absent. Quantitative estimation revealed that *Zinziber officinale* contained 0.87% of alkaloids and 0.935% of flavonoids, while saponins were present in a concentration of 0.226%.

The results shown in Table 1 revealed no significant alterations between untreated and vehicle control groups (Groups 1, 2). Also oral administration of aqueous ginger extract for 30 days in mice caused no significant alterations except significant ($p \leq 0.05$) reduction in glutathione content in the liver (Group 3), as compared to the untreated and vehicle controls (Groups 1, 2). The level of lipid peroxidation was significantly higher ($p \leq 0.05$) in paraben-treated mice than that in the controls. As compared to the controls (Groups 1, 2, 3), the paraben treatment for 30 days (Groups 4, 5) caused significant ($p \leq 0.05$), reduction in the activities of catalase, superoxide dismutase and glutathione peroxidase, as well as in the levels of glutathione and total ascorbic acid in the liver of mice. Thus, paraben treatment caused an increase in lipid peroxidation by decreasing the antioxidative defence mechanism of the cell (Table 1).

Oral administration of aqueous ginger extract along with paraben for 30 days (Groups 6, 7) significantly ($p \leq 0.05$) ameliorated paraben-induced lipid peroxidation. This could be due to the significantly ($p \leq 0.05$) higher activities of superoxide dismutase, catalase and glutathione peroxidase and higher levels of glutathione and total ascorbic acid in the liver of animals receiving aqueous ginger extract plus paraben (Groups 6, 7), as compared with those given paraben alone (Groups 4, 5).

The results clearly indicate an increase in lipid peroxidation in the liver of paraben-treated mice as compared to the controls. Paraben causes lipid peroxidation (4) and also induces severe cell injury accompanied by a significant decrease in cellular levels of both glutathione (GSH) and protein (24).This also involves inactivation of critical cellular enzymes. Nakagawa and Moore (25) have also reported that when butylparaben (0.05, 0.10, or 0.25 mM) was added to the isolated rat mitochondria, it increased the rate of state 4 oxygen consumption and inhibited the rate of state 3 oxidation. Methyl paraben-treated human skin keratinocytes and exposure to UV-B light significantly increased cell death, oxidative stress, NO

production, lipid peroxidation and activation of transcription factors (26). Our earlier studies have reported the antioxidative effect of ginger extract on paraben induced lipid peroxidation in *in vitro* study. (27).

Oral administration of aqueous ginger extract along with paraben for 30 days (Groups 6, 7) caused a significant ($p \leq 0.05$) amelioration in paraben-induced lipid peroxidation by increasing the antioxidative activity of the cells. Ginger with anticancer potential is also good source of antioxidants and therefore may be capable of preventing tissue damage by ROS (28). Ginger (*Z. officinale;* 1% w/w) significantly lowered lipid peroxidation by ameliorating the activities of the antioxidant enzymes: superoxide dismutase, catalase and glutathione peroxidase in rats (29). Feeding with ginger (*Zingiber Officinale* Rosc.) to rats fed the normal diet and those fed the high fat diet lowered levels of TBARS and hydroperoxides and raised the activities of SOD and CAT and levels of reduced GSH in the aorta, liver, kidney and intestine significantly, compared to rats fed an unsupplemented normal or high fat diet, respectively (30). The aqueous extracts of ginger (*Zinziber officinale*) inhibited lipid peroxidation by 65, 72 and 66%, respectively, and inhibited the formation of diene, triene and tetraene conjugates in human erythrocyte membrane (31). Hong et al. reported that supplementation with ginger can reduce free radical-mediated oxidative stress to the cells. The crude gingerol extract was found to have antioxidant activity (32).

It is concluded that oral administration of aqueous ginger extract along with paraben significantly ameliorates paraben-induced lipid peroxidation by increasing the antioxidative defence mechanism of the cells.

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