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NATURAL DRUGS

ANTIOXIDATIVE EFFECT OF RHIZOME OF *ZINZIBER OFFICINALE* ON PARABEN INDUCED LIPID PEROXIDATION: AN *IN VITRO* STUDY

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Abstract: Antioxidative effect of aqueous extract of rhizome of Ginger (*Zinziber officinale)* was examined on *p*-hydroxybenzoic acid (paraben) induced lipid peroxidation. Addition of paraben (25-150 μg/mL) to liver and kidney homogenates significantly increases H_20_2 induced lipid peroxidation *in vitro*. Effect was dose dependent up to 100 μg/mL concentration. An addition of aqueous extract of ginger significantly reduced paraben (100 μg/mL) induced lipid peroxidation in liver and kidney homogenates. The effect was concentration dependent.

Keywords: Paraben, ginger extract, lipid peroxidation.

Synthetic methyl, ethyl and propyl parabens are developed from benzoic acid and are considered effective and economical. They are inexpensive to use as both cosmetic and food grade preservatives. A report published in the Journal of American College of Toxicology estimated that parabens could be found in over 13000 cosmetic products (1). Since 1984 many things in cosmetology changed. However, recently this preservative system has come into question as these substances have been found in cancerous tissues, especially breast cancer tissue (2).

The determination and toxicological characterization of products of the reaction between *p*-hydroxybenzoic acid esters and superoxide radicals are very important because of the frequent use of parabens in cosmetics and possible generation of $O^{\cdot}_2{}^{-}$ in the skin. Parabens may induce oxidative stress in the skin (3).

Ginger (*Zinziber officinale)* is known to possess antioxidant activity (4). Water and alcoholic extracts of ginger have been dose dependly inhibited oxidation of fatty acid (5). Ginger extracts also showed selective anticancer activity (6).

Lipid peroxidation is a complex process that damages the cell structure and function (7). Peroxidation of membrane lipids initiates the loss of membrane integrity, membrane bound enzyme activity and cell lysis (8, 9).

The present *in vitro* study was undertaken to evaluate the lipid peroxidation inducing effect of paraben and its amelioration by aqueous ginger extract.

EXPERIMENTAL

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 twice filtered through Whatman filter paper no. 1. The filtrate was collected and allowed to dry. Liver and kidney of healthy Swiss strain female albino mice weighing 30-35 g were used for experimental purpose.

A standard method of assessing lipid peroxidation is the thiobarbituric acid test (11). The tissue was homogenized in phosphate buffered saline (PBS) to prepare 10% (w/v) tissue homogenate. 0.2 mL of homogenate (without prior centrifugation to remove tissue debris) was then added to the tubes containing varying concentration of paraben (*p*-hydroxybenzoic acid) (0 to 150 μg/mL), 0.2 mL of 8.1% sodium dodecyl sulfate (SDS), 1.5 mL of 20% acetic acid solution (adjusted to pH 3.5 with 1 M NaOH) and 1.5 ml of 1% thiobarbituric acid (TBA) solution. Paraben was purchased from HI Media Laboratories Limited, Mumbai, India. Paraben was dissolved in 1 M saline (0.9% NaCl).

Another set of tubes containing 100 μg of paraben and 0-0.5 mL of ginger extract (concentra-

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tion 0-100 µg/mL) were added. 0.2 mL of 8.1% sodium dodecyl sulfate (SDS) 1.5 mL of 20% acetic acid solution (adjusted to pH 3.5 with 1 M NaOH) and 1.5 mL of 1% thiobarbituric acid (TBA) solution were prepared.

Reactions were initiated by the addition of 10 mM H_2O_2 and the mixture was incubated at 37°C for 30 min with intermittent shaking. Blank tube was prepared by substituting TBA with distilled water. The tubes were then heated at 95°C for 60 min, cooled and 10% trichloroacetic acid (TCA) was added. Solution was mixed and centrifuged at 1000 g for 15 min.

The aliquot of the resulting supernatant fraction was read against blank on Systronics 118 UV-Vis spectrophotometer (Systronics, Ahmedabad, India) at 532 nm. Protein was determined acc. to Lowry et al. (12) using bovine serum albumin as standard. All chemicals used were of analytical grade. Data were expressed as mean ± S.E.M. Statistical analysis was performed using the Student t-test.

RESULTS

Results shown in Figures 1 and 2 clearly indicate that paraben increases H_20_2 induced lipid peroxidation in liver and kidney homogenates. At 100 µg/mL paraben concentrations, maximum lipid peroxidation was observed in both liver and kidney homogenates. Further increase in paraben concentrations caused lesser lipid peroxidation as compared to 100 µg/mL, though it was always significantly higher than the control. The lipid peroxidation was comparatively higher in kidney homogenate than that of liver homogenate.

Results shown in Figures 3 and 4 indicate that concurrent addition of ginger extract (0-100 µg/mL) along with paraben (100 µg/mL) significantly retarded paraben-induced lipid peroxidation. An addition of only aqueous ginger extract (control) did not cause significant increase in lipid peroxidation. An addition of 100 µg/mL paraben caused significant increase in lipid peroxidation. However, addition of paraben along with ginger aqueous extract significantly retarded paraben induced lipid peroxidation. The effect was concentration-dependent with maximum retardation at 100 µg/mL concentration of aqueous ginger extract. Retardation was significant as compared to toxin treated ones (Figs. 3 and 4).

DISCUSSION AND CONCLUSION

At 100 µg/mL paraben concentrations, maximum lipid peroxidation was observed in both liver and kidney homogenates (Figs. 1 and 2). It might be due to the direct action of paraben on the plasma membrane causing lipid peroxidation, membrane permeability alteration and cell lysis. Nakagawa and Moore (13) have reported that *p*-hydroxybenzoate ester induces cytotoxicity in rat hepatocytes due to mitochondrial membrane permeability transition. Parabens may induce oxidative stress in the skin after conversion to GSH conjugates of hydroquinone by reacting with $O^{\cdot}_2{}^{-}$ (3).

The results indicate that lipid peroxidation was concentration dependent with maximum retardation at 100 µg/mL concentration of aqueous ginger extract (Figs. 3 and 4). It could be due to antioxidative properties of ginger extracts. Ginger contains antioxidant components that act within the cell

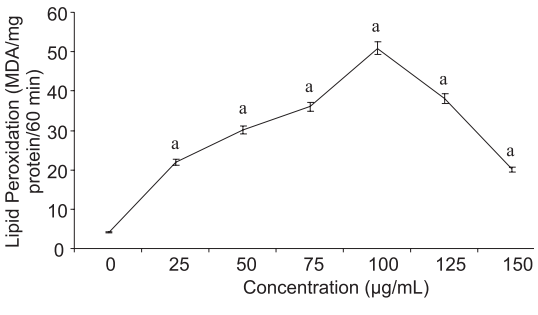

Figure 1. Effect of paraben on lipid peroxidation in liver homogenate.
Values are mean ± S.E.M., n = 10
Significant at level:
[a] $p < 0.001$ (as compared to control)

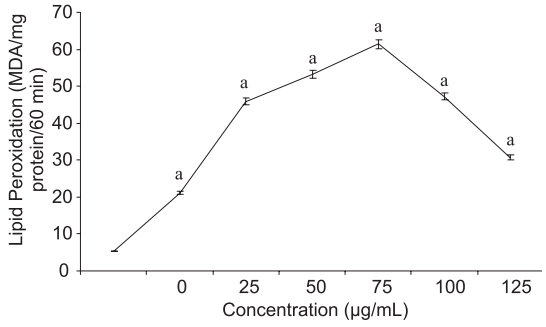

Figure 2. Effect of paraben on lipid peroxidation in kidney homogenate.
Values are mean ± S.E.M., n = 10
Significant at level:
[a] $p < 0.001$ (as compared to control)

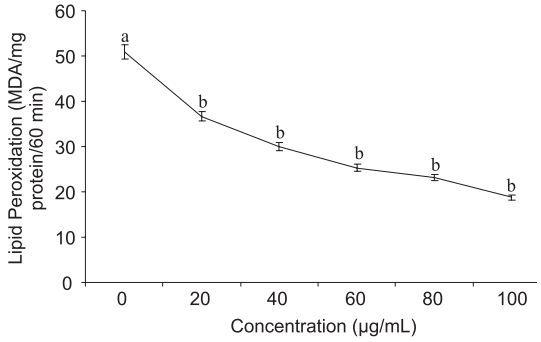

Figure 3. Effect of aqueous ginger extract on paraben induced lipid peroxidation in liver homogenate.
Values are mean ± S.E.M., n = 10
Significant at level:
[a] $p < 0.001$ (as compared to control)
[b] $p < 0.001$ (as compared to paraben)

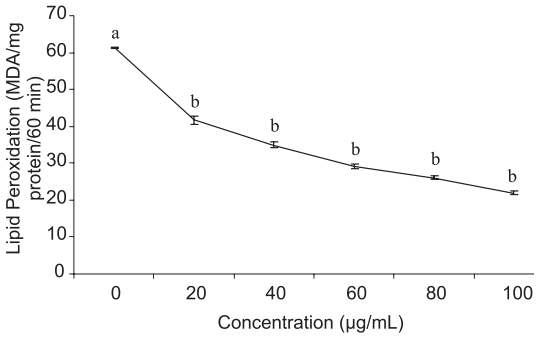

Figure 4. Effect of aqueous ginger extract on paraben induced lipid peroxidation in kidney homogenate.
Values are mean ± S.E.M., n = 10
Significant at level:
[a] $p < 0.001$ (as compared to control)
[b] $p < 0.001$ (as compared to paraben)

membrane and slow lipid peroxidation *in situ* (14). Aqueous extract of ginger is known to possess antioxidant activity (15) and significantly lowered the lipid peroxidation by maintaining the activities of superoxide dismutase, catalase and glutathione peroxidase (16).

In conclusion, ginger aqueous extract is an effective reductant for reduction of lipid peroxidation and cytotoxicity of paraben.

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