Parabens are alkyl esters of p-hydroxybenzoic acid widely used in foodstuff, cosmetics, toiletries and pharmaceuticals. Parabens are carcinogenic (1) and hepatotoxic in nature. Our earlier studies have shown that addition of paraben to saline suspension of RBC caused swelling and eventual bursting of cell at higher concentrations and at lower concentration morphological alteration occurs. The effect could be due to paraben-induced oxidative stress and lipid peroxidation (2).

Ginger (Zingiber officinale Roscoe, Zingiberaceae) has been widely used as a dietary spice, as well as in traditional oriental medicine and possesses potential in chemopreventive activities. Ginger extracts showed selective anticancer activity (3). It has been implicated as one of the promising chemopreventive agents against colon and skin cancer (4). Ginger is also known to possess antioxidant activity (5) and significantly lowered the lipid peroxidation by maintaining the activities of superoxide dismutase, catalase and glutathione peroxidase (6).

The present investigation is an attempt to evaluate the ameliorative effect of an aqueous extract of ginger, on paraben-induced hemolysis in vitro.

**EXPERIMENTAL**

The extract was prepared according to WHO protocol CG-06 (7). Dried ginger was obtained from market and made to fine powder. 5 g of finely ground ginger powder and 100 mL of double distilled water was stirred with a magnetic stirrer for 1.5 h. The mixture was twice filtered with normal and then through Whatman filter paper no. 1. The supernatant was collected and allowed to dry.

Human venous blood was collected in EDTA bulbs from well-nourished healthy adults (25-30 years old). It was diluted with saline (0.9% NaCl) and centrifuged at 1000 g for 10 min. RBC pellets were washed twice and suspended in fresh saline so as to get cell density of 2 ◊ 10^4 RBC/mL. For examining hemolysis and its amelioration by aqueous extract of ginger four sets of tubes were prepared.

1. Control tubes containing 2.0 mL of RBC suspension and 2 mL of normal saline.
2. Antioxidant control tube containing 100 µg/mL of aqueous ginger extract added to 2.0 mL of RBC suspension.
3. Toxin treated tubes – 150 µg/mL paraben and 2.0 mL of RBC suspension.
4. Tubes containing 150 µg/mL of paraben and varying concentration of aqueous ginger extract (5-100 µg/mL) in 2.0 mL of RBC suspension.

The total volume of each tube was made up to 4 mL with additional saline. All tubes were incubated at 37°C for 4 h with intermittent shaking.
Required concentration of aqueous ginger extract and paraben were prepared in saline.

Absorbance of the supernatants obtained after centrifuging the incubated tubes at 1000 g for 10 min, were read spectrophotometrically at 540 nm (8). Percent hemolysis was calculated by the formula:

\[
\text{Percent Hemolysis} = \frac{\text{Absorbance of individual tubes}}{\text{Absorbance with 100 % hemolysis}} \times 100
\]

100% hemolysis was prepared by adding 2 mL of distilled water to 2 mL of RBC suspension (9). Percent retardation with different concentration of antioxidant was calculated with the formula (10):

\[
\text{Percent Retardation} = \frac{A - B}{A} \times 100
\]

Where A = paraben induced hemolysis

B = hemolysis caused by concurrent addition of paraben and antioxidant.

Student ‘t’ test was used for statistical analysis of the data.

RESULTS

In control and antidote treated tube the cells remain settled in the bottom of the tubes with clear ambient supernatant and RBC appeared as intended spheres or biconcave discs. Incubation of RBC suspension with 150 µg/mL of paraben causes pronounced swelling and cell lysis (79% hemolysis). Incubation of RBC suspension with paraben (150 µg/mL) in the presence of 5-100 µg/mL of aqueous ginger extract caused reductions in paraben-induced hemolysis. The effect was concentration dependent with maximum retardation at 40-60 µg/mL concentration of ginger extract. Further increase in concentration caused lesser retardation as compared to 40-60 µg/mL concentration, however, retardation was significant as compared to toxin treated one (Table 1).

DISCUSSION AND CONCLUSION

The results shown in Table 1 clearly indicate that 150 µg/mL concentration of paraben caused swelling and eventual bursting of cells. 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 (11) have also reported that p-hydroxybenzoate ester induces cytotoxicity in rat hepatocytes due to mitochondrial membrane permeability transition.

The results shown in Table 1 also indicate that concurrent addition of ginger extract in paraben significantly retards paraben-induced hemolysis. It could be due to antioxidative properties of ginger extracts. Ginger contains antioxidant component(s)

<table>
<thead>
<tr>
<th>Paraben µg/mL</th>
<th>Aqueous ginger extract µg/mL</th>
<th>% Hemolysis</th>
<th>% Retardation</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>2.11±0.356</td>
<td>-</td>
</tr>
<tr>
<td>0</td>
<td>100</td>
<td>1.78±0.355</td>
<td>-</td>
</tr>
<tr>
<td>150</td>
<td>0</td>
<td>78.18±2.00</td>
<td>-</td>
</tr>
<tr>
<td>150</td>
<td>5</td>
<td>50.58±0.86</td>
<td>35.30</td>
</tr>
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<td>150</td>
<td>10</td>
<td>41.51±1.731</td>
<td>46.90</td>
</tr>
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<td>150</td>
<td>20</td>
<td>31.12±1.60</td>
<td>60.19</td>
</tr>
<tr>
<td>150</td>
<td>30</td>
<td>23.00±1.03</td>
<td>70.58</td>
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<td>75.13</td>
</tr>
<tr>
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<td>18.91±0.62</td>
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</tr>
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</tr>
<tr>
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<td>34.82</td>
</tr>
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<td>90</td>
<td>26.83±1.89</td>
<td>65.68</td>
</tr>
<tr>
<td>150</td>
<td>100</td>
<td>22.52±1.80</td>
<td>71.19</td>
</tr>
</tbody>
</table>

Values are mean ± S.E.M.; n = 7

a* = p < 0.001 or as compared to control
b* = p < 0.001 or as compared to treated
Aqueous ginger extract ameliorates paraben induced cytotoxicity

that act within the cell membrane and slow lipid peroxidation in situ (12). The aqueous extract of ginger was also found to inhibit the formation of diene, triene and tetraene conjugates in human erythrocyte membrane (13).

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

REFERENCES


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