

GINGER EXTRACT AMELIORATES PARABEN INDUCED BIOCHEMICAL CHANGES IN LIVER AND KIDNEY OF MICE

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Abstract: The present investigation was undertaken to evaluate the effect of paraben (p-hydroxybenzoic acid) on acidic, basic, and neutral proteins content, as well as carbohydrate and cholesterol contents in liver and kidney of mice. Adult female albino mice were orally administrated with 2.25 and 4.5 mg of paraben in 0.2 mL of olive/animal/day for thirty days. The results revealed dose dependent, significant reduction in acidic, basic, and neutral protein, carbohydrate contents and an increase in cholesterol content of the investigated liver and kidney. Oral administration of aqueous extract of *Zinziber officinale* (3 mg/animal/day) along with paraben for thirty days caused significant amelioration in all the protein types, carbohydrate and cholesterol of liver and kidney.

Keywords: protein, ginger, paraben, liver, kidney

Parabens are a group of chemicals widely used as preservatives in food and cosmetic and therapeutic products. Paraben is the common name for this class of chemicals, however, they are also known by other names such as esters of p-hydroxybenzoic acid. A preferential use of methyl > ethyl > propyl > butyl > benzyl paraben in various groups of cosmetic products were reported and 77% of cosmetics have been found to contain paraben (1). Parabens also have a long history of use in a variety of pharmaceutical products intended for either injection, inhalation, oral, topical, rectal or vaginal administration (2, 3). Parabens are preservatives that have been identified as estrogenic and disruptive of normal hormone functions (4).

Our earlier studies have shown that addition of paraben to saline suspension of RBC caused swelling and eventual bursting of cell and ginger aqueous extract is an effective reductant for percentage hemolysis and cytotoxicity of paraben (5).

Recently, intensive researches on biological function of natural antioxidants have been carried out with numerous plant material worldwide, including those used as foods (6). It is also well known that natural antioxidants including phenolic or thiol compounds could protect against damages caused by reactive oxidants by various biological mechanisms in living cells (7).

Ginger (*Zingiber Officinale Roscoe*) is one of the world's best known spices, and it has also been universally used throughout history for its health

benefits. Ginger extract possesses antioxidative characteristic, since it can scavenge superoxide anion and hydroxyl radicals (8). Ginger acts as a hypolipidemic agent in cholesterol-fed rabbits (9, 10).

The present study was undertaken to evaluate the possible ameliorative effect of aqueous ginger extract on paraben-induced damage in the protein types, sugar and cholesterol of liver and kidney.

MATERIAL AND METHODS

Paraben was purchased from HI Media, Mumbai, India. All the chemicals used were of analytical grade. All the reactions were run in freshly prepared double distilled water.

Shade dried ginger (*Zinziber officinale*) was purchased from local market and aqueous extract was prepared according to WHO protocol CG-06 (11). 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 with Whatman filter paper no. 1. The filtrate was collected and allowed to dry.

Young adult inbred Swiss stain 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 cycles at 25±2°C. Animal feed was prepared as per the formulation given by National

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Institute of Occupational Health, Ahmedabad, India and was confirmed to be free of any toxins. Guidelines for care and use of animals in Scientific Research 1991 published by Indian National Science Academy, New Delhi, India, were followed.

Seventy animals were divided into seven groups and 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) and *Zinziber officinale* aqueous extract (3 mg/animal/day), respectively, for 30 days and served as a pretreatment control. Animals of group 4 and 5 were orally administrated with paraben in 2.25 mg (low dose; LD) and 4.5 mg (high dose; HD) of paraben in 0.2 mL of olive/animal/day for 30 days. Group 6 and 7 animals were orally treated with paraben along with aqueous ginger extract (3 mg/animal/day) for 30 days.

The dose of paraben (p-hydroxybenzoic acid) was based on LD₅₀ value (12). Females are comparatively more sensitive than males, therefore they were used in the present study. Olive oil was obtained from Figaro, Madrid, Spain. All the treatments were given orally using a feeding tube attached to a hypodermic syringe for 30 days.

The acidic, basic and neutral proteins were extracted separately by the method of Shashi and Singh (13). Determination of acidic, basic and neutral proteins was done spectrophotometrically by the method described by Lowry et al. (14) using bovine serum albumin as standard.

The concentration of cholesterol was estimated in the liver and kidney of controls and all treated groups of mice by the method of Zlatki et al. (15). The concentration of total carbohydrate was done by the method of Seifter et al. (16). Concentration of reducing sugar was estimated by dinitrosalicylic acid (DNSA) method of Van Bezeij (17).

STATISTICAL ANALYSIS

The results were expressed as mean $\pm$ SEM. The data were statistically analyzed using one way analysis of variance (ANOVA) followed by Tukey test. The levels of significance were accepted with $p < 0.05$. Comparisons of p values between different groups were performed.

RESULTS

The effect of paraben (p-hydroxybenzoic acid) and paraben plus aqueous ginger extract treatment on biochemical parametres in liver is shown in Table 1. No significant difference in protein types,

carbohydrate, and cholesterol contents were observed between different groups of controls.

Oral administration of paraben for 30 days caused, as compared with controls (Groups 1-3), significant ($p < 0.05$) dose dependent reduction in proteins and carbohydrate contents. On the other hand, cholesterol content was significantly ($p < 0.05$) higher, as compared to controls, in the liver of paraben treated mice. Treatment with aqueous ginger extract along with paraben caused significant ($p < 0.05$) amelioration, as compared with paraben alone treated, in liver of mice (Groups 6, 7).

Amelioration was almost complete in all parameters in low dose paraben treated group (Group 6). However, amelioration was comparatively lower in high dose paraben treated (Group 7) but values were still significantly ($p < 0.05$) different from the controls.

Table 2 shows the effect of paraben-treated with and without ginger aqueous extract treatment on the biochemical changes in the kidney of mice. There was no significant change among untreated (Group 1) vehicle (Group 2) and antidote (ginger aqueous extract) (Group 3) controls.

Oral administration of paraben for 30 days caused significant reduction, as compared with vehicle control, in proteins and carbohydrate contents, in kidney of mice and cholesterol content was significantly ($p < 0.05$) higher.

Treatment with aqueous ginger extract along with paraben caused significant ($p < 0.05$) almost complete amelioration in proteins, carbohydrate and cholesterol contents as compared to paraben alone treated group (Groups 4, 5).

DISCUSSION

The present study clearly indicates that oral administration of paraben for 30 days caused significant reduction in acidic, basic and neutral proteins and carbohydrate content and increase in cholesterol content in liver and kidney of mice (Table 1, 2). Paraben causes lipid peroxidation (18) that indirectly affects the protein synthesis. Reactive oxygen species (ROS) induced lipid peroxidation disrupts the structural integrity of the protein and hence destroys them. This also involves inactivation of critical cellular enzymes, which are involved in protein synthesis. Nakagawa and Moore (19) also have reported that p-hydroxybenzoate ester induces cytotoxicity in rat hepatocytes due to mitochondrial membrane permeability transition.

Administration of ginger aqueous extract alone did not cause, as compared to vehicle control group,

Table 1. Effect of paraben on liver biochemical parameters and its amelioration by aqueous ginger extract.

Parameter	Control			Paraben Treated			Paraben Treated + Aqueous ginger extract	
	Group I Control	Group II Vehicle Control	Group III Antidote Control	Group IV Low Dose	Group V High Dose	Group VI LD + Antidote	Group VII HD + Antidote	
Acidic protein	6.72 ± 0.07	6.59 ± 0.08	6.70 ± 0.02	3.40 ± 0.03 ^{abcdefg}	2.27 ± 0.01 ^{abcdefg}	5.90 ± 0.02 ^{abcdehg}	5.03 ± 0.06 ^{abcdehfg}	
Basic protein	4.12 ± 0.05	4.03 ± 0.01	4.14 ± 0.01	1.74 ± 0.01 ^{abcdefg}	0.81 ± 0.01 ^{abcdefg}	3.72 ± 0.05 ^{abcdehg}	2.91 ± 0.01 ^{abcdehfg}	
Neutral protein	1.17 ± 0.01	1.19 ± 0.01	1.14 ± 0.04	0.51 ± 0.02 ^{abcdefg}	0.31 ± 0.01 ^{abcdefg}	1.01 ± 0.02 ^{abcdehg}	0.828 ± .01 ^{abcdehfg}	
Cholesterol	0.80 ± 0.04	0.81 ± 0.01	0.81 ± 0.08	1.20 ± 0.07 ^{abcdefg}	1.30 ± 0.01 ^{abcdefg}	0.94 ± 0.01 ^{abcdehg}	1.04 ± 0.08 ^{abcdehfg}	
Reducing sugar	1.74 ± 0.006	1.67 ± 0.10	1.82 ± 0.05	0.63 ± 0.07 ^{abcdefg}	0.41 ± 0.01 ^{abcdehfg}	1.15 ± 0.07 ^{abcdehg}	0.89 ± 0.01 ^{abcdehfg}	
Total sugar	6.70 ± 0.10	6.71 ± 0.09	6.37 ± 0.04	3.40 ± 0.01 ^{abcdefg}	1.82 ± 0.04 ^{abcdefg}	5.97 ± 0.01 ^{abcdehg}	4.51 ± 0.01 ^{abcdehfg}	

Values are mean ± S.E.M; n=10
^a as compared to group I; ^b p< 0.05; ^c as compared to group II; ^d p< 0.05 ^e as compared to group III; ^f p< 0.05; ^g as compared to group V; ^h as compared to group VI; ⁱ p< 0.05; ^jas compared to group VII; ^kp<0.05.

Table 2. Effect of paraben on kidney biochemical parameters and its amelioration by aqueous ginger extract.

Parameter	Control			Paraben Treated			Paraben Treated + Aqueous ginger extract	
	Group I Control	Group II Vehicle Control	Group III Antidote Control	Group IV Low Dose	Group V High Dose	Group VI LD + Antidote	Group VII HD + Antidote	
Acidic protein	6.05 ± 0.01	5.92 ± 0.01	6.04 ± 0.02	3.04 ± 0.15 ^{abcdefg}	2.54 ± 0.01 ^{abcdefg}	5.43 ± 0.02 ^{abcdehg}	4.82± 0.03 ^{abcdehfg}	
Basic protein	3.84 ± 0.02	3.78 ± 0.01	3.8 ± 0.01	1.65 ± 0.01 ^{abcdefg}	0.78 ± 0.01 ^{abcdefg}	3.51± 0.01 ^{abcdehg}	2.61± 0.01 ^{abcdehfg}	
Neutral protein	1.00 ± 0.01	1.01 ± 0.04	1.03 ± 0.01	0.47 ± 0.01 ^{abcdefg}	0.26 ± 0.01 ^{abcdefg}	0.85 ± 0.01 ^{abcdehg}	0.78 ± 0.01 ^{abcdehfg}	
Cholesterol	0.60 ± 0.01	0.56 ± 0.04	0.61 ± 0.08	0.78 ± 0.08 ^{abcdefg}	0.95 ± 0.05 ^{abcdefg}	0.65 ± 0.01 ^{abcde}	0.80 ± 0.06 ^{abcdehfg}	
Reducing sugar	0.65 ± 0.05	0.66 ± 0.01	0.63 ± 0.01	0.25 ± 0.08 ^{abcdefg}	0.19 ± 0.01 ^{abcdefg}	0.48 ± 0.01 ^{abcdehg}	0.41 ± 0.06 ^{abcdehfg}	
Total sugar	0.73 ± 0.07	0.79 ± 0.01	0.79 ± 0.01	0.34 ± 0.08 ^{abcdefg}	0.16 ± 0.07 ^{abcdefg}	0.63 ± 0.07 ^{abcdehg}	0.45 ± 0.04 ^{abcdehfg}	

Values are mean ± S.E.M; n=10
^a as compared to group I; ^b p< 0.05; ^c as compared to group II; ^d p< 0.05 ^e as compared to group III; ^f p< 0.05; ^g as compared to group IV; ^h as compared to group V; ⁱ as compared to group VI; ^j p< 0.05; ^kas compared to group VII; ^kp<0.05.

any significant changes in different protein parameters in liver and kidney of mice. However, ginger aqueous extract along with paraben treatment caused significant increase in acidic, basic, and neutral protein and carbohydrate content and also decreased cholesterol content in liver and kidney of paraben treated mice. Feeding rats ginger significantly elevated the activity of hepatic cholesterol-7 α -hydroxylase, the rate-limiting enzyme in bile acids biosynthesis, thereby stimulating cholesterol conversion to bile acids, resulting in elimination of cholesterol from the body (20). In addition, a pure constituent from ginger [E-8 beta, 17-epoxyabd-12-ene-15,16-dial (ZT)], was shown to inhibit cholesterol biosynthesis in homogenated rat liver (21). All the indigenous materials with antitubercular potential of ginger are also good source of antioxidants and therefore may be capable of preventing tissue damage by ROS (22). Decrease in ROS production will reduce the protein deprivation in all the animals. Ginger has dominative protective effect on DNA damage induced by H₂O₂ (23).

It is concluded that oral administration of ginger aqueous extract ameliorates paraben-induced changes in acidic, basic and neutral proteins, carbohydrate and cholesterol contents of liver and kidney.

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