

A STUDY ON TOXICITY OF GASOLINE AND GM-10 ON LIVER OF MICE AND IT'S AMELIORATION BY BLACK TEA EXTRACT

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Abstract: The aim of present study is to investigate the ameliorative effect of black tea extract on gasoline and GM-10 induced toxicity in liver of mice. Eighty healthy male mice weighing 38-40 g approximately were divided into eight groups which included untreated control and various treated groups. Mice were treated with Gasoline 462 mg/kg/day and GM-10 low dose (206 mg/kg/day) and high dose (412 mg/kg/day) subcutaneously for 30 days. Black tea extract was given as 2 g/100 mL drinking water (2% w/v) instead of pure drinking water. All the animals were sacrificed on 31st day by cervical dislocation and livers were isolated and weighed. Parameters such as lipid peroxidation, catalase, superoxide dismutase, glutathione peroxidase, glutathione and total ascorbic acid were studied. The results revealed dose-dependent toxicity of gasoline and GM-10 on liver. Administration of black tea extract ameliorates this toxicity of gasoline and GM-10 in liver of mice. This proves the effective ameliorative effect of black tea extract.

Keywords:- gasoline, GM-10, black tea extract, liver, amelioration

Occupational health and safety, a field that involves many disciplines, is of major concern in environmental health. Occupational exposure is also a vital source of information on environmental hazards. Workplace exposures are usually more intense than those which occur in the non-occupational environment. Biological monitoring for occupational exposure to alternative liquid fuel and environmental impact assessment of emission are matters of great concern regarding the risk to human health.

Gasoline

Gasoline is one of the indispensable energy sources and is the primary product of most petroleum refineries. An exposure to liquid gasoline occurs by unintentional or intentional ingestion, accidental skin contact or by misuse of the solvent. The misuse of gasoline, especially to clean and degrease floors, tools and machine parts, represents the single most important health risk from gasoline for the general public. Persons who unintentionally ingest gasoline while siphoning and those who intentionally inhale gasoline vapors to obtain euphoric effects, risk serious health consequences (1).

The skin, a major environmental interface to the human body, is repeatedly exposed to a broad array of exogenous chemicals potentially capable of causing toxicity. After gasoline application to skin, a decrease in glutathione concentration, glutathione-S-transferase activity and lipid peroxidation were observed in skin, liver and brain (2).

Methanol

Methanol is a colorless liquid with essentially no odor and very little taste. Methanol as a fuel has a lower density, thus allowing more air into the engine. This decreases the fuel to air ratio and possibly lowers carbon monoxide emissions.

The majority of the available information on methanol toxicity in human beings is related to acute rather than chronic exposure. The toxic effects after repeated or prolonged exposure to methanol are believed to be qualitatively similar by less severe than those induced by acute exposure (3).

Gasoline : methanol mixture

The new Energy Development Organization (NEDO) in Japan and Health Effects Institute

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(HET), USA have initiated programs to evaluate the human health following exposure to automotive gasoline : methanol blend vapor.

Poon et al. (4) studied short-term inhalation toxicity of methanol, gasoline and methanol : gasoline blend in rats. Depression in weight gain and reduced feed consumption were observed in male rats and increased relative liver weight was detected in rats of both sexes exposed to gasoline or methanol : gasoline mixture.

Joshi and Verma (5) studied the effect of gasoline : methanol exposure on the lungs of rats. The results showed significantly higher total cells, neutrophils and lymphocyte counts, increased acid phosphatase activity and significantly lowered macrophages count in broncho-alveolar lavage fluid (BALF).

Tea (*Camellia sinensis*)

Tea is second only to water in worldwide popularity as a beverage. Consumption of tea has been associated with many health benefits including the prevention of cancer and heart diseases (6).

Oral administration of green or black tea powder inhibited the lipid peroxidation of liver induced by *tert*-butyl hydroperoxide in rats (7), while in kidney, the antioxidant effect was observed only for the green tea fed group. Serafini et al. (8) showed that ingestion of tea produced a significant increase in human plasma antioxidant capacity.

The present study is an attempt to investigate gasoline and GM-10 induced toxic changes in liver of Swiss strain male albino mice (*Mus musculus*) and the possible amelioration by black tea extract.

MATERIALS AND METHODS

Animals

Young adult inbred Swiss strain male albino mice (*Mus musculus*) weighing 38-40 g were obtained from Alembic Ltd., Baroda, India. Animals were provided with animal feed and water *ad libitum* and maintained under 12 h light/dark cycles at 26°C. Animal feed was prepared as per the formulation given by the National Institute of Occupational Health, Ahmedabad, India.

Chemicals

Gasoline was procured from an authorized outlet of Indian Oil Corporation Ltd., Ahmedabad, India. Methanol was procured from Sisco Research Laboratories Pvt. Ltd., Mumbai, India. All other chemicals used in present study were of analytical grade.

Animal studies

Eighty animals were divided into eight groups and caged separately. Group I (control) animals were maintained without any treatment. Animals of Group II received black tea extract 2 g/100 mL drinking water (2% w/v) instead of pure drinking water for 30 days. Group III animals were subcutaneously administered with 462 mg/kg body weight/day gasoline for 30 days. Animals of Group IV and V were subcutaneously administered gasoline : methanol (90 : 10, GM-10) blend in doses 206 mg/kg/day (low dose) and 412 mg/kg/day (high dose) for 30 days. Animals of Group VI, VII and VIII were treated with gasoline and GM-10 as mentioned for Groups III, IV, and V, respectively, and were additionally given black tea extract 2 g/100 mL drinking water (2% w/v) instead of pure drinking water for 30 days. The doses of gasoline and GM-10 high dose in this experiment were the same in order to evaluate the comparative toxicity between the two. Black tea solids (Lipton Yellow Label of Hindustan Lever Ltd., Mumbai, India) and deionized water were used to produce a 2% tea infusion. The effective dose of black tea extract was based on work done earlier (9).

On completion of the treatment, the animals were sacrificed by cervical dislocation. Livers of animals were quickly isolated, blotted free of blood and weighed to the nearest mg on a balance.

Lipid peroxidation was measured by the method of Ohkawa et al. (10), catalase by the method of Luck (11), superoxide dismutase activity by the method of Kakkar et al. (12), glutathione peroxidase by the method of Paliga and Valentine (13), glutathione by the method of Grunert and Phillips (14) and total ascorbic acid by the method of Roe and Kuether (15). For all the parameters a minimum of 10 replicates were used and the data were statistically analyzed using one way analysis of variance (ANOVA) followed by Tukey test. The level of significance was accepted with $p < 0.05$. Comparisons of p-values between different groups were performed.

RESULTS

Table 1 shows the effect of gasoline and GM-10 alone as well as along with black tea extract on lipid peroxidation, activities of catalase, superoxide dismutase and glutathione peroxidase, as well as glutathione and total ascorbic acid contents in the liver of mice. Except significant reduction in glutathione and total ascorbic acid contents in the liver of black tea extract treated mice (Group 2) no significant

Table 1. Effect of black tea extract on gasoline and GM-10 induced lipid peroxidation and antioxidative defense mechanism in liver of mice.

Parameters	Experimental Groups							
	1	2	3	4	5	6	7	8
	Control	Black Tea Extract	Gasoline	GM-10 Low Dose	GM-10 High Dose	Gasoline+Black Tea Extract	GM-10 Low Dose + Black Tea Extract	GM-10 High Dose + Black Tea Extract
Lipid peroxidation (nmoles MDA/mg protein/60 min.)	2.79 ± 0.14	2.68 ± 0.13	3.84 ^{abefgh} ± 0.16	3.25 ^{abcdeh} ± 0.08	3.73 ^{abefgh} ± 0.08	2.82 ^{cde} ± 0.15	2.51 ^{cde} ± 0.11	2.70 ^{cde} ± 0.08
Catalase activity (µmoles H ₂ O ₂ consumed/mg protein/min)	44.60 ± 0.52	44.54 ± 0.63	41.71 ^{abefgh} ± 0.45	43.73 ^{abce} ± 0.46	42.02 ^{abefgh} ± 0.47	43.61 ^{abefgh} ± 0.44	44.39 ^{cdef} ± 0.45	44.29 ^{ce} ± 0.45
Superoxide dismutase activity (units/mg protein)	3.83 ± 0.18	3.89 ± 0.18	2.91 ^{ab} ± 0.12	3.23 ^a ± 0.16	2.83 ^{ab} ± 0.11	3.36 ± 0.17	3.36 ± 0.17	3.27 ± 0.16
Glutathione peroxidase activity (nmoles NADPH consumed/mg protein/min)	0.506 ± 0.014	0.497 ± 0.025	0.423 ^{afgh} ± 0.022	0.489 ± 0.023	0.449 ^{gh} ± 0.019	0.503 ^c ± 0.026	0.551 ^{ce} ± 0.021	0.538 ^{ce} ± 0.018
Glutathione (mg/100 mg tissue weight)	49.30 ± 2.19	38.22 ^a ± 1.50	40.41 ^{afgh} ± 1.64	43.75 ^{abh} ± 1.55	42.88 ^{abh} ± 1.02	44.82 ^{abeh} ± 1.14	46.62 ^{bc} ± 1.22	49.90 ^{bcdef} ± 1.34
Total ascorbic acid (mg/g tissue weight)	1.81 ± 0.08	1.47 ^a ± 0.08	1.37 ^{afgh} ± 0.07	1.57 ± 0.04	1.37 ^{afgh} ± 0.06	1.80 ^{ce} ± 0.09	1.78 ^{ce} ± 0.08	1.78 ^c ± 0.08

Values are the means ± S.E.M.; n = 10; ^a as compared to Group I; p < 0.05; ^b as compared to Group II; p < 0.05; ^c as compared to Group III; p < 0.05; ^d as compared to Group IV; p < 0.05; ^e as compared to Group V; p < 0.05; ^f as compared to Group VI; p < 0.05;

^g as compared to Group VII; p < 0.05; ^h as compared to Group VIII; p < 0.05

alterations were observed between control (Group 1) and black tea extract treated group (Group 2).

The level of lipid peroxidation was significantly ($p < 0.05$) higher in gasoline and GM-10 treated groups than that of control (Group 1). The effect was dose-dependent. As compared to control (Group 1), gasoline and GM-10 treatment for 30 days caused significant dose-dependent reduction in catalase, superoxide dismutase and glutathione peroxidase (only in gasoline), as well as level of glutathione and total ascorbic acid in the liver of mice. Thus gasoline and GM-10 treatment caused a dose-dependent increase in lipid peroxidation by decreasing the antioxidative defense mechanism of the cell.

Administration of black tea extract (orally) along with gasoline and GM-10 (subcutaneously) significantly ($p < 0.05$) mitigates gasoline and GM-10 induced lipid peroxidation. This could be due to higher activities of catalase, superoxide dismutase, glutathione peroxidase, as well as glutathione and ascorbic acid contents. Superoxide dismutase amelioration was significant in gasoline and high dose GM-10 treated groups receiving tea extract (Group VI and VIII) as compared to gasoline and GM-10 high dose alone treated groups (Groups III and V).

DISCUSSION

Gasoline and GM-10 administration caused hyperactivity for short duration in mice. Edematous swelling was observed at the site of subcutaneous injection in GM-10 high dose and gasoline treated groups. Joshi (16) have also reported edematous swelling after subcutaneous administration of GM-10.

The present study clearly indicates that an increase in lipid peroxidation is due to significant reduction in the activities of enzymatic antioxidants such as catalase, superoxide dismutase and glutathione peroxidase as well as non-enzymatic antioxidants such as glutathione and total ascorbic acid in the liver of gasoline and GM-10 treated mice as compared to the controls. Oxidative stress is mainly due to increased formation of reactive oxygen species (17). Superoxide dismutase protects cell from oxidative damage by breaking down a potentially hazardous free radicals superoxide ($O_2^{\cdot-}$) to H_2O_2 and O_2 . The H_2O_2 produced can then be decomposed enzymatically by catalase and glutathione peroxidase (GSH-PX). GSH-PX not only decomposes H_2O_2 but can also interact with lipid peroxidation. Thus significant reduction in these enzyme activities (Table 1) could be responsible for increased lipid peroxidation due to gasoline and GM-10.

Glutathione content is decreasing in our study after gasoline and GM-10 treatment which suggests its rapid oxidation. Glutathione has a beneficial effect by virtue of possessing -SH groups which help to protect biological membranes, which are readily susceptible to injury by peroxidation (18).

Our present study shows reduction in ascorbic acid levels in the liver after 30 days of gasoline and GM-10 treatment. During free radical scavenging action, ascorbic acid is transformed into L-dehydroascorbate. Reduced glutathione is required for the conversion of L-dehydroascorbate back to ascorbate. The fall in the level of reduced glutathione decreases the conversion of L-dehydroascorbate to ascorbate and this probably explains the lowered level of ascorbic acid in the gasoline and GM-10 treated mice. Breimer (19) reported that free radicals produced in the biological membranes rapidly react with α -tocopheryl radicals. The cytosolic GSH and ascorbic acid help in regeneration of α -tocopheryl.

Administration of black tea extract in group 2 animals caused, as compared with control, significant decrease in non-enzymatic antioxidant such as glutathione and ascorbic acid. However, treatment did not cause any significant change in lipid peroxidation and in enzymatic antioxidants such as superoxide dismutase, catalase and glutathione peroxidase. Black tea extract contains many polyphenols with antioxidative property which are continuously administered to this group of animals. Thus significant reduction in endogenous non-enzymatic antioxidants such as glutathione and ascorbic acid, might be due to higher exogenous antioxidants due to black tea administration.

Oral administration of black tea extract for 30 days along with gasoline and GM-10 (given subcutaneously) caused significant amelioration in gasoline and GM-10 induced lipid peroxidation by increasing the antioxidative activity of the cells. Activities of enzymatic antioxidants (superoxide dismutase, catalase and glutathione peroxidase) as well as non-enzymatic antioxidants (glutathione and ascorbic acid) were higher as compared to gasoline and GM-10 alone treated groups.

Thus, the investigation suggests that black tea extract 2 g/100 mL drinking water (2% w/v) effectively ameliorates dose-dependent Gasoline and GM-10 induced toxicity in liver of mice.

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