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BLACK TEA INFUSION AMELIORATES ENZYMATIC CHANGES INDUCED BY SUBCUTANEOUS EXPOSURE OF GASOLINE AND GM-10 IN MICE

Manjeet Dave[1], Ramtej Jayram Verma[2]
[1]Govt. P.G. College, Bareli
[2]University School of Sciences, Gujarat University, Ahmedabad

ABSTRACT

The present study was carried out to examine the ameliorative effect of black tea infusion on gasoline and GM-10 induced enzymatic changes in kidney of mice. Eighty healthy adult Swiss strain male albino mice weighing 32-35 gm were divided into eight groups including untreated control and various treated groups. Treated groups were subcutaneously administered with gasoline (412 mg/kg/day) and GM-10 low dose (206 mg/kg/day) and high dose (412 mg/kg/day) for 30 days. Black tea infusion (2%) was orally administered alone and along with gasoline and GM-10 through drinking water. All experimental animals were sacrificed on 31st day by cervical dislocation; kidney were isolated and used. Activities of succinic dehydrogenase, adenosine triphosphatase, acid phosphatase and alkaline phosphatase were assayed in kidney.The results revealed that subcutaneous administration of gasoline and GM-10 caused dose-dependent, significant enzymatic alterations in kidney of mice. Oral administration of black tea infusion along with subcutaneous treatment with gasoline and GM-10 significantly ameliorates all enzymatic changes induced by gasoline and GM-10 in kidney of mice.

Keywords:
Gasoline, Gm-10, Black Tea Extract, Kidney.

INTRODUCTION

The environmental health deterioration due to the usages of automobiles is well known and faced by nearly all the countries in the world, For the environmental conservation many countries have introduced CO emission reduction programmed that include the use of oxyfuels such as methanol or ethanol mixture, usually as blend with gasoline in which quantity of ethanol/methanol varies from 10 to 85% (GM or GE 10 to 85).

Both green and black tea has shown cancer chemo preventive activity against ultraviolet light, chemically induced and genetic models of carcinogenesis. Therefore, the present investigation was designed to study gasoline and GM-10 induced enzymatic changes in kidney of mice and its possible amelioration by black tea infusion.

MATERIALS AND METHODS

Swiss strain healthy adult male albino mice (Mus musculus) weighing 32-35 gm were were divided into eight groups, each group containing 10 animals. Group 1 (control) animals were not given any

treatment. Animals of group 2 were treated with 2% (w/v) black tea infusion in pure drinking water for 30 days. Group 3 animals were subcutaneously treated with gasoline (412 mg/kg body weight/day) for 30 days. Animals of group 4 and 5 were subcutaneously treated with gasoline: methanol (90:10 v/v, GM-10) blend in doses of 206 mg/kg/day (low dose) and 412 mg/kg/day (high dose) for 30 days. Animals of groups 6, 7 and 8 were treated with gasoline and GM-10 as mentioned for groups 3, 4, and 5 respectively along with 2% black tea infusion in pure drinking water for 30 days. Doses of gasoline and GM-10 high dose were kept same so that the comparative evaluation of the toxicity of both can be done. Doses of gasoline and GM-10 were based on LD50 values.

On completion of the treatment, animals were sacrificed by cervical dislocation. Kidneys from all the animals were quickly isolated, blotted free of blood and used. Activities of succinic dehydrogenase (Beatty el al 1966), adenosine triphosphatase (Quinn and White 1968), acid phosphatase (Bessey et al., 1946) and alkaline phosphatase (Bessey et al., 1946) were assayed. Protein content was estimated by the method of Lowry et al. (1951) using bovine serum albumin as standard.

Data were expressed as mean ± S.E.M. All the data obtained were statistically analyzed using one-way Analysis of variance (ANOVA) followed by Tukey test. The levels of significance was accepted with $p<0.05$.

RESULTS

There was no significant change between control (group 1) and black tea extract treated group (group 2). Subcutaneous administration of gasoline and GM-10 caused dose-dependent, significant ($p<0.05$) reduction in activities of succinic dehydrogenase and adenosine triphosphatase, as compared to control. On the other hand, activities of acid and alkaline phosphatases were significantly ($p<0.05$) higher, as compared to control, in the kidney of gasoline and GM-10 treated mice 2A-C). The effect was dose-dependent. Oral administration of black tea infusion along with gasoline and GM-10 (groups 6, 7, 8) caused significant amelioration in these enzyme activities in kidney (Table 2A-C) of mice as compared with gasoline and GM-10 alone treated groups (groups 3, 4, 5).

Table 2A: Effect of black tea infusion on subcutaneous exposure of gasoline and GM-10 induced enzymatic changes in kidney of mice

Parameters	**Experimental groups**			
	1	**2**	**3**	**6**
	Control	**Black tea infusion**	**Gasoline**	**Gasoline+black tea infusion**
Succinic dehydrogenase activity[1]	95.92 ±2.85	100.18 ±1.55	76.90[abf] ±3.66	84.60 [abc] ±1.18
Adenosine triphosphatase activity[2]	0.396 ±0.009	0.368 ±0.006	0.327 [abf] ±0.008	0.438 [bc] ±0.069
Acid phosphatase activity[3]	1.590 ±0.028	1.620 ±0.032	1.870 [abf] ±0.028	1.780[abc] ±0.038
Alkaline phosphatase activity[3]	0.823 ±0.170	0.804 ±0.032	0.962 [abf] ±0.028	0.888[abc] ±0.038

Values are Means ± S.E.M.; n=10

Activities are expressed as: [1]µg formazon formed/mg protein/15 min; [2]µ moles i.p. released/mg protein/30 min;
[3]µ moles p-nitrophenol released/mg protein/30 min)

[a] as compared to Group 1 : p<0.05; [b] as compared to Group 2 : p<0.05;
[c] as compared to Group 3 : p<0.05; [f] as compared to Group 6 : p<0.05

Table 2B: Effect of black tea infusion on subcutaneous exposure of gasoline and GM-10 induced enzymatic changes in kidney of mice

Parameters	**Experimental groups**			
	1	**2**	**4**	**7**
	Control	**Black tea infusion**	**GM-10 low dose**	**GM-10 low dose + black tea infusion**
Succinic dehydrogenase activity[1]	95.92 ±2.85	100.18 ±1.55	83.73 [abg] ±1.21	92.43 [bd] ±2.61
Adenosine triphosphatase activity[2]	0.396 ±0.009	0.368 ±0.006	0.347[ag] ±0.015	0.400 [d] ±0.009
Acid phosphatase activity[3]	1.590 ±0.028	1.620 ±0.032	1.730 [abg] ±0.030	1.590[d] ±0.023
Alkaline phosphatase activity[3]	0.823 ±0.170	0.804 ±0.032	0.957[abg] ±0.014	0.847 [d] ±0.013

Values are Means ± S.E.M.; n=10

Activities are expressed as: [1]µg formazon formed/mg protein/15 min; [2]µ moles i.p. released/mg protein/30 min;
[3]µ moles p-nitrophenol released/mg protein/30 min)

[a] as compared to Group 1 : p<0.05; [b] as compared to Group 2 : p<0.05;
[d] as compared to Group 4 : p<0.05; [g] as compared to Group 7 : p<0.05

Table 2C: Effect of black tea infusion on subcutaneous exposure of gasoline and GM-10 induced enzymatic changes in kidney of mice

Parameters	**Experimental groups**			
	1	**2**	**5**	**8**
	Control	**Black tea infusion**	**GM-10 high dose**	**GM-10 high dose + black tea infusion**
Succinic dehydrogenase activity[1]	95.92 ±2.85	100.18 ±1.55	75.45 [abh] ±1.14	86.24 [abe] ±1.65
Adenosine triphosphatase activity[2]	0.396 ±0.009	0.368 ±0.006	0.322 [abh] ±0.009	0.445 [abe] ±0.088

Acid phosphatase activity[3]	1.590 ±0.028	1.620 ±0.032	2.010 [abh] ±0.032	1.720 [abe] ±0.028
Alkaline phosphatase activity[3]	0.823 ±0.170	0.804 ±0.032	0.966 [abh] ±0.016	0.881 [be] ±0.018

Values are Means ± S.E.M.; n=10

Activities are expressed as: [1]µg formazon formed/mg protein/15 min; [2]µ moles i.p. released/mg protein/30 min;
[3]µ moles p-nitrophenol released/mg protein/30 min)

[a] as compared to Group 1 : $p<0.05$; [b] as compared to Group 2 : $p<0.05$;
[e] as compared to Group 5 : $p<0.05$; [h] as compared to Group 8 : $p<0.05$

DISCUSSION

Oral administration of gasoline and GM-10 for 30 days caused significant reduction in the activities of succinic dehydrogenase and adenosine triphosphatase in kidney of mice. The effect was comparatively more pronounced in gasoline and GM-10 high dose group than that of low dose. Reduction in succinic dehydrogenase activity clearly indicates decreased aerobic metabolism which might be the result of reduced oxygen transport to tissues.

Reduction in adenosine triphosphatase activity in kidney suggests reduced utilization of ATP produced in the cell.

Acid phosphatase activity was significantly increased in kidney of gasoline and GM-10 treated mice. Therefore, rise in acid phosphatase activity in kidney of mice could be due to increased leakage in lysosomal enzymes as well as lysis of the cells.

Gasoline and GM-10 treatment for 30 days caused significant increase in the alkaline phosphatase activity in the kidney of mice. Reasons for increase in alkaline phosphatase activity might be through its *de novo* synthesis or a decrease in its turnover (Castranova et al., 1988).

Treatment with black tea extract along with gasoline and GM-10 significantly ameliorates succinic dehydrogenase activity in gasoline and GM-10 high dose group. It could be due to presence of antioxidants in black tea infusion (Du Toit et al, 2001).

Thus, the present study indicates that black tea infusion significantly ameliorates gasoline and GM-10 induced toxicity in kidney of male mice.

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