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Alterations in Serum Biochemical Parameters in Response to Gasoline Inhalation and the Protective Effects of Green Tea and Curcumin

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Abstract: Herbal medicines derived from plant extracts are being increasingly utilized to treat a wide variety of clinical disease. More attention has been paid to the protective effects of natural antioxidants against drug-induced toxicities especially whenever free radical generation is involved. Flavonoids have been found to play important roles in the non-enzymatic protection against oxidative stress, especially in case of cancer. This study aimed to investigate the alterations in biochemical parameters in serum which measure liver functions, kidney functions, serum lipids and proteins due to inhalation of gasoline and the protective effects of some natural products against gasoline toxicity. Green tea extract and powdered curcumin were chosen as antitoxicity natural products. CD1 mice were taken as experimental model. Mice were exposed to gasoline vapor 2 h/day for 3 weeks in inhalation chamber. The concentration of gasoline is 9375 ppm and the concentration of benzene is 100 fold less than gasoline in equilibrium with pure benzene being 93.75 ppm. The results concluded that: 1-Liver functions did not affect in all animal groups. 2-Increase in globulin concentration in serum was observed in gasoline group, this was lowered in the other groups. 3-Triglycerides in serum were increased by gasoline and returned near to normal control by green tea extract and curcumin addition to diet. 4-Growth rate per day was reduced with gasoline inhalation and ameliorated with curcumin, but hepatosomatic and spleenosomatic indices were not affected in all groups.

Key words: Gasoline, green tea, curcumin

INTRODUCTION

Gasoline is a refined product of petroleum consisting of a complex mixture of hydrocarbons. A generic mixture contains about 54% of paraffins and isoparaffins (alkanes from C_4 to C_{12}), 36% of aromatics (principally benzene, toluene, ethylbenzene and xylene), 6% of olefins (or alkenes), 5% of naphthenics hydrocarbons (or saturated cyclic hydrocarbons) and <1% of other compounds. Many of the toxicological effects associated with exposure to gasoline can be attributed to specific components of the mixture: e.g., benzene (ATSDR, 1995).

Some populations as automobile mechanics, service station, filling station, workers and taxi drivers are exposed to benzene through their contact with gasoline vapor and engine exhaust and by multiple routes. Automobile mechanics represent a population of workers exposed to modest levels of benzene through their contact with gasoline and engine exhausts. Although the concentration of benzene in gasoline is typically <1% (v/v) in the USA (Wallace, 1996), thermolytic dealkylation of alkylbenzenes raises the level of benzene in car exhausts to ~5% of total hydrocarbon emissions (Wallace, 1996). Mechanics' benzene exposures have recently been reported to range from 0.01 to 13.6 mg/m^3, with the vast majority of measurements well below the current OSHA standard of 1 ppm (3.2 mg/m^3) (Nordlinder and Ramnas, 1987; Popp *et al*., 1994; Mannino *et al*., 1995; Hotz and Lauwerys, 1997; Javelaud *et al*., 1998).

Herbal medicines derived from plant extracts are being increasingly utilized to treat a wide variety of clinical disease (Gupta *et al*., 2004). More attention has been paid to the protective effects of natural antioxidants against drug-induced toxicities especially whenever free radical generation is involved (Frei and Higdon, 2003). Flavonoids have been found to play important roles in the non-enzymatic protection against oxidative stress (Okada *et al*., 2001; Babich *et al*., 2005), especially in case of cancer. Flavonoids are group of polyphenolic compounds that occur widely in fruit, vegetables, tea, cocoas and red wine (Arts *et al*., 1999; Bearden *et al*., 2000; Matito *et al*., 2003). Flavonoids, including flavones, flavanone, flavonols, flavanols and isoflavones, are polyphenolic compounds which are widespread in foods and beverages and possess a wide range of biological activities (Harborne and Williams, 2000), of which antioxidation has been extensively explored (Bors *et al*., 1994; Terao *et al*., 1994; Ioku *et al*., 1995; Croft, 1998; Pietta, 2000; McPhail *et al*., 2003; Goupy *et al*., 2003; Vaya *et al*., 2003).

Green tea consumption has been inversely associated with the development and progression of cardiovascular diseases (Cheng, 2006; Stangl *et al*., 2006) and associated with a lower incidence of coronary artery disease. The protective effect of green tea in cardiovascular diseases is thought to stem from its

antioxidant activity (Higdon and Frei, 2003; Zaveri, 2006). Shen *et al.* (1998) studied the ability of tea polyphenols to lower serum cholesterol and triglyceride in aged rats and showed that 2% tea polyphenols lowered serum cholesterol with 21.6% and increased the ratio of HDL-C to total cholesterol by 30%.

Lee *et al.* (2005) studied the long-term effects of green tea consumption on atherosclerotic markers in smokers and concluded that green tea ingestion resulted in decrease of plasma soluble p-selectin and oxidized LDL in plasma. The extract of green tea attenuated blood pressure increase in spontaneously hypertensive rats, an affect attributed to its antioxidant properties (Negishi *et al.*, 2004). It also could lower blood pressure in rats through the inhibition of angiotensin I-converting enzyme activity (Wang and Wang, 1991; Ke *et al.*, 2000; Lin and Line-Shia, 2001; Lin *et al.*, 2000).

Erba *et al.* (2005) investigated the effect of addition of two cups of green tea containing about 250 mg of total catechins to a controlled diet in a group of healthy volunteers against a control group, a part from triacylglycerol level which was significantly higher in the green tea group. However, in both control and experimental group the triglyceride level, total cholesterol and HDL was not modified by green tea consumption.

Oral intake of green tea extract by human volunteers increased resistance of plasma LDL to oxidation *in vivo*, an effect that may lower the risk of artherogensis (Miura *et al.*, 2000). In apolipoprotein E-deficient mouse model of atherosclerosis, green tea extract in drinking water prevented the development of atherosclerosis without affecting plasma lipids (Miura *et al.*, 2001). Similarly, EGCG at a dose 10 mg/kg given intraperitoneally inhibited the developing atherosclerotic plaque in apolipoprotein E-deficient mice but no effect on established lesions (Chyu *et al.*, 2004).

Plants containing flavonoids have been used to treat diabetes in Indian medicine, the green tea flavonoid has been shown to have insulin-like activities (Waltner-Law *et al.*, 2002) as well as insulin-enhancing activity (Anderson and Polansky, 2002). However, epigallocatechin gallate, which is the principal catechin in green tea, differs from insulin in that it affects several insulin-activated kinases with slower kinetics. Furthermore, epigallocatechin regulates genes that encode gluconeogenic enzymes and protein-tyrosine-phosphorylation by modulating the redox state of the cell (Waltner-Law *et al.*, 2002). Thus epigallocatechin gallate may be an antidiabetic agent.

Recent studies provide scientific evidence regarding the potential pharmacological, prophylactic or therapeutic use of Curcumin, as anti-inflammatory, anti-carcinogenic, anti-tumoral, anti-viral, antifungal, anti-parasitic, anti-mutagen, anti-infectious, anti-hepatotoxic and anti-oxidant compound (Chen *et al.*, 2006; Aggarwal *et al.*, 2007; Ciftci *et al.*, 2010, 2011a,b, 2012a,b; Shehzad *et al.*, 2011).

Pulla Reddy and Lokesh (1992) observed that curcumin is capable of scavenging oxygen free radicals, such as superoxide anions and hydroxyl radicals, which are the initiators of lipid peroxidation. The lipid peroxidation has a main role in the inflammation, in heart diseases and in cancer (Jayaprakasha *et al.*, 2005). Naik *et al.* (2004) demonstrated the protective effect of curcumin against the cytotoxic effects of ethanol by measuring lipid peroxidation in terms of thiobarbituric acid reactive substances and are expressed in μM of malondialdehyde formed/100 gm tissue. They found that the amount of lipid peroxidation was increased by ethanol only with two folds compared to control, but when liver cells pre-treated with curcumin the level of lipid peroxidation lowered to reach control level.

MATERIALS AND METHODS

Experimental animals: Sixty male mice (*Mus musculus*) weighting 20-25 g was purchased from the Egyptian Organization for Serological and Vaccine Production, Egypt, were used as experimental animals throughout the present work. The animals were housed individually in plastic cages and acclimated for 1 week before gasoline-fume exposure. Food and water were offered *ad libitum*. Animals were maintained at 22±2°C at normal light/dark cycle.

Preparation of green tea extract: Green tea (*Camellia sinensis*) was purchased from Shanghai tea import and export Corporation, China. The green tea extract was made according to Maity *et al.* (1998), by soaking 15 gm of instant green tea powder in 1 L of boiling water for 5 min. The solution was filtered to obtain 1.5% green tea extract; this solution was provided to mice as their sole source of drinking water.

Preparation of curcumin in the diet: The dried ground rhizomes of *Curcuma longa* were purchased from local market in Cairo, Egypt, grinded, powdered and added to the diet of mice, 30 gm to 1 kg of diet to form concentration of 3% (Conney *et al.*, 1997).

Inhalation of gasoline: A glass cubic box its length is 70 cm, width is 70 cm and high is 70 cm, was manufactured to make as gasoline inhalation chamber, there are two orifices in both right and left sides of the box in the upper portion of the box to make aeration, each orifice 5 cm in diameter covered with wire mesh to prevent mice escaping. At a 10 cm distance from the bottom of the box, a wire mesh shelf 70 x 70 cm was fixed to put the mice on it. Under this shelf, a 200 ml cans containing 150 ml of gasoline were placed in the exposure chamber and the animals were allowed to inhale the fumes evaporating from the cans. The gasoline, which evaporated during the time of inhalation was about 80 ml/2 h. The time of exposure was 10.00 to

12.00 am and the cans were withdrawn and the inhalation stopped. The experimental fume gasoline inhalation was exceeded for successive three weeks as 2 h/day/three weeks.

Gasoline: The Egyptian commercial unleaded gasoline (octane 90) was purchased from a filling station. Gasoline is a petroleum-derived liquid mixture consisting mostly of more than 300 individual hydrocarbons primarily (in volume) of paraffins (30-90%), cycloparaffins (1-35%), olefins (0-20%) and aromatic (5-55%), distilling in the approximate range of 30-220°C. Composition of gasoline varies with the source of the crude oil, refinery processes, conditions and the blending of refinery streams in the gasoline boiling range to meet performance criteria as well as regulatory requirements (Roberts *et al*., 2001). Volatile organic compound emissions from gasoline storage showed that total organic compounds per cubic meter gasoline loaded is 35 g/m^3 saturated vapor at 25°C.

Gasoline dose: Based on analysis reported by Johnson *et al*. (1990) the concentration in equilibrium with gasoline is 9375 ppm. Benzene is 100-fold less than in equilibrium with pure benzene being 93.75 ppm. This dose of benzene is in equilibrium with gasoline in the inhalant mice cages in the current study. However, gasoline fraction differs from whole gasoline by containing far less aromatic, longer chain and longer aliphatic hydrocarbons. Analysis of workplace exposure to gasoline vapors revealed that C4-C5 length hydrocarbons constitute from 67 to 74% by weight of the typical vapor (Halder *et al*., 1986).

Animal groups: After an acclimation period for 1 week, animals were classified into six groups, each group consists of ten mice as follow:

1: Control group, received only the ordinary mice diet and drink water without any additions and kept 2 h daily in the inhalation chamber without gasoline for three weeks
2: Green tea group, received ordinary diet, drink green tea extract (1.5%) as a sole source of drinking water and kept two hours daily in the inhalation chamber without gasoline for three weeks
3: Curcumin group, these animals received powdered dried ground rhizomes of *Curcuma longa* (turmeric) in the diet (3%) and kept two hours daily in the inhalation chamber without gasoline for three weeks
4: Gasoline inhalation group, this is the intoxicated group with gasoline inhalation, these mice were kept 2 hours daily in an inhalation chamber with gasoline for three weeks. This group drinks water and eat the ordinary diet.
5: Gasoline and green tea group, these animals exposed to gasoline 2 h daily in an inhalation chamber for three weeks and received green tea extract (1.5%) eat the ordinary diet
6: Gasoline and curcumin group, this group exposed to gasoline in the inhalation chamber, 2 h daily for three weeks and received powdered dried ground rhizomes of *Curcuma longa* in their ordinary diet along the time of the experiment and drink water

Blood collection: Twenty four hours after stopping gasoline inhalation, animals were anaesthetized by diethyl ether, dissected and blood was collected by heart puncture with syringe (3 ml capacity). The required amount of blood was collected in tubes and the blood allowed to coagulate in water bath at 37°C for 30 min. Serum was separated by centrifugation in cooling centrifuge (Hettich, Germany) at 3000 x g for 15 min, transported into another dry and clean Eppendorf tubes and was kept in deep freezer at-20°C for biochemical analysis.

Determination of liver function tests:

Serum alanine aminotransferase (ALT) activity: Serum ALT was measured according to Reitman *et al*. (1957) method by using the kit of Randox Company, United Kingdome.

Serum aspartate aminotransferase (AST) activity: Serum AST was measured according to Reitman *et al*. (1957) method by using the kit of Randox Company, United Kingdome.

Serum alkaline phosphatase (ALP) activity: ALP was determined according to the method of Kaplan and Righetti (1955) by using Teco Diagnostics kit (U.S.A).

Serum total proteins: Total serum proteins in was determined by using the kit of Biosystem Company, Spain, according to the method of Gornall *et al*. (1949).

Albumin: Serum albumin was measured according to the method of Doumas *et al*. (1971) by using the kit of Diamond-Diagnostics, Egypt.

Globulin: Serum globulin was calculated by subtracting albumin from total protein.

Determination of kidney function tests

Serum urea: Urea level in serum was determined using the method of Talk and Schubert (1965), by kit of Biosystem Company, Spain.

Serum uric acid: Uric acid in serum was determined according to the method of Barham and Tinder (1972), by using the kit of Biosystem Company, Spain.

Determination of serum lipids

Total cholesterol: Total cholesterol in serum was determined according to the method of Allain *et al.* (1974), by using the kit of Biosystem Company, Spain.

Triglycerides: Triglycerides in serum was determined according to the method of Bucolo and David (1973), by using the kit of Biosystem Company, Spain.

Statistical analysis: Data are expressed as mean±SD. The level of statistical significance was taken at $p<0.05$, using one way analysis of Variance (ANOVA) test followed by Dunnett test to detect the significance of differences between each group and control. All analysis and graphics were performed by using, INSTAT and graphPad Prism software version 4.

RESULTS

Liver function tests: In this study liver function tests were measured in serum of CD1 mice (ALT, AST, ALP), these which illustrated in Table 1. Gasoline had not any significant effect on all liver function tests in all groups which intoxicated with gasoline alone or protected with green tea or curcumin. There were a remarkable reduction in AST activity by addition of curcumin alone to the animal's diet (3%) by 46.35% compared to control ($p<0.01$). Green tea with or without gasoline inhalation did not show any significant changes neither in ALT nor AST compared to control. Alkaline phosphase (ALP) also did not show any significant changes in all animal groups.

Serum proteins: By measuring proteins in the serum samples (total proteins, albumin, globulins, A/G ratio) as illustrated in Table 1 were noticed that the changes in serum proteins were mainly in globulins fraction, it increased significantly by gasoline intoxication ($p<0.01$) by -73.57% compared to control, consequently, total proteins also increased by -31.61% ($p<0.01$) and A/G ratio were decreased by -39.41% ($p<0.01$), on the other hand, albumin did not show any significant changes in all animal's groups compared to control.

Co-administration of green tea extract or curcumin with gasoline intoxication caused reduction of globulin by (-12.45 and -9.55%) and in total proteins by (-6.08 and -5.04%), respectively but these changes were not significant. A/G ratios were not improved by addition of green tea or curcumin but they still lower than control by -39.22 and-30.2%, respectively.

Kidney function tests: Serum urea and uric acid were measured as kidney function tests. Gasoline intoxication caused a highly significant reduction in urea level in blood compared to control by -32.95% ($p<0.01$), this was not affected by green tea extract addition, but was affected by curcumin addition by 51.69% compared to gasoline alone group ($p<0.01$). Neither green tea alone nor curcumin alone had any effect on serum urea levels (Table 1).

Serum uric acid level was increased significantly in gasoline+green tea group by 30.18% compared to control ($p<0.05$), but all other groups had not any significant changes (Table 1).

Plasma lipids: In this study the levels of cholesterol and triglycerides were measured as illustrated in Table 1. Cholesterol did not show any significant changes in all animal's groups but triglycerides levels when analyzed by ANOVA test showed a highly significant difference ($p<0.01$), this test was followed by Dunnett test between each group and control, gasoline caused a degree of increase (36.72%) ($p<0.01$) compared to control.

Green tea extract alone and curcumin alone did not cause significant changes in serum triglycerides level. Co-administration of green tea or curcumin caused lowering in triglycerides level to reach near to normal. The percentage of differences between gasoline+green tea group and gasoline alone group is -20.21% and between gasoline+curcumin group and gasoline alone group is -25.88%, these differences were highly significant ($p<0.01$).

Body and organs weights: Body weights were recorded at beginning and the end of experiment, growth rate was calculated and by statistical analysis ANOVA was found that gasoline inhalation caused suppression of growth significantly, on the other hand the treated group with curcumin did not show any significant changes compared to control. All the parameters of hepatosomatic index and spleenosomatic index in all groups did not change compared to control group.

DISCUSSION

Serum biochemical parameters: It has been suggested that the quinine forms of benzene metabolites, for example, p-benzoquinone, mediate benzene toxicity because of their ability to covalently bind to proteins. Benzene is not a liver toxin probably because of the failure to establish significant concentrations of quinones; hydroquinone is maintained in the reduced form by high concentrations of reducing enzymes such as NQ01, whereas in bone marrow high levels of peroxidatic activity are more likely to yield reactive metabolites from hydroquinone (Snyder, 2002), this discus the present results in liver function tests on mice inhaled benzene for three weeks, where no significant changes occurred for ALT, AST and ALP as a result of gasoline intoxication.

In the present study green tea caused marked depression in hypertriglyceridemia induced by benzene inhalation. Koo and Noh (2007) discussed this results that green tea and its catechins effectively lower the

Table 1: Serum Biochemical parameters in CD1 mice exposed to gasoline inhalation and effect of green tea or curcumin

Animal groups	Control Mean±SD	Green tea Mean±SD	Curcumin Mean±SD	Gasoline Mean±SD	Gasoline+ green tea Mean±SD	Gasoline+ Curcumin Mean±SD
ALT (U/L)	25.25±6.185	22±4.69	18.8±2.168	23.6±8.355	24.2±3.899	21±4.32
AST(U/L)	38.40±7.701	35.40±4.615	20.60±2.608**	40.80±2.775	41.40±9.044	36.60±8.503
Alkaline phosphatase (U/L)	29.55±8.19	35±7.246	36.71±5.823	36±4.183	38±4.809	35.83±8.202
Total protein (gm/dl)	6.291±0.459	5.793±0.782	6.192±0.843	8.28±0.993**	7.776±0.648**	7.862±0.48**
Albumin (gm/dl)	3.271±0.548	3.304±0.496	3.46±0.602	3.26±0.152	3.058±0.377	3.44±0.311
Globulin (gm/dl)	2.906±0.598	2.323±0.486	2.729±0.8	5.044±0.975**	4.416±0.671**	4.562±0.389**
A/G ratio	1.086±0.227	1.177±0.194	1.287±0.221	0.658±0.12**	0.66±0.102**	0.758±0.111**
Urea (mg/dl)	61.75±10.02	63.5±13.2	60.13±11.53	41.4±5.177**	40.6±5.771**	62.8±11.3
Uric acid (mg/dl)	8.58±1.42	6.88±1.06	8.27±1.85	9.52±1.45	11.17±0.88*	8.66±1.35
Cholesterol (mg/dl)	128.8±20.06	131.9±15.79	129.0±18.08	134.2±11.12	127.4±26.83	143±7.583
Triglycerides (mg/dl)	149.2±18.03	127.8±13.70	141.8±27.66	204±28.21	144.4±20.21**	151.2±19.33

(*) Significant difference compared to control group (p<0.05), (**) Highly significant difference compared to control group (p<0.01)

Table 2: Growth rate, Hepatosomatic index and spleenosomatic index in CD1 mice exposed to gasoline inhalation and effect of green tea or curcumin

Animal groups	Control Mean±SD	Green tea Mean±SD	Curcumin Mean±SD	Gasoline Mean±SD	Gasoline+ green tea Mean±SD	Gasoline+ Curcumin Mean±SD
Growth rate (g/day)	0.949±0.055	0.89±0.098	0.967±0.208	0.819±0.069*	0.814±0.059*	1.036±0.057
Hepatosomatic index	0.0671±0.0149	0.0619±0.0133	0.0687±0.0102	0.0707±0.0131	0.0623±0.0097	0.0754±0.0025
Spleenosomatic index	0.00816±0.00279	0.00772±0.000986	0.0069±0.0022	0.00787±0.00242	0.00952±0.00091	0.00904±0.00134

(*) Significant difference compared to control group (p<0.05), (**) Highly significant difference compared to control group (p<0.01)

intestinal absorption of lipids. Among the green tea catechins, EGCG is the most potent inhibitor of lipid absorption. The potent inhibitory effect of EGCG appears to be associated with its ability to form complexes with lipids and lipolytic enzymes, thereby interfering with the luminal processes of emulsification, hydrolysis, micellar solubilization and subsequent uptake of lipids. EGCG appears to be more effective in lowering the absorption of lipids of extreme hydrophobicity, such as cholesterol and α-tocopherol, with little or a moderate effect on less hydrophobic lipids such as retinol and fatty acid.

At present, it remains debatable whether the reduction in coronary heart disease (CHD) risk in humans associated with green tea consumption is attributable to the prevention of LDL oxidation or to the antioxidant potential of green tea or its catechins (Hodgson *et al*., 2002); however, evidence from animal studies clearly indicates that green tea or its catechins lower the blood levels of cholesterol in cholesterol-fed rats, mice and hamsters, as well as the plasma levels of triglyceride in hamsters fed a high-fat diet and in rats fed a high fructose diet (reviewed in Koo and Noh, 2007).

Treatment of curcumin in the present study reduced the level of triglycerides in plasma which increased as a result of gasoline intoxication. Rukkumani *et al*. (2003) suggested that hypocholesterolemic effect of curcumin is due to the increased HDL formation, which transports the excess cholesterol from extrahepatic tissues to liver where it is catabolized. Curcumin also decreases the absorption of cholesterol. Curcumin increases 7α-hydroxylase activities; the main enzyme involved in the conversion of cholesterol to bile acid and thus facilitates biliary cholesterol excretion. The exact mechanisms by which curcumin lowers other lipid levels are not known, however, studies have shown that some of the spices play a vital role in lipid metabolism, due to their active principle. The spices are known to affect bile acid excretion and thereby influence lipid levels. The decreased levels of phospholipids and triglycerides may also be due to the decreased free fatty acids synthesis by curcumin, which may suppress the enzymes involved in free fatty acids synthesis.

Conclusion: The results concluded that, exposure of mice to gasoline vapor did not affect on liver function. On the other hand this exposure leads to increase in globulin and triglycerides, which improved by co-administration of green tea or curcumin.

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