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Effect of *Terminalia Arjuna* Stem Bark on Antioxidant Status in the Lungs of Rats exposed to Benzo(a)pyrene

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Abstract

Background - Free radicals and associated oxidative stress induced by Benzo[a] pyrene are implicated in eliciting pathological changes in lung toxicity. Since ancient times Terminalia arjuna bark (chhal e Arjun), an indigenous plant used in ayurvedic and unani Medicine, primarily as a cardiotonic as well as in other diseases also.
Material and methods - The present study examined the effect of ethanolic extract (150 and 300 mg/kg body weight) of Terminalia arjuna stem bark in Benzo[a] pyrene induced lung toxicity.
Result - The extract produced significant reduction in lipid peroxidation (LPO). The effect of oral T. arjuna at the dose of 300 mg/kg body weight was more than the 150 mg/kg body weight. The extract also causes a significant increase in glutathione peroxidase, glutathione-s-transferase glutathione reductase and reduced glutathione in lung of Benzo[a] pyrene induced lung toxicity in rats.
Conclusion - The result indicates that the extract exhibit the antioxidant activity through amendment of oxidative stress in Wistar rats.

Keywords – Arjun Stem bark, GPx, MDA, Benzopyrene

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Introduction

Lung cancer is one of most widespread cancers in the world, and death due to lung cancer is expected to stay on very elevated for several years to appear. Since lung cancer does not show any symptoms in early stage of the illness, most of the patients with this cancer are diagnosed with metastasis. Therefore search for extrapolative indicators of lung cancer is a significant clinical issue [1-3]. Cancer arises mainly as a result of exposure of individuals to carcinogenic agents in what they breathe in or eat and drink, or during exposures at their work place. Relatively than inherited genetic characteristics, other factors participate in the etiology of cancer which includes personal habits like tobacco use, dietary patterns, and occupational exposure to carcinogens [4]. Overproduction of free radicals causes imbalance in homeostatis between oxidants and antioxidants in the body. This imbalance leads to oxidative stress which can be consider the basis such as ageing and various human diseases like atherosclerosis, stroke, diabetes, cancer and neurodegenerative diseases, such as Alzheimer's and Parkinsonism [5]. Smoking is a most important risk factor for lung cancer [6] and one of the most important causes of cancer death in developed countries [7]. Relative risk for lung cancer is augmented 20-fold in smokers, through a non-linear relationship among the number of cigarettes smoked and lung cancer risk [8]. Loads of chemicals present in cigarette smoke are suspected to be mutagenic or carcinogenic [9,10]. Polycyclic aromatic hydrocarbons, present in the tar fraction of cigarette to be persuasive carcinogens, particularly benzo[a]pyrene BaP, methylcholanthrene and anthracenes [11–13]. These chemicals are produced throughout combustion of organic material and consequently are also present in major levels in the environment from furnace gases and combustion engine emissions. Polycyclic aromatic hydrocarbons like BaP, present in environmental pollution and cigarette smoke, converted into carcinogenic subsequent to metabolic activation by CYP/AHH to BaP diol epoxide (BPDE). *Terminalia arjuna* is a medicinal plant belongs to *Combretaceae* family. It is an important cardiotonic plant described in the Ayurveda [14]. In recent times the antioxidant activity of the chloroform extract of *T. arjuna* in diabetic rats have been reported [15].

In this study we have investigated the antioxidant potential of ethanolic extract (50%) of T. arjuna stem bark on lung of Benzo-a-pyrene induced lung toxicity in rats.

Materials and methods

Chemicals

Benzo[a]pyrene, reduced glutathione (GSH), oxidized glutathione (GSSG), nicotinamide adenine dinucleotide phosphate reduced (NADPH), flavin adenine dinucleotide (FAD), bovine serum albumin (BSA), and bisbenzamide were obtained from Sigma (St Louis, Missouri, USA). All the antibodies, chemicals, and reagents used were of the highest purity and standard commercially available.

Animals

Male rats of Wistar strain were used in this study. Animals were obtained from the Central Animal house Facility of Medical College. All procedures using animals were reviewed and approved by the Institutional Animal Ethical Committee. The rats were approximately 8 weeks old at starting of study (weights in the range of 150–200 g). They were housed in polypropylene cages in groups of six rats per cage and were kept in a room maintained at 25±2 ◦C with a 12 h light/dark cycle, and were allowed to acclimatize for one week before the experiments. They were given free access to standard laboratory animal feed (Hindustan Lever Ltd., Bombay, India) and water ad libitum.

Treatment regimen

To study the effect of pretreatment of animals with Terminalia arjuna on B [a] P induced lung epithelial damages, 24 male Wistar rats were randomly allocated to four groups of six rats in each. The animals of Groups I and II served as control group and toxicant group respectively.

Groups I and II received vehicle (0.15 M NaCl) orally (5 ml/kg b.wt., once daily, for 21 days). Group III received pretreatment with Terminalia arjuna by gavages once daily for 21 days at a dose of 150 mg/kg b.wt. Group IV received Terminalia arjuna once daily for 21 consecutive days at a dose of 300 mg/kg b.wt. On days 19, 20 and 21, 1 h after the treatment with Terminalia arjuna or vehicle the animals of Groups II–IV were administered with B(a)P (5 mg/kg b.wt. in 1% gelatin in 0.15 M NaCl). All the animals were sacrificed 24 h after last treatment.

PMS preparation

Postmitochondrial supernatant (PMS) preparation was carried out by the method described by Nafees et al. Liver was removed and cleaned with ice-cold saline (0.85% sodium chloride). Liver tissues were homogenized in chilled phosphate buffer (0.1M, pH 7.4) using a homogenizer (Remi Process Plant And Machinery Ltd, Mumbai, Maharashtra, India) and were centrifuged at 3000 r min1 for 10 min at 4°C in a Cooling Centrifuge (Remi Process Plant And Machinery Ltd) to separate the nuclear debris. The aliquot thus obtained was centrifuged at 12,000 r min1 for 20 min at 4°C to obtain PMS, which was used as the source of enzymes [16].

GSH estimation

1 ml of 10% PMS was mixed with 1 ml of 4% sulfosalicylic acid, incubated at 4°C for a minimum time period of 1 h, and then centrifuged at 4°C at 1200g for 15 min. Briefly, the reaction mixture having 0.4 ml supernatant, 2.2 ml phosphate buffer (0.1 M, pH 7.4), and 0.4 ml 5, 50-dithiobis-(2-nitrobenzoic acid) (DTNB; 4 mg ml1) making a total volume of 3.0 ml. The yellow color developed was read immediately at 412 nm on spectrophotometer (Lambda EZ201,Perkin Elmer, Waltham, MA, USA). The GSH concentration was expressed as nanomoles of DTNB conjugate formed per gram tissue [17].

GR activity

The reaction mixture consisted of 1.65 ml phosphate buffer (0.1 M, pH 7.6), 0.1 ml NADPH (0.1 mM), 0.05 ml GSSG (1 mM), 0.1 ml ethylenediaminetetraacetic acid (EDTA; 0.5 mM), and 0.1 ml 10% PMS in a total volume of 2 ml. Enzyme activity was assessed at 25°C by measuring the disappearance of NADPH at 340 nm and was expressed as nanomole of NADPH oxidized per minute per milligram protein using molar extinction coefficient of 6.22 x103 M-1 cm-1 [18].

GPx activity

Total of 2 ml volume consisting of 0.1 ml EDTA (1 mM), 0.1 ml sodium azide (1 mM), 1.44 ml phosphate buffer (0.1 M, pH 7.4), 0.05 ml GR (1 IU ml1), 0.05 ml reduced GSH (1 mM), 0.1 ml NADPH (0.2 mM), 0.01 ml hydrogen peroxide (H2O2; 0.25 mM), and 0.1 ml 10% PMS was used. The depletion of NADPH at 340 nm was recorded at 25°C. The activity of the enzyme was expressed as nanomole of NADPH oxidized per minute per milligram protein with the molar extinction coefficient of 6.22 x 103 M-1 cm-1[18].

Assay for GST activity

The reaction mixture comprised 0.025 ml CDNB (1 mM), 0.2 ml GSH (1 mM), 1.475 ml phosphate buffer (0.1 M, pH 6.5) and 0.3 ml PMS 10% in a total volume of 2.0 ml. The changes in the optical density were recorded at 340 nm, and the activity of the enzyme was calculated as nanomoles of CDNB conjugate formed per minute per milligram of protein using a molar extinction coefficient of 9.6 x 103 M-1 cm-1[19].

Assay for Quinone reductase activity

The 3-mL reaction mixture consisted of 2.13 ml Tris-HCl buffer (25 mM, pH 7.4), 0.7 mL BSA, 0.1 mL FAD, 0.02 mL NADPH (0.1 mM), and 50 µl (10%)PMS. The reduction of DCPIP was recorded calorimetrically at 600 nm and enzyme activity was calculated as nmol of DCPIP reduced min-1mg protein-1 using molar extinction coefficient of 2.1 × 104 M-1cm-1 [18] .

Assay for LPO

The reaction mixture in a total volume of 1 ml contained 0.58 ml phosphate buffer (0.1 M, pH 7.4), 0.2 ml microsomes, 0.2 ml ascorbic acid (100 mM), and 0.02 ml ferric chloride (100 mM). The reaction mixture was incubated at 37°C in a shaking water bath for 1 h and the reaction was stopped by adding 1 ml of 10% trichloroacetic acid. Following the addition of 1 ml 0.67% thiobarbituric acid, all tubes were placed in boiling water bath for 20 min and then shifted to crushed ice-bath before centrifuging at 2500g for 10 min. The malondialdehyde (MDA) formation in each sample was detected as optical density observed at 532 nm. The results were expressed as nanomole of MDA formed per minute per gram of tissue using molar extension coefficient 1.56 x 105 M-1 cm-1 [20].

Estimation of protein

The protein concentration in all samples was determined by the method of Lowry et al., 1951, using bovine serum albumin (BSA) as standard [21].

Statistical analysis

Differences between groups were analyzed using analysis of variance (ANOVA) followed by Dunnet's multiple comparisons test. All data points are presented as the treatment groups mean ±Standard error of the mean (S.E.).

Results

T. arjuna pretreatment decreased malondialdehyde (MDA) formation

MDA formation was measured to demonstrate the oxidative damage on LPO of Benzo [a] pyrene induced lung injury in rats. A significant increase of the MDA formation was found in the Benzo [a] pyrene treated group when compared with control. We have observed that pre-treatment with T. arjuna at both D1and D2 leads to the significant prevention of membrane damage when compared to 2-AAF treated group. No significant difference was found in the MDA level between control and only D2 groups. (Table 1)

Table 1- Results of pretreatment of T. arjuna on antioxidant enzymes like reduced glutathione, glutathione –S-transferase and glutathione reductase

Treatment regimen per group	**GSH** (n mol CDNB Conjugate formed /g tissue)	**GST** (n mol CDNB conjugate formed/min/mg protein)	**GR** (n mol NADPH Oxidized/min/ mg protein)
Group I (control)	0.521±0.005	191.18 ± 63.60	301.2±6.02
Group II (only B[a]P)	0.128±0.01****	481.32 ±8.89*	90.13 ±7.11*****
Group III (D1+ B[a]P)	0.221±0.04$^{\#}$	398.70 ±44.7^{NS}	158.32±18.35$^{\#}$
Group IV (D2 + B[a]P)	0.493±0.03$^{\#\#}$	281.74± 31.11$^{\#}$	281.32±20.61$^{\#\#\#}$
Group V (only D2)	0.502±0.004	201.31 ± 18.49	299.14± 10.21

Results represent mean ± SE of six animals per group. Prophylactic treatment with T. arjuna at both doses significantly modulated the alterations induced by B [a] P $^{\#\#\#}$P< 0.001 and $^{\#\#}$P< 0.05, compared to corresponding value for saline treated control*****P < 0.001, $^{\#\#}$P<0.01, $^{\#}$P < 0.05, *****P < 0.1 and NS= not significant, compared with the corresponding value for treatment with B[a]P. T. arjuna, D1= 150 mg / kg b. wt. D2 = 300 mg / kg b. wt.

T. arjuna pretreatment increased the hepatic reduced glutathione (GSH) level

Protective effect of T. arjuna on GSH level was marked. The level of GSH was depleted

significantly in Benzo [a] pyrene treated group as compared to control group. B. purpurea pre-treatment increased its level significantly in III and IV groups as compared to Benzo [a] pyrene treated group. T. arjuna alone pre-treated group exhibited no significant changes in GSH level as compared to control group (Table 2).

Table 2- Results of pretreatment of T. arjuna on quinone reductase, glutathione peroxidase and lipid peroxidation

Treatment regimen per group	**Quinone reductase** nmol NADPH oxidized /min/mg protein)	n mol NADPH Oxidized/min/ ng protein)	**LPO** (nmol MDA formed/ hr/g tissue)
Group I (control)	271.97 ± 4.83	322.23±21.01	0.8 ± 0.02
Group II (only B[a]P	102.10 ± 3.88****	110.13±11.05*****	6.41 ± 0.10****
Group III (D1+ B[a]P	191.39 ± 6.02$^{\#}$	187.20±21.01$^{\#}$	3.23 ± 0.19$^{\#}$
Group IV (D2 + B[a]P	219.01 ± 15.32$^{\#\#}$	296.80±31.21$^{\#}$	1.31 ± 0.02$^{\#\#}$
Group V (only D2)	232.01 ± 1.91	312.30±15.45	1.56± 0.01

Results represent mean ± SE of six animals per group. B[a]P treatment leads to significant depletion in the activities of antioxidant enzymes in II group as compared to I group ($^{**}p < 0.01$ and $^{*}P < 0.05$). Pretreatment of T. arjuna restored activity of these enzymes in the III & IV groups significantly as compared to B[a]P treated II group ($^{\#\#}p< 0.01$) and ($^{\#}p < 0.05$). T. arjuna D1= 150 mg / kg b. wt. D2 = 300 mg / kg b. wt.

T. arjuna pre-treatment ameliorates the activity of hepatic glutathione peroxidase (GPx)

Administration of Benzo [a] pyrene was found to deplete lung antioxidant enzyme GPx significantly as compared to control. Pre-treatment with T. arjuna before 2-AAF administration was found significantly effective in restoring the enzyme activity at dose two. We have observed that there is no significant difference in the activity of the antioxidant enzyme between control and only T. arjuna treated group.

T. arjuna pre-treatment ameliorates the glutathione reductase (GR) activity

Administration of Benzo [a] pyrene was found to deplete the activity of lung antioxidant enzyme GR significantly as compared to control. Pretreatment with T. arjuna before Benzo [a] pyrene administration was found significantly effective in restoring the enzyme at both, dose one and dose two. We have observed that there is no significant difference in the activity of the antioxidant enzyme between control and only T. arjuna treated group.

T. arjuna pre-treatment ameliorates the glutathione-S-transferase (GST) activity

Administration of Benzo [a] pyrene was found to deplete the activity of lung antioxidant enzyme GST significantly as compared to control. Pretreatment with T. arjuna before Benzo [a] pyrene administration was found not significant at dose one and significantly effective in restoring the enzyme at dose two. We have observed that there is no significant difference in the activity of the antioxidant enzyme between control and only T. arjuna treated group.

T. arjuna pre-treatment ameliorates the activity of quinone reductase (QR)

Administration of Benzo [a] pyrene was found to deplete lung antioxidant enzyme QR significantly as compared to control. Pretreatment with T. arjuna before Benzo [a] pyrene administration was found significantly effective in restoring enzyme at both, dose one and dose two. We have observed that there is no significant difference in the activity of the enzymes between control and only T. arjuna treated group.

Discussion and conclusion

Oxidative stress occurs in all the stages of the progress of cancer with the genesis of other diseases. Antioxidant defense enzymes consists of glutathione peroxidase (GPx), glutathione reductase (GR) and reduced glutathione (GSH), protect the cellular macromolecules against oxidative damage by detoxifying carcinogens both by destroying their reactive centres or by conjugating with endogenous ligands facilitating their excretion [22]. In the present study, we have demonstrated T. arjuna inhibit several aspects of Benzo[a] pyrene induced lung toxicity. Due to loss of cellular proliferation control there is a loss of lipid peroxidation in preneoplastic cellular membranes [23]. T. arjuna ameliorated Benzo[a]pyrene -induced inhibition of the activities of antioxidant enzymes, viz., glutathione peroxidase, glutathione reductase and phase-II metabolising enzymes such as glutathione-S-transferase and quinone reductase. Our study shows induction of lung glutathione-S-transferase and quinone reductase activity by T. arjuna pretreatment. Several chemopreventive compounds are well-known to degrade electrophilic metabolites by induction of quinone reductase and glutathione-S-transferase activities[24]. One of the major enzymes of xenobiotics metabolism is quinone reductase which protects the cells against mutagenicity and carcinogenicity resulting from free radicals and toxic oxygen metabolites by carrying out two-electron reductions. It has been reported that there is a correlation between the induction of quinone reductase activity and prevention of cancer[25].There was also dose-dependent decrease in the Benzo[a]pyrene mediated susceptibility of microsomal membrane induced lipid peroxidation through decreased production of free radicals as revealed by ameliorated malondialdehyde levels. Reduced glutathione known to be a first line of defense neutralizes the hydroxyl radical and plays a key role against inflammatory responses and oxidative stress. A significant restoration of glutathione and dependent enzymes, namely GR and GPx, to normal levels in the dose dependent manner in T. arjuna pretreated groups is found. This was evident from the significant increase in antioxidant armoury. Thus, our data suggest that T. arjuna inhibits Benzo[a]pyrene-induced lung toxicity by reducing the oxidative damage in rats.

References

1.Pei XP, Nakanishi Y, Takayama K, Bai F, Hara N. Benzo[a]pyrene activates the human p53 gene through induction of nuclear factor kappaB activity. J Biol Chem. 1999; 274(49):35240-6.

2. Sangrajrang S, Sornprom A, Chernrungroj G, Soussi T. Serum p53 antibodies in patients with lung cancer: correlation with clinicopathologic features and smoking. Lung Cancer. 2003; 39:297-301.

3. Lopez-Gonzales JS, Prado-Garcia H, Aguilar-Cazares D, Molina- Guarneros JA, Morales-Fuentes J, Mandoki JJ. Apoptosis and cell cycle disturbances induced by coumarin and 7-hydroxicoumarin on human lung carcinoma cell lines. Lung Cancer. 2004;43:275-83.

4. World Health Organization. Policies and managerial guideliness for national câncer control programs. Rev Panam Salud Publica. 2002;12(5):366-70.

5. Raghavan B, Krishnakumari S. Effect of terminalia arjuna stem bark on antioxidant status in liver and kidney of alloxan diabetic rats. Indian J Physiol Pharmacol. 2006; 50(2):133–142.

6. Smith RA, Glynn TJ. Epidemiology of lung cancer. Radiol Clin North Am. 2000;38(3): 453–470.

7.Goodman GE. Prevention of lung cancer. Crit Rev Oncol Hematol.2000; 33(3): 187–197.

8.Vineis P, Kogevinas M, Simonato L, Brennan P, Boffetta P. Levelling-off of the risk of lung and bladder cancer in heavy smokers: an analysis based on multicentric case control studies and a metabolic interpretation. Mutat Res. 2000; 463(1): 103–110.

9. Smith CJ, Hansch C. The relative toxicity of compounds in mainstream cigarette smoke condensate. Food Chem Toxicol. 2000;38(7): 637–646.

10.Smith CJ, Perfetti TA, Mullens MA, Rodgman A, Doolittle DJ.“IARC group2B carcinogens” reported in cigarette mainstream smoke. Food Chem Toxicol. 2000;38(9):825–848.
11.Smith LE, Denissenko MF, Bennett WP, Li H, Amin S, Tang M, Pfeifer GP. Targeting of lung cancer mutational hotspots by polycyclic aromatic hydrocarbons. J Natl Cancer Inst. 2000 ;92(10): 803–811.
12. Hengstler JG, Van der Burg B, Steinberg P, Oesch F. Interspecies Differences in Cancer Susceptibility and Toxicity. Drug Metab Rev.1999; 31: 917–970.
13. Petruzzelli S, Celi A, Pulera N, Baliva F, Viegi G, Carrozzi L, et al. Serum antibodies to benzo[a]pyrene diol epoxide–DNA adducts in the general population: effects of air pollution, tobacco smoking, and family history of lung diseases. Cancer Res. 1998;58(18): 4122–4126.
14. Tripathi VK, Singh B, Tripathi, RK, Upadhyay and Pandey VB. Terminalia arjuna its present status. Oriental J of Chem. 1996;12(1): 1–16.
15. Manonmani G, Anbarasi K, Balakrishna K, Veluchamy G, Shyamala Devi CS. Effect of Terminalia arjuna on the antioxidant defense system in alloxan induced diabetes in rats. Biomedicine. 2002; 22: 52–61.
16.Nafees S, Ahmad ST, Arjumand W, Rashid S, Ali N ,Sultana S. Carvacrol ameliorates thioacetamide-induced hepatotoxicity by abrogation of oxidative stress, inflammation and apoptosis in liver of Wistar rats. Hum Exp Toxicol. 2013;32(12):1292-304
17.Zunino F, Praseti G, Micheloni A, Cavalleti E, Sala F, Tofanetti O. Protective effect of reduced glutathione against cisplatin induced renal and systemic toxicity and its influence on the therapeutic activity of the antitumor drug. Chem Biol Interact. 1989; 70(1-2): 89–101.
18. Nafees S, Ali N, Rashid S, Hasan SK, Sultana S. Chemopreventive Effect of Bauhinia Purpurea Against Chemically Induced Hepatocarcinogenesis via Amelioration of Oxidative Damage, Cell Proliferation and Induction of Apoptosis in Wistar Rats. Toxicol Int. 2013;20(2): 117-125
19. Sultana S, S Nafees S, Khan AQ. Perillyl alcohol as a protective modulator against rat hepatocarcinogenesis via amelioration of oxidative damage and cell proliferation. Hum ExpToxicol. 2013; 32(11): 1179-1192
20. Samir M, el Kholy NM. Thiobarbituric acid reactive substances in patients with laryngeal cancer. Clin Otolaryngol Allied Sci. 1999; 24(3): 232–234.
21.Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem. 1951; 193: 265–275.
22. Nafees S, Ahmad ST, Arjumand W, Rashid S, Ali N, Sultana S. Modulatory effects of gentisic acid against genotoxicity and hepatotoxicity induced by cyclophosphamide in Swiss albino mice. J Pharm Pharmacol. 2012; 64(2): 259–267.
23. Nafees S, Ahmad ST, Arjumand W, Ali N, Rashid S , Sultana S. Hibiscus Rosa Sinensis alleviates Thioacetamide induced Acute Hepatotoxicity in Wistar Rats. Int J Drug Dev & Res. 2013; 5(1): 143-153.
24.Nelson SD. Mechanisms of the formation and disposition of reactive metabolites that can cause acute liver injury. Drug Metab Rev. 1995; 27:147–177.
25.Ibrahim SS. Protective effect of hesperidin, a citrus bioflavonoid, on diabetes induced brain damage in rats. J app Sci Res. 2008; 4(1):84–95.
26. Wu G, Fang YZ, Yang S, Lupton JR, Turner ND. Glutathione metabolism and its implications for health. J Nutr. 2004;134(3): 489–492.