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Antioxidant activity of Smoke Shield in-vitro and in-vivo

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Abstract

Smoke Shield is a proprietory formulation containing extract of turmeric (*Curcuma longa*), obtained by supercritical carbon dioxide gas extraction and post-supercritical hydroethanolic extraction, together with extracts of green tea and other spices whose presence synergistically increases the activity of turmeric. This study evaluates the antioxidant potentials of Smoke Shield in-vitro and in experimental animals, as well as in human models. Smoke Shield was found to scavenge superoxide radicals generated by photoreduction of riboflavin (50% inhibitory concentration = 91 μg mL^{-1}) and hydroxyl radicals generated by Fenton reaction (50% inhibitory concentration = 95 μg mL^{-1}) and reduced lipid peroxidation. Administration of Smoke Shield to mice was found to elevate antioxidant enzymes such as catalase and superoxide dismutase in blood as well as in liver and kidney. Glutathione-*S*-transferase activity was found to be significantly elevated in liver and kidney of animals treated with Smoke Shield. Glutathione levels were also significantly elevated in blood. Glutathione reductase was significantly elevated in kidney. Administration of Smoke Shield decreased the lipid peroxidation in serum, liver and kidney, as well as reduced the levels of conjugated dienes and hydroperoxides. Administration of Smoke Shield to smokers was found to increase the superoxide dismutase and glutathione in blood and decrease glutathione peroxidase. Smoke Shield inhibited phase I enzymes as represented by aniline-hydroxylase and aminopyrene-demethylase in-vitro. These results indicate that Smoke Shield has potent antioxidant activity, could inhibit phase I enzymes and increase detoxifying enzymes, which makes it an effective chemoprotective herbal formulation.

Introduction

Reactive oxygen species (ROS), which are formed in the body as a result of normal metabolic reactions, exposure to ionizing radiations and cigarette-smoke, environmental pollutions and by the influence of several xenobiotics, are implicated in the causation of several diseases including cancer (Gracy et al 1999). ROS appear to have broader significance in the production of tissue injury under conditions of oxidative stress, a state of imbalance between pro-oxidants and antioxidants in which the former predominate. ROS damage the biomolecules such as DNA, proteins, carbohydrates and lipids and affect enzyme activity and genetic machinery. The body possesses a number of mechanisms to remove the free radicals formed. For example, the integrated antioxidant system, which can scavenge free radicals, has an important role in the removal of free radicals in biological systems (Sun 1990). However, when the normal level of antioxidant system is not enough for the eradication of free radical mediated injury, administration of antioxidant compounds has a potential role to play.

Several antioxidants of plant origin have been screened for their ability to scavenge free radicals and are used as effective protective agents against oxidative stress. Turmeric (*Curcuma longa*) and its major ingredient, curcumin, have been shown to possess antioxidant activity (Soudamini & Kuttan 1989). Similar results have been obtained for green tea extract, which is a strong inhibitor of lipid peroxidation induced by free radicals (Komari et al 1993). Recently, New Chapter Inc. USA has introduced a patent pending formulation called Smoke Shield, designed to protect persons from the health damaging effects produced by smoke of various origins. Ingredients in Smoke

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Shield include turmeric, green tea, cloves, ginger, parsley, peppermint and rosemary, which act synergistically. Certain of the key herbs in the formula are separately hydroethanolically extracted, which ensures the maximum representation of the protective plant constituents. The turmeric in Smoke Shield, for instance, delivers both the value of the turmeric oils together with the protective action of the soluble curcuminoids. The quality of the herbs in Smoke Shield is assured by key marker compounds and is devoid of any organic solvent residues.

In this study, we have critically evaluated the antioxidant potential of this formulation in-vitro, as well as in mice and in human smokers.

Materials and Methods

Chemicals

Smoke Shield was supplied by New Chapter Inc. (USA). Aminopyrene was purchased from Sigma Chemicals (St Louis, MO). Nitroblue tetrazolium (NBT), NADPH, 1-chloro-2,4-dinitrobenzene (CDNB), glutathione (GSH) and 5,5′dithiobis (2-nitrobenzoic acid) (DTNB) were purchased from Sisco Research Laboratory (Mumbai). Thiobarbituric acid (TBA) was obtained from HiMedia (Mumbai). All other chemicals and reagents used were of analytical reagent grade.

Preparation of drug

The contents of a Smoke Shield capsule were dissolved in dimethyl sulfoxide (DMSO) (1 g/100 mL) for in-vitro studies. Due to the toxicity of DMSO, for studying in-vivo antioxidant activity in animals the drug was suspended in 0.1% carboxymethylcellulose. For human trials, volunteers were given 2 capsules (0.663 g) daily for one month.

Determination of in-vitro antioxidant activities of Smoke Shield

The superoxide scavenging activity of Smoke Shield was determined by the method of McCord & Fridovich (1969). In this method, superoxides generated by photoreduction of riboflavin reduce nitroblue tetrazolium salt, which gives a blue colour that was measured at 530 nm. The reaction was repeated in the presence of Smoke Shield. The percent inhibition for different concentrations of Smoke Shield as compared with controls were plotted and the concentration needed to produce 50% inhibition (IC50) was calculated from the plot. Lipid peroxidation was induced in rat liver homogenate by incubating with Fe^{2+}-ascorbate for 1 h (Bishayee & Balasubramanian 1971) and lipid peroxide formation was determined by the estimation of thiobarbituric acid reacting substances (TBARS) by the method of Ohakawa et al (1979). The reaction was carried out in the presence of different concentrations of Smoke Shield and inhibition of lipid peroxidation was plotted and the concentration needed for 50% inhibition was calculated. 1,1,3,3-Tetramethoxy propane was used as standard, which can produce TBARS. Hydroxyl radicals were generated by Fenton reaction by incubating with Fe^{3+}–ascorbate–EDTA–H_2O_2 system (Elizabeth & Rao 1990). The hydroxyl radicals attack deoxyribose, which eventually results in the formation of TBARS (Ohakawa et al 1979). Experiments were performed in the presence of various concentrations of Smoke Shield and inhibition was plotted against different concentrations of Smoke Shield and concentration needed for 50% inhibition were calculated from the plot.

Inhibition of aniline hydroxylase activity

Aniline hydroxylase activity was measured by the method of Mazel (1971). The reaction mixture consisted of phosphate buffer (0.5 mL, 150 mM, pH 7.4) aniline (0.1 mL, 120 mM) and enzyme (1–1.5 mg protein) and different concentrations of Smoke Shield in a final volume of 1.5 mL. The assay mixture was incubated at 37 °C for 2 h and 0.5 mL of 20% trichloroacetic acid (TCA) was then added to stop the reaction. The contents were mixed and centrifuged and the supernatant was estimated for *p*-aminophenol. For this, 1 mL of the TCA supernatant was treated with 0.5 mL of 10% Na_2CO_3 solution and 1 mL of 20% phenol in 0.2 M NaOH, for the development of colour. The absorbance of controls and samples were then read at 630 nm. Fifty percent inhibition was then calculated by plotting a graph with different concentrations of Smoke Shield against absorbance.

Inhibition of aminopyrene-*N*-demethylase activity

Aminopyrene-*N*-demethylase activity was assayed according to the method of Mazel (1971). The reaction mixture consisted of phosphate buffer (0.5 mL, 0.15 M, pH 7.4), $MgCl_2$ (0.1 mL, 75 mM), NADPH (0.1 mL, 5 mM), aminopyrene (0.1 mL, 120 mM), semicarbazide hydrochloride (0.1 mL, 120 mM), enzyme (1–1.5 mg protein) and different concentrations of Smoke Shield in a final volume of 1.5 mL. The assay mixture was incubated at 37 °C for 2 h. The reaction was then stopped by the addition of 0.5 mL of 10% $ZnSO_4$ and 0.5 mL of a saturated solution of $Ba(OH)_2$ and the contents were centrifuged. The supernatant was taken for estimation of formaldehyde after heating in the water bath at 60 °C for 30 min. For colour development, the absorbance was measured at 412 nm and percent inhibition was calculated.

Effect of Smoke Shield on antioxidant system in-vivo

Swiss albino mice used in this study were purchased from the National Centre for Laboratory Animal Sciences (Hyderabad). They were housed in ventilated cages in air-controlled rooms and fed with normal mice diet (Sai Durga Feeds, Bangalore, India) and allowed free access to water. All the experiments were carried out after getting ethical clearance from Institutional Animal Ethics Committee.

Mice were divided into 4 groups of 6 and they were given Smoke Shield at different doses: Group I, normal control; Group II, Smoke Shield 0.5 g kg^{-1} daily for 30 days; Group III, Smoke Shield 1 g kg^{-1} for 30 days; Group IV, Smoke Shield 2.5 g kg^{-1} for 30 days.

The drug was administered orally for a period of one month. At the end of the experiment mice from all the groups were sacrificed by cervical dislocation. Blood was collected by heart puncture immediately and liver and kidney were excised and thoroughly washed in ice-cold saline (4 °C, 0.9%). Liver and kidney homogenates were prepared in ice-cold Tris-HCl buffer (0.1 M, pH 7.4) and cytosolic samples of liver and kidney homogenates were prepared by centrifuging at 10 000 rev min^{-1} for 30 min at 4 °C. The blood, serum, liver and kidney homogenates were used for the biochemical analysis.

Biochemical analysis

Superoxide dismutase activity of blood and liver and kidney tissues of mice treated with Smoke Shield for 30 days was determined by the nitroblue tetrazolium reduction method of McCord & Fridovich (1969). Values were expressed as U (g haemoglobin (Hb))$^{-1}$ in the case of blood and U (g protein)$^{-1}$ in the case of liver and kidney. Catalase activity in blood and tissues was determined by the method of Aebi (1947), by measuring the rate of decomposition of hydrogen peroxide at 240 nm. A decrease in absorbance was observed after the addition of H_2O_2 to the reaction mixture containing either the tissue homogenate or the erythrocyte sediment used as the source of catalase. Units of activity were determined from the E_{max} of H_2O_2. Reduced glutathione (GSH) activity in blood and tissues was measured by the method of Moron et al (1979), based on the reaction with 5-5′dithiobis (2-nitrobenzoic acid). Values were calculated from a standard graph of GSH treated with the same reagent. Glutathione peroxidase (GPX) activity in blood and tissues was determined by the method of Paglia & Valentine (1967) based on the degradation of hydrogen peroxide in the presence of reduced glutathione. Reduction of GSH concentration was determined by reacting with 5-5′dithiobis (2-nitrobenzoic acid) and values were calculated from a standard plot of GSH. Glutathione-*S*-transferase activity (GST) of cytosolic liver and kidney samples was estimated by the method of Habig et al (1974), based on the rate of increase in conjugate formation between GSH and 1-chloro-2,4-dinitrob enzene. The absorbance was measured at 340 nm for 5 min for an interval of 1 min. The readings were taken against a reagent blank and activity was calculated. The activity of glutathione reductase in blood and cytosolic fraction of liver and kidney tissues was determined by Racker's method (Racker et al 1955), based on the amount of NADPH consumed during the conversion of oxidised glutathione to reduced glutathione. The decrease in absorbance/min was noted and followed at 1-min intervals for 5 min at 340 nm and the concentrations were calculated from the E_{max} of NADP. Lipid peroxidation levels in liver and kidney were estimated using the TBA method of Ohakawa et al (1979) by using 1,1,3,3 tetramethoxy propane as standard against absorbance. Lipid peroxidation in serum was done by the TBA method as modified by Yoshioka et al (1979), using TCA and TBA. Hydroperoxides and conjugated dienes in tissues were determined by the method of John & Steven (1978). In both tests, the samples were first extracted in chloroform and methanol and the lower layer was taken to dryness. The remaining lipid residue was dissolved in 1.5 mL cyclohexane and the absorbance was taken at 233 nm. For the estimation of hydroperoxides, the lipid residues were treated with hydrogen iodide and cadmium acetate. The absorbance was then measured at 353 nm and concentrations were calculated from E_{max}. Haemoglobin was estimated by the cyanmethaemoglobin method using Drabkin's solution (Carman 1993) and the protein estimated by Lowry's method (Lowry et al 1951). Results are presented as means ± s.d. Statistical analysis was performed by one-way analysis of variance test followed by Dunnet's test and Kruskal Wallis' test, if necessary.

Effect of Smoke Shield on the antioxidant system in smokers

To study the effect of Smoke Shield on the antioxidant system in smokers, twenty subjects were selected. Individual written consent was obtained before starting the experiment. Ethical clearance from the Institutional Human Ethics Committee was procured. They were divided into two groups of ten smokers (control) in each group. The blood samples were collected before the treatment from nonsmokers and smokers. The smoker group was treated with Smoke Shield (2 capsules daily) for one month. This dose was selected as per the instruction of the manufacturer. After one month, blood was drawn again and the effect of the drug on the antioxidant system was evaluated by analysing catalase (Aebi 1947), superoxide dismutase (McCord & Fridovich 1969), GPX (Paglia & Valantine 1967) and GSH (Moron et al 1979) activity in erythrocytes. Both Student's *t*-test and paired *t*-test were used in analysing the results in smokers.

Results

Smoke Shield was found to scavenge superoxide and hydroxyl radicals and inhibited tissue lipid peroxidation in-vitro in a concentration-dependent manner (Figure 1). The concentration of the Smoke Shield needed for 50% scavenging of superoxide generated by photoreduction of riboflavin was 91 μg mL^{-1} and that of turmeric, which is an active constituent of Smoke Shield, was 400 μg mL^{-1}. Degradation of deoxyribose mediated by hydroxyl radicals generated by the Fe^{3+}–ascorbate–EDTA–H_2O_2 system was inhibited by the addition of Smoke Shield. The concentration of Smoke Shield needed for 50% inhibition was 95 μg mL^{-1}, indicating that the Smoke Shield could inhibit the hydroxyl radical formation very effectively. The concentration of Smoke Shield needed for 50% inhibition of lipid peroxidation was found to be 134 μg mL^{-1} and that of turmeric was found to be 200 μg mL^{-1}.

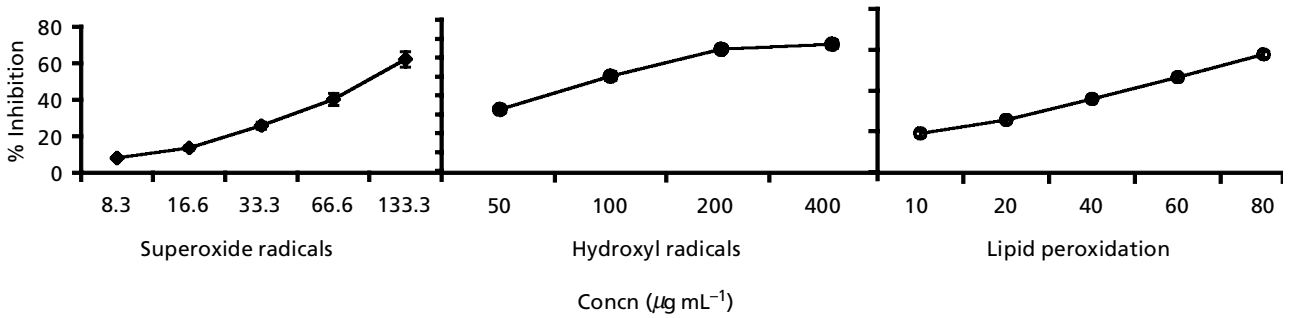

Figure 1 Inhibition of superoxide, hydroxyl radicals and lipid peroxidation by Smoke Shield (in-vitro).

Aminopyrene demethylase and aniline hydroxylase, which are phase I enzymes present in the microsomal P450 system, are implicated in the activation of carcinogens into their ultimate form. Smoke Shield inhibited the activity of both these enzymes in-vitro in a concentration-dependent manner (Figure 2) and the concentration needed for 50% inhibition was found to be 320 μg mL^{-1} for aniline hydroxylase and for aminopyrene-*N*-demethylase could not be calculated.

The effect of Smoke Shield on the antioxidant system in blood and serum of mice after treatment for one month is given in Table 1. Superoxide dismutase was found to be significantly elevated in mice treated with 1.0 or 2.5 g kg^{-1} Smoke Shield daily (Groups II and III, respectively) ($P < 0.001$), while in mice treated with 0.5 g kg^{-1} (Group I) the increase was not significant. Catalase was also found to be elevated after the treatment with Smoke Shield (Groups I, II and III) when compared with the normal controls ($P < 0.001$). GSH was elevated after the treatment with Smoke Shield ($P < 0.001$) in the Groups II or III, but the increase was not significant in the Group I. Glutathione reductase was not altered in any of the treated groups when compared with the normal controls. GPX was found to be decreased in all three treatment groups but the value was significant only in Group III, who received a dose of 2.5 mg kg^{-1} daily ($P < 0.005$). Lipid peroxidation, which is measured as the malonaldehyde formed (MDA), was found to be decreased in all the three treatment groups after the Smoke Shield administration, but the values were not significant.

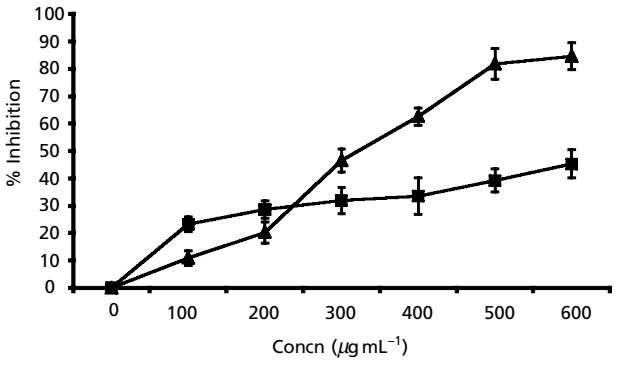

Figure 2 Inhibition of phase I enzymes (▲, aniline; ■, aminopyrene-*N*-demethylase) by Smoke Shield.

Table 2 shows the effect of Smoke Shield on the antioxidant system in mice liver after treatment for one month. Catalase was found to be elevated in the treated groups when compared with the normal controls. Superoxide dismutase showed an increase in the liver but the value was not statistically significant. GST was elevated in the treated groups ($P < 0.001$) and the increase was concentration dependent. Glutathione reductase showed a marked decrease in the treated groups ($P < 0.001$). GPX showed a slight decrease in its activity and was significant only in Group II (that received 1 mg kg^{-1} Smoke Shield daily). Lipid peroxidation in the tissue was decreased after Smoke Shield treatment. GSH, hydroperoxides and conjugated dienes were unaltered by Smoke Shield administration.

Table 3 shows the effect of Smoke Shield administration for one month on the antioxidant system of the mouse kidney. The catalase level in the kidney increased after the Smoke Shield administration in a concentration-dependent manner ($P < 0.001$). Superoxide dismutase was found to be slightly elevated (this increase was significant in Group I, $P < 0.005$). GST increased after Smoke Shield treatment (in Group III this was found to be significant, $P < 0.005$). GPX was elevated in the treated groups (only significant in Group II, $P < 0.005$). Elevation was also observed in the case of glutathione reductase and was concentration dependent and significant in Groups II and III ($P < 0.001$). Reduced glutathione (GSH) was found to be slightly elevated in all the 3 groups when compared with the normal. A significant decrease was observed in lipid peroxidation in all the three treatment groups after the drug administration ($P < 0.001$). Conjugated dienes showed a slight decrease, as was the case with hydroperoxides in all the 3 treated groups.

Table 4 shows the effect of Smoke Shield administration on the antioxidant system in smokers. The increased blood catalase in smokers was not altered by Smoke Shield administration. Blood superoxide dismutase, which was decreased in smokers ($P < 0.001$), was elevated after Smoke Shield treatment ($P < 0.005$). Similarly, GPX, which was significantly increased in smokers ($P < 0.001$), was found to be significantly decreased ($P < 0.005$) after

Table 1 Effect of Smoke Shield administration (one month) on the antioxidant system in mice (serum and blood).

Parameters	Control	Group I ($0.5\,g\,kg^{-1}$)	Group II ($1\,g\,kg^{-1}$)	Group III ($2.5\,g\,kg^{-1}$)
SOD (U (g Hb)$^{-1}$)	218.18 ± 32.28^{C}	252.15 ± 31.09^{C}	313.56 ± 9.33^{B}**	384.38 ± 55.79^{A}**
Catalase (U (g Hb)$^{-1}$)	5.91 ± 1.48^{D}	8.19 ± 0.48^{C}**	10.82 ± 0.54^{B}**	12.99 ± 0.86^{A}**
GSH (nmol mL^{-1})	34.16 ± 1.83^{B}	38.5 ± 3.08^{B}	49.66 ± 1.86^{A}**	48.33 ± 6.28^{A}**
Glutathione reductase (U (mg protein)$^{-1}$)	14.63 ± 1.68	15.36 ± 1.78	14.87 ± 1.14	14.75 ± 1.17
GPX (U (g Hb)$^{-1}$)	5.50 ± 0.77^{A}	3.94 ± 0.60^{BC}	4.60 ± 0.97^{AB}	3.37 ± 0.83^{C}*
Lipid peroxidation (nmol (mL serum)$^{-1}$)	0.77 ± 0.43	0.55 ± 0.12	0.45 ± 0.15	0.46 ± 0.22

Values are expressed as means ± s.d. (n = 6). SOD, superoxide dismutase; GSH, glutathione; GPX, glutathione peroxidase. **$P < 0.001$, *$P < 0.005$ vs control (Dunnet's test); differences between groups (denoted by A, B, C, and D) were compared by one-way analysis of variance. A, B C and D indicate the statistical notation used to represent the results of ANOVA. The same letter for two or three results indicates that they are not statistically significant (the values are homogenous); different letters indicate the results are statistically significant (the values are heterogenous).

Table 2 Effect of Smoke Shield administration (one month) on the antioxidant system in mice (liver).

Parameters	Control	Group I	Group II	Group III
SOD (U (g protein)$^{-1}$)	339.60 ± 144.04	405.73 ± 222.39	397.29 ± 204.85	596.63 ± 238.33
Catalase (U (g protein)$^{-1}$)	15.52 ± 2.61	19.63 ± 2.56	19.48 ± 2.47	19.71 ± 4.12
GST (nmol (mg protein)$^{-1}$)	345.87 ± 48.18^{C}	481.33 ± 67.75^{B}**	90.43 ± 108.59^{A}**	642.12 ± 67.18^{A}**
GPX (U (mg protein)$^{-1}$)	10.34 ± 2.94	6.58 ± 3.37	5.53 ± 4.01*	8.93 ± 2.31
Glutathione reductase (U (mg protein)$^{-1}$)	76.65 ± 14.97^{A}	37.96 ± 8.00^{C}**	58.70 ± 16.43^{B}**	43.99 ± 9.00**
GSH (nmol mL^{-1})	16.33 ± 2.16	15.33 ± 1.03	16.00 ± 1.09	14.83 ± 3.18^{C}
Lipid peroxidation (nmol (mg protein)$^{-1}$)	0.077 ± 0.035	0.066 ± 0.035	0.051 ± 0.018	0.045 ± 0.025
Conjugated dienes (mM/100 g tissue)	8.15 ± 1.77	7.11 ± 2.60	9.59 ± 0.92	7.99 ± 1.50
Hydroperoxides (mM/100 g tissue)	9.40 ± 2.26	10.67 ± 2.57	9.27 ± 1.78	8.87 ± 1.86

Values are expressed as means ± s.d. (n = 6). SOD, superoxide dismutase; GST, glutathione-*S*-transferase; GPX, glutathione peroxidase; GSH, glutathione. **$P < 0.001$, *$P < 0.005$ vs control (Dunnet's test); differences between groups (denoted by A, B, C, and D) were compared by one-way analysis of variance. A, B C and D indicate the statistical notation used to represent the results of ANOVA. The same letter for two or three results indicates that they are not statistically significant (the values are homogenous); different letters indicate the results are statistically significant (the values are heterogenous).

Table 3 Effect of Smoke Shield administration (one month) on the antioxidant system in mice (kidney).

Parameters	Control	Group I	Group II	Group III
SOD (U (g protein)$^{-1}$)	186.01 ± 61.78^{C}	372.23 ± 102.21^{A}*	218.65 ± 55.11^{BC}	283.08 ± 54.34^{B}
Catalase (U (g protein)$^{-1}$)	8.62 ± 1.61^{C}	11.38 ± 1.72^{B}**	12.90 ± 1.13^{B}**	15.28 ± 2.01^{A}**
GST (nmol (mg protein)$^{-1}$)	223.21 ± 40.69^{C}	292.33 ± 101.82^{BC}	357.29 ± 92.11^{AB}	452.86 ± 117.31^{A}*
GPX (U (mg protein)$^{-1}$)	1.95 ± 0.53^{B}	3.50 ± 1.25^{AB}	4.35 ± 1.55^{A}*	3.81 ± 1.89^{A}
Glutathione reductase (U (mg protein)$^{-1}$)	107.30 ± 17.98^{C}	129.15 ± 19.34^{B}	143.72 ± 12.09^{AB}**	153.62 ± 18.45^{A}**
GSH (nmol mL^{-1})	16.00 ± 1.09	17.33 ± 3.72	18.80 ± 0.98	19.33 ± 2.58
Lipid peroxidation (nmol (mg protein)$^{-1}$)	0.250 ± 0.060^{A}	0.070 ± 0.013^{B}**	0.065 ± 0.006^{B}**	0.068 ± 0.005^{B}**
Conjugated dienes (mM/100 g tissue)	4.03 ± 1.50	4.04 ± 1.17	3.47 ± 1.09	3.89 ± 1.10
Hydroperoxides (mM/100 g tissue)	3.28 ± 0.49	3.03 ± 0.53	2.45 ± 0.77	2.36 ± 0.91

Values are expressed as means ± s.d. (n = 6). SOD, superoxide dismutase; GST, glutathione-*S*-transferase; GPX, glutathione peroxidase; GSH, glutathione. **$P < 0.001$, *$P < 0.005$ vs control (Dunnet's test); differences between groups (denoted by A, B, C, and D) were compared by one-way analysis of variance. A, B C and D indicate the statistical notation used to represent the results of ANOVA. The same letter for two or three results indicates that they are not statistically significant (the values are homogenous); different letters indicate the results are statistically significant (the values are heterogenous).

Table 4 Effect of Smoke Shield administration (one month) on the antioxidant system in man.

Group	Catalase (K (g Hb)$^{-1}$)	SOD (U (g Hb)$^{-1}$)	GPX (U L^{-1})	GSH (nmol (mg Hb)$^{-1}$)
I Non-smokers	110.6 ± 24.7	684.3 ± 117.2	319.7 ± 140.9	16.79 ± 1.52
II Smokers (before treatment)	128.4 ± 88.8	460.9 ± 176.5**	599.5 ± 123.2**	14.40 ± 1.03
III Smokers (after treatment)	130.6 ± 18.0	528.3 ± 160.9*	463.5 ± 94.1*	15.50 ± 1.15

Values are mean ± s.d. Average of 10 subjects in each group. SOD, superoxide dismutase; GPX, glutathione peroxidase; GSH, glutathione. $^{**}P < 0.001$, $^{*}P < 0.005$, Group II was compared with Group I; Group III was compared with Group I and Group II.

Smoke Shield treatment. Glutathione showed only a slight decrease in smokers and treatment with Smoke Shield produced a slight increase.

Discussion

The results presented in this paper point to the significant antioxidant potential of Smoke Shield, a herbal preparation. This preparation, formulated by New Chapter (USA), contains several spices that have been reported to be antioxidant, antimutagenic and anticarcinogenic. For example, turmeric, which has been used in the culinary arts in many Asian countries, has curcumin as the major ingredient along with turmeric oils and water-soluble antioxidant peptides and proteins that have been shown to reduce cancer incidence in several experimental systems (Bhide et al 1991). In Smoke Shield, turmeric is first extracted with CO_2 gas under supercritical conditions and later by post-critical hydroethanolic extraction to extract hydrophilic materials. This dual extraction process protects the antioxidants and other pharmacologically active substances in turmeric. The preparation also contains spices such as green tea, clove, ginger, parsley, peppermint and rosemary. The antioxidants present in this extract synergistically act to produce the desired high efficient activity of this formulation.

Smoke Shield is mainly aimed at reducing smoke-related damage in the body. In the present life-style one cannot avoid exposure to smoke coming not only from smoking but also from automobile exhaust, charboiling of meat, wood burning, etc. Smoke not only contains carbonaceous particles but also several cancer causing agents, such as polycyclic aromatic hydrocarbons and nitrosamines. Most of the carcinogenic substances need to be metabolised to their ultimate form by microsomal-P450-mediated oxidation (Cooper et al 1965). Antioxidants present in Smoke Shield have a significant role in preventing the oxidation and reducing the mutagenic and carcinogenic response mediated by xenobiotic materials. The polyherbal antioxidant composition of Smoke Shield will be highly effective in inhibiting the oxidative steps leading to the activation of carcinogens. Smoke Shield has also been found to inhibit enzymes responsible for the activation of xenobiotics, as represented by aniline hydroxylase and aminopyrene demethylase. Thirdly, Smoke Shield has been shown to produce a significant increase in GST activity, which can detoxify the carcinogens effectively. We have earlier reported the antimutagenicity of Smoke Shield against a variety of natural and synthetic mutagens in-vitro and in-vivo (unpublished data).

Smoke Shield had been found to inhibit oxygen free radicals, such as superoxides and hydroxyl radicals, and lipid peroxidation in-vitro. Moreover, the preparation was found to increase the activity of enzymes that can scavenge oxygen radicals, such as superoxide dismutase, catalase and GPX and increased the cellular glutathione levels. It was also found that Smoke Shield could inhibit the lipid peroxides in serum and tissues. Oxygen radicals attack high-molecular-weight compounds and in the case of lipids they produce peroxidative damage. When an oxygen radical attacks the membrane lipid it can produce structural damage to the cell, as well as to the tissue. In fact, the aetiology of several diseases has been shown to be due to the initial damage caused by free radicals (Wilson 1998). Here, we have also tested the effect of Smoke Shield in smokers. The decreased antioxidant potential produced in the smokers, as seen from the values of GPX and superoxide dismutase, was found to be increased by administration of Smoke Shield.

Smoke Shield may thus be a highly useful formulation not only to reduce smoke-related damage but also as a chemoprotective agent. The ingredients in Smoke Shield are used in the daily diet as spices or food additives and hence their non-toxicity is well documented. The formulation is, therefore, an effective prophylactic agent for several chronic diseases.

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