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PHYSIOLOGICAL AND MOLECULAR HAZARDS OF COOKING OIL FUMES: THE PROTECTIVE ROLE OF SAGE AND ROSEMARY

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ABSTRACT

Cooking oil fumes (COF) are the main cause of indoor air pollution in our houses. The study aimed to investigate the effects of COF on the lung of rats. Animals were subdivided into four groups; control group; oil fumes group, rats were exposed to the COF for 15 min/day for one month; oil fumes+rosemary extract group, rats were exposed to the COF and orally administered with 300 mg/kg b.wt. of rosemary extract. The oil fumes+sage extract group, rats were exposed to the COF and orally administered with 300 mg/kg b.wt. of sage extract. Serum tumor markers such as arginase and alpha-L-fucosidase were significantly increased in the oil fumes group. The activity of glutathione related enzymes and Cu/Zn-dependent superoxide dismutase were decreased in the lung of oil fumes group. Moreover, the gene expression of those enzymatic antioxidant genes was considered by using specific primers and RT-PCR. Administration of watery extracts of sage and rosemary ameliorates the biochemical and molecular changes induced by COF in the lung tissues.

INTRODUCTION

Cooking oil fumes (COF) are generated during cooking in Western-style deep-frying and of course in Arabic kitchens. The biological effects of COF on human lung epithelial CL3 cells, namely is the inhibition of cell growth and induction of oxidative stress. So, daily exposure to COF might be an important risk factor in the etiology of lung cancer; this may be due to the mutagenicity or carcinogenicity of COF. Furthermore, the exposure to polycyclic aromatic hydrocarbons or possibly other compounds in COF may cause oxidative DNA damage (Lai *et al.,* 2013). Under normal conditions, in human and animal body, reactive oxygen species (ROS) can be neutralized by antioxidant defense systems, including antioxidant enzymes such as superoxide dismutase (SOD) (Wang *et al.,* 2011), glutathione peroxidase (GSH-Px), and catalase (CAT) (Fang *et al.,* 2002). An imbalance between the amounts of ROS and antioxidant defenses is characteristic of lung diseases. ROS damage has been implicated in the pathogenesis of a number of injury and disease states (Chiang *et al.,* 1997). The proinflammatory properties of ROS in the lung and liver include endothelial cell damage and lipid peroxidation.

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Electrophilic foreign compounds (xenobiotics) may be detoxified in a reaction catalyzed by a group of enzymes named glutathione-S-transferases (GST), by which they are conjugated with glutathione (GSH) (Strange *et al.,* 2001). Glutathione is involved in the conjugation and detoxification of several types of compounds that cause toxicity and carcinogenesis (Termato *et al.,* 1996), as well known that GSH plays an important role in the defense mechanism of the lung (Elsaid, 2006).In normal conditions, cells cope with free radicals using such enzymatic and nonenzymatic defenses as SOD, GST, CAT, GSH-Px and GSH (Bakan *et al.,* 2003).The available information on the genotoxic and mutagenic activity of COF includes the data in cooks and homemakers that show the induction of 8-oxoguanine DNA glycosylase 1, which is a DNA repair enzyme that removes 8-hydroxydeoxyguanine and in the same time it makes a reduction in the levels of GSH in the liver cells (Dung *et al.,* 2006). Moreover, COF had been found to produce DNA adduct, and be a potent inducer of DNA damage, and also able to disturb the cell cycles (Cao *et al.,* 2013). The glutathione-S-transferase mu (GSTM1) gene, which encodes GSTm, has three identified alleles (a, b and null) (Yang *et al.,* 2002). Genotypes associated with lung cancer prognosis have been reported, but not with lung cancer risk. A few studies have reported an association between GSH pathway genes and lung cancer risk in never smokers, but the results are inconsistent (Lin *et al.,* 2003).

Rosmarinic acid is highly polar than carnosic acid and carnosol, therefore it was mostly located in the continuous aqueous phase derived from rosemary. Pigment oxidation was mainly due to myoglobin reacting with quinones,formed by hydroxyl oxidation of rosmarinic acid, carnosol and carnosic acid (Herndndez-Herndndez *et al.,* 2009).Many species of **S**alvia**,** including sage, have been used as traditional herbal medicine against a variety of diseases (Eidi and Eidi, 2009). The plant has a wide range of biological activities, such as antioxidative properties (Hohmann *et al.,* 1999). The strongest active constituents of sage are within its essential oil, which contains cineole, borneol, and thujone. Sage leaf contains tannic acid, oleic acid, ursonic acid, ursolic acid, cornsole, cornsolic acid, fumaric acid, chlorogenic acid, caffeic acid, niacin, nicotinamide, flavones, flavonoid glycosides and estrogenic substances. Upon of these bases, the study aimed to investigate the biochemical and molecular changes of COF on lungs, of rats and to study the protective role of sage and rosemary in alleviation of these hazards. Administration of extracts to the rats occurred after oil fumes exposure.

MATERIALS AND METHODS

Herbs extracts: Crude aqueous extracts were prepared from rosemary (*Rosmarinus officinalis L.*, family *Lamiaceae)*, sage (*Salvia officinalis L*., family *Lamiaceae*). Briefly, 5g of each herb separated, ground in a blender and was then incubated in 100ml of boiling distilled water for 20min. The extracts were filtered once by four layers of cheesecloth and once by a single layer of Whatmann filter paper. Finally, each extract was centrifuged at 6000g for 10min, and the supernatant was decanted and fractioned into 10ml aliquots and freeze-stored at -20°C. Just prior to each experiment, an aliquot of the extract was thawed. Cooking oil fumes exposure: Animals were housed in a room chamber of volume (18 m^3), with dimensions (2m width, 3m length and 3m height). For production of fumes, about 250 ml of fresh corn cooking oil was daily preheated on digital hot plate to approximately 280±20°c. Consequently, rats inhaled oil fumes (15min/d) for 30days.

Animal grouping: Twenty male *Sprague Dawley* strain, adults, weighing180–200g, were obtained from the Experimental Animal Unit, College of Science, King Khalid University, Saudi Arabia were used this study. All rats received food and water *ad libitum* and were kept in a room with the temperature regulated to 22±1°C.The experiment was approved by the Animal Ethical Committee, College of Science, King Khalid University. The animals were subdivided randomly into four groups.The control group, rats received no treatment; the oil fumes group; rats were exposed to the cooking oil fumes for 15min/day for one month. The oil fumes+rosemary extract group; the rats were exposed to the cooking oil fumes for 15min/day and orally administered with 300mg/kg b.wt. of rosemary extract for the same experimental period. The oil fumes+sage extract group; the rats were exposed to the cooking oil fumes for 15min/day and orally administered with 300mg/kg b.wt. of sage extract for the same experimental period. At the end of the experimental period, rats were decapitated and blood samples were centrifuged at 3000 rpm for 15min, then sera kept in clean Eppendorf tubes on -20°C until biochemical analysis.

Preparation of tissue homogenate: At the end of 30 days, a piece of lung was taken freshly from each animal on ice. Homogenates of the tissues were prepared in 1.0ml of phosphate buffer (pH, 7.4) per 100mg of tissue by using an electrical tissue homogenizer. The samples were spun at 3000 rpm. at 4°C for 15min, and the supernatant was used for the biochemical analysis. For RNA extraction, samples from rat lung were removed, rinsed with ice cold saline solution, weighed then directly frozen by dropping into dry ice and stored at -80°C for further investigations.

Biochemical analysis

Determination of malondialdehyde concentration**:** T**o** analyse lipid peroxidation in lung, samples 2-Thiobarbituric Acid-Reactive Substances (TBARS) were measured according to Ohkawa *et al.,*(1979). Determination of Glutathione (GSH) concen-tration: Total GSH content was measured according to the method described by Beutler *et al.,* (1963) using glutathione reduced colorimetric method. Determination of superoxide dismutase (SOD) activity: SOD activity was determined in the sample according to the method of Nishikimi *et al.,* (1972).Determination of glutathione peroxidase (GSH-Px) activity: GSH-Px activity in the sample was measured according to the Paglia and Valen-tine's method (1967). Determination of Glutathione reductase (GSH-R) activity: GSH-R activity was measured as described by Goldberg and Spooner (1983).

Determination of Glutathione-S-transferase (GST) activity: The method of Habig *et al.,* (1974) was used. It depends on measuring the conjugation of reaction 1-chloro-2,4-dinitrobenzene (CDNB) with reduced glutathione. Determination of total antioxidant capacity (TAC): TAC was measured as described by Koracevic *et al.,* (2001). Determination of serum arginase: Serum arginase estimation was based upon the colorimetric determination of urea by condensation with diacetyl monoxime in an acid medium in the presence of ferric chloride and carbazide according to the method of Marsh *et al.,* (1965). Determination of serum α-l-fucosidase: The assay used for α-l-fucosidase, was based on the enzymatic cleavage of the synthetic substrate p-nitrophenyl a -l-fucopyranoside to p-nitrophenyl and l-fucose L.The yellow color of p-nitrophenyl in an alkaline medium was measured quantitatively at 405nm (Zietke *et al.,* 1972).

Gene expression

RNA extraction:To determine mRNA expression levels of GST, GSH-Px, SOD (Cu/Zn-dependent superoxide dismutase), and CAT total RNA was isolated from lung using RNeasy Mini kit,(Qiagen) according to the manufacturers' protocol. Following the photometrical determination of RNA concentration and purity at 260nm and 280nm. Complementary DNA (cDNA): According to the manufacturers' protocol of Omniscript RT Kit-*Qiagen*, USA; total RNA (2µg) was transcribed into cDNA in a 20µL final volume of reaction buffer [2µL 10x Buffer; 2µL dNTP mix (5mM each); 1µL RNase inhibitor (10U/µL); 2µL oligo dT primers (100µM); 1µL Omniscript reverse transcriptase and the corresponding amount of RNA (100ng)] by incubation for 1h at 37°C.

The reaction was stopped by incubation at 99°C for 5min. cDNA's were stored at -20°C until usage.Amplification of transcripts by Polymerase Chain Reaction: According to the manufacturers' protocol of GoTaq® DNA Polymerase Promega, USA. For rat GST, GSH-Px, Cu/Zn-SOD and CAT, PCR was performed with 100ng of the synthesized cDNA and specific primers for a given gene of interestas the following. In a typical PCR, a fresh master mix was prepared by mixing (per sample) 2.5µl 10x PCR buffer, 1µl $MgCl_2$ (25mM), 2µl dNTP mix (2.5mM of each dNTP), 0.5µl Taq DNA polymerase (final concentration 2.5U/ml), and 17µl water.

To the resulting 23µl per sample, 1µl of a pre-made mix of forward and reverse primers (5µM each; final concentration 200nM) and 1µl cDNA template was added, for a final reaction volume of 25µl. The thermal cycler was started with initial denaturation at 94°C for 3min, denaturation at 94°C for 30sec, annealing at 58°C for 30sec and extension at 72°C for 60sec. These steps were repeated for 30 cycles. Last extension was carried out at 72°C for 5min and then finally reaction was kept at 4°C. After the reaction was completed, PCR tubes were stored at -20°C until further use. All PCR amplifications were performed on a Primus 25 advanced thermocycler (Germany). From each PCR reaction, 5µL was electro-phoresed in a 1% agarose gel in Tris-borate-EDTA (TBE) buffer.

GST-F:5'CCTCACCCTTTACCAATCTA3', GST-R:5'TTCGTCCACTACTGTTTACC3';
Cu-Zn/SOD-F:5'TCTAAGAAACATGGCGGTCC3', Cu-Zn/SOD-R:5'CAGTTAGCAGGCCAGCAGAT3';
GSH-Px-F:5'CTCTCCGCGGTGGCACAGT3', GSH-Px-R:'CCACCACCGGGTCGGACATAC3' and
CAT-F: 5'GCGAATGGAGAGGCAGTGTA3', CAT-R: 5'GAGTGACGTTGTCTTCATTAGCACTG3'.

Statistical analysis

The biochemical data were expressed as mean±SD and statistical and correlation analyses were performed using the one-way ANOVA followed by a post-hoc least significant difference (LSD) test. P values < 0.05 were considered as statistically significant. Statistical analyses were performed with the statistical package for the social sciences for Windows (SPSS, version 15.0, Chicago, IL, USA).

RESULTS

Biochemical results

In Table 1, rats exposed to COF showed a significant increase in the activity of SOD in lung tissues when compared to the control group.

Table 1. Lung superoxide dismutase (SOD), total antioxidant capacity (TAC) and lipid peroxidation in different animal groups

Parameters	Control	Oil Fumes	Oil fumes+ Rosemary	Oil fumes+ Sage
SOD (U/g tissue)	15.3±4.6	47.6±0.8***	43.8±1.5$^{***, II}$	39.3±0.9$^{***, III}$
TAC (U/ g tissue)	351.5±22.4	239.7±16.7***	246±36***	278.1±21.2^{***,I}
MDA (n mol/g tissue)	102.5±8.3	148.4±13.2***	135.1±7.6$^{***, I}$	124±7.6$^{***, III}$

Values were expressed as means ±SD of five animals in each group.
*** = P<0.001, when all groups compared with control group.
I = P<0.05, II= P<0.01, III = P<0.001, when all oil fumes+rosemary and oil fumes+sage groups compared with oil fumes group.

Table 2. Lung glutathione contents and their relative antioxidant enzymes in different animal groups

Parameters	Control	Oil Fumes	Oil fumes+ Rosemary	Oil fumes+ Sage
GST (U/ g tissue)	318±20.7	196.1±20.7***	289.5±11.2^{III}	296.9±21.3^{III}
GSH-Px (U/ g tissue)	207.4±11.9	118.7±20.5***	167.1±5.5$^{***, III}$	148.8±25.2$^{***, II}$
GSH-R (U/ g tissue)	109.6±4.5	51.1±4.4***	55.2±2.6$^{***, III}$	74.9±6.4***
GSH (U/ g tissue)	657.5±23.4	419.1±12.8***	492.3±5.1$^{***, III}$	521.1±10.1$^{***, III}$

Values were expressed as means ±SD of five animals in each group.
*** = P<0.001 (very high significant), when all groups compared with control group.
II = P<0.01, III = P<0.001, when all oil fumes+rosemary and oil fumes+sage groups compared with oil fumes group.

Table 3. Serum tumor markers

Parameters	Control	Oil Fumes	Oil fumes+ Rosemary	Oil fumes+ Sage
Arginase (U/l)	141.4±9	321.1±6.9***	242.1±11.1^{***,III}	205.8±11.9^{***,III}
Alpha-L- Fucosidase (U/l)	3.1±0.2	7.5±0.8***	4.1±0.6$^{***,, III}$	5.2±0.2$^{***, III}$

Value Values were expressed as means ±SD of five animals in each group.
*** = P<0.001 (very high significant), when all groups compared with control group.
III = P<0.001 (very high significant), when all oil fumes+rosemary and oil fumes+sage groups compared with oil fumes group.

The DNA was visualized and photographed using the BioRad Gel documentation system. The sequences of the primers were listed in the following:

However, rats exposed to COF along with rosemary or sage showed a significant improvement in the imbalance of SOD activity compared to the COF exposed groups.

Reversely, the total antioxidant capacity was depleted significantly in the exposed groups as compared to the control one. Oral administration of watery extracts of rosemary or sage could restore the total antioxidant capacity in the lung when compared to oil fumes group. Lipid peroxidation (measured by MDA level) showed a significant increase in cooking oil fumes group when compared to the control group.

As indicated in table 2 the enzymatic antioxidants such as glutathione-S-transferase (GST), glutathione peroxidase (GSH-Px) and glutathione reductase (GSH-R) and non-enzymatic antioxidant such as glutathione (GSH) were adversely changed lung of all groups when compared with the control. The decrement of the above enzymes may be due to the significant decrease in GSH of lung tissue of the COF exposed rats. In Table 3, a significant increase in arginase and alpha-L-fucosidase in the COF exposing groups as compared to the control group. Co-administration of rosemary or sage showed marked improvement in these tumor markers in the treated groups when compared with oil fumes froup.

Molecular results

Figure (1): Gel electrophoresis of the RT-PCR product of Cu-Zn SOD gene (a), GSTgene (b), GSH-Px gene (c) and CAT gene (d) in lung tissue in different animal groups. The differences in band density (amplicones) of Cu-Zn SOD, GST, GSH-Px and CAT genes between the control, oil fumes and oil fumes treated groups were observed after RT-PCR reactions (Fig. 1). The gene expression of Cu-Zn SOD was significantly appeared in oil fumes group and nearly comes down in co-administered with rosemary or sage when compared with control (Fig. 1).The gene expression of GST enzyme was non significantly observed in the lung of oil fumes group (Figure 1, b). The mRNA expressions of GST were significantly appeared in oil fumes co-administered with rosemary or sage when compared with oil fumes (Fig. 1, b). The expression of GSH-Px gene had been observed in the lung (Fig. 1, c). In lung, the variations in band density between oil fumes group and other corresponding treated groups were observed after RT-PCR reactions for CAT when compare with control one (Fig. 1, d).

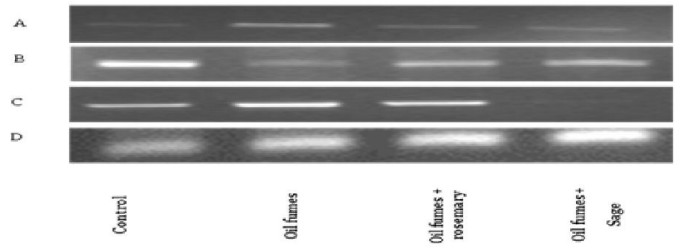

Figure 1. Gel electrophoresis of the RT-PCR product of Cu-Zn SOD gene (a), GSTgene (b), GSH-Px gene (c) and CAT gene (d) in lung tissue in different animal groups

DISCUSSION

The exposure to COF during cooking was confirmed to be associated with cancer and lung diseases because they cause oxidative damage to nucleic acids, proteins, amino acids and lipids (Murata *et al.,* 2003; Elsaid, 2006).

Hydrophobic interaction between PAHs and blood serum albumin plays an important role in PAH transportation in the body. Activated carcinogens bind covalently to nucleic acids and proteins and therefore, quantitation of the PAH–DNA adduct formed in the body may be used as a measure of the biologically active dose of the metabolically activated PAH (Wang *et al.,* 2011). The increment of lipid peroxidation was more pronounced in the lung of oil fumes when compared with the control. Oral administration of aqueous extracts of rosemary and sage (300mg/kg b.wt.) was able to alleviate the lipid peroxidation in the lung homogenate due to their antioxidant activity. Under normal physiological conditions, low concentrations of lipid peroxidation products are found in tissues and cells. In the presence of oxidative stress induced by PAHs more lipid peroxidation products are formed due to the attack of reactive oxygen species that peroxides the polyunsaturated fatty acids in cell membranes (Hanzalova *et al.,* 2010).

Cellular antioxidant enzymes such as SOD, GST, GSH-Px, GSH-R and CAT normally challenge the oxidative stress in the lung tissue. The data in this study showed an increase in MDA concentration in lung tissue, which may be related to the increased in ROS. Cao *et al.* (2013) suggested that COF exposure could increase the level of MDA and decreased activities of SOD and GSH in the AEC II cells. The current study showed a significant increase in the activity of SOD in groups of rats exposed to COF. This exhibition could be attributed to an enhanced production of ROS during metabolism of PAHs in the lung to mitigate this condition. The activites of SOD was increased as Cu-Zn SOD gene was more expressed as in Fig. (1).

Administration of aqueous extracts of rosemary and sage is potent agents as they alleviate the changes of the SOD enzyme when compared to the oil fumes group. The detoxification of PAHs occurs by converting them into a variety of reactive metabolites among which epoxides have a special importance according to Singh *et al.,*(2008).The enzymes involved in their detoxification are classified into two categories: phase 1 enzymes, which catalyze oxidative reactions, and phase 2 enzymes, which catalyze conjugative reactions of oxidized PAHs with endogenous compounds such as sulfuric acid, glucuronic acid and GSH. Phase 1 metabolism involves the following steps. At first, the PAHs are transformed by cytochrome P450-dependent monooxygenases to various arene oxides which then may rearrange to form phenols (Singh *et al.,* 2008). Secondly, primary metabolites may be further oxidized to e.g. trans-dihydrodiol epoxides, the ultimate carcinogens. The latter may covalently bind to nucleophilic sites of DNA. In phase 2 metabolism phenols, phenol dihydrodiols, quinones, and dihydrodiols are conjugated to form sulphates or glucuronides.

The resulting sulfates, glucuronides or protein adducts are detoxifying products of hazardous PAHs metabolites. DNA adducts, however, can lead to muta-tion and initiation of cancer cells if they are not repaired (Wang *et al.,* 2011). The formed epoxide, which is successively hydrolyzed to form a diol then dihydroxy-epoxides can be formed, which in turn may decompose to strong electrophilic compounds capable to react with e.g. DNA (Singh *et al.,* 2008).

Since GSH is required to maintain the normal reduced state of cells and to counteract all the deleterious effects of oxidative stress. The key enzyme that detoxifies the synthesized epoxides is GST (phase II enzyme) that binds epoxides to GSH and forms epoxide-GSH conjugates (Yang *et al.,* 2004). The inhibition of GST in oil fumes group may be due to the increase of the reactive metabolites of PAHs in the lung cells. The molecular study was documented the inhibition of the activity of GST enzyme in the different groups, as showed by the low density of amlicone of GST expressed gene in oil fumes group as compared to the control one. Moreover, the decrease in the activity of GSH-Px in the investigated tissues in the exposing COF groups could be due to the decrease of the reduced form of GSH (Singh *et al.,* 2008). The decrease in the reduced form of GSH in lung tissue after the exposure to COF may be due to the inhibition of GSH-R activity. Moreover, oxidative stress is responsible for an increase in the accumulation of the reactive oxygen species in cells, which may subsequently lead to an increase in the expression of genes encoding antioxidant enzymes (Michiels *et al.,*1994). Variances in band density of amplicones of CAT between rats in the control group and oil fumes groups; administration with rosemary or sage may be differentially expressed in cells exposed to oxidative stress compared with the control one.

The DNA adducts may be the main reason and act as hotspots in mutation of the investigated CAT and other genes that deflect their expression. The decrease in total antioxidant capacity of all tissues in the oil fumes can lead reactive metabolites of PAH cruelty to attack the biomolecules such as DNA. These DNA adducts can then, in case no reparation mechanisms start, cause tumorigenesis. This may explain the significant increase in arginase and alpha-L-fucosidase activity in oil fumes group. Oral administration with rosemary or sage has anticancer action as they restoring the level of tumor markers nearly to the control group.

Conclusions

Corn cooking oil fumes have cytotoxic, genotoxic and carciongenic action. They induce the biochemical changes representing by the deflection of the activity of the antioxidant enzymes in the lung tissue.The oxidative stress was highly increased as indicated by the increase in lipid peroxidation. In addition, the gene expression of SOD, GST, GSH-PX, and CAT enzymes were adversely affected by exposing rats to the oil fumes. Corn cooking oil fumes significantly elevate the tumor markers in the sera of rats, such as arginase and alpha-L-fucosidase; this means that corn oil fumes have carcinogenic action. Oral administration of water extracts of rosemary and sage was able to restore the balance in the antioxidants and oxidants status.

Conflict of interest

The authors declared no conflict of interest.

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