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Hypericum Perforatum Extract inhibits Cigarette Smoke Induced Lung Inflammation

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Abstract: Hypericum perforatum, is a plant which blooms between july and september at farms, borders of roads and woods, top of hills and grasslands, whose anti-inflammatory effects are shown in various studies. It is shown that cigarette smoke causes increase in inflammation mediators on lung. Due to this reason, we planned a study to examine the protective effects of Hypericum perforatum aganist smoke exposure induced lung damage in mice. For this purpose, mice exposured to smoke during 2 mounth and 70 mg/kg Hypericum perforatum extract per a day and cyclooxygenase-2, cytosolic phospholipase A2($cPLA_2$) and inducible nitric oxide synthase (iNOS) enzymes in their lungs are analyzed by using ELISA method. Smoke exposure increased the expression of cyclooxygenase-2 (COX-2), $cPLA_2$ and iNOS enzymes. Hypericum perforatum administration to mice that are exposured to smoke previously decreased the rate of increase of the $cPLA_2$, cyclooxygenase-2 and iNOS In conclusion, our study shows that the smoke exposre causes inflammation induced lung damage and the use of Hypericum perforatum extract can be helpful against this toxic effect.

Keywords: Cigarette smoke, Lung, Hypericum perforatum, cyclooxygenase-2, cytosolic phospholipase A2 and inducible nitric oxide synthase.

1. Introduction

CIGARETTE SMOKING is a risk factor in the pathogenesis of chronic obstructive pulmonary disease (COPD), which is characterized by abnormal inflammatory responses in the lungs [1]. Moreover, the harmful effects of cigarette smoke in lung inflammation may result from increasing cyclooxygenase (COX)-2 expression in the lungs [2]. COX-2 regulates airway inflammatory responses [3].

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Cigarette smoke also increases cPLA2 which hydrolyzes membrane phospholipids to produce arachidonic acid (AA). Ecosanoids which are generated from AA have been defined in airway secretion of asthmatics in situ [4]. Eicosanoids play an important role in the inflammatory pathogenesis, including asthma and COPD [5]. constitutive enzyme cyclooxygenase (COX)-1 or the inducible COX-2 converts AA to PGs, such as PGE2 by the in some cell types [6, 7]. The PLA2 superfamily is defined of three main types of lipolytic enzymes which are secretory PLA2, the 85 kDa cytosolic group IV PLA2 (cPLA2), and a calcium-independent group VI PLA2 in mammalian cells [8]. Although several subtypes of PLA2 have been defined, cPLA2 is the only one that shows specificity for AA, and its role in mediating agonist-induced AA release for eicosanoid production in various cell types is well studied [9]. cPLA2 has also role in acute lung injury induced by sepsis [10] and bronchial reactivity associated with anaphylaxis [11]. Furthermore, increase in cPLA2 activity induces increase synthesis of PGE2 in various cell types [12, 13]. Tracheal smooth muscle cells also synthesize these eicosanoids that have role on physiological or pathological actions. These results show that cPLA2 plays an important role in mediating AA release for production of eicosanoid by inflammatory cells and airway resident cells. Moreover various study shown that cigarette smoke increased iNOS levels in lungs. NO is synthesized from L-arginine by nitric oxide synthase (NOS), which are defined as three isoforms [14]. enhanced expression or activity of inducible NOS (iNOS) and endothelial NOS (eNOS) causes Increased NO production and nitrosative stress in COPD [15].

Hypericum perforatum is a plant which blooms between july and september at farms, borders of roads and woods, top of hills and grasslands, that is used to cure some diseases by the local people. This plant contains Flavonoids, Phenolic acids, Naphtodianthrones, Phloroglucinols, Volatile fatty acids and Saturated fatty acids [16-18].

However anti-inflammatory effects of this plant are shown in various studies. Inhibitory effects of perforatum on LSP induced cyclooxygenase-2 and iNOS enzymes are shown in a study [19]. Besides that, hyperforin, which is one of the bioactive compounds of perforatum, is shown to be a strong inhibitor of cyclooxygenase-1 and 5-lipooxygenase [20]. Furthermore, perforatum application also reduces the prostoglandin synthesis which plays an important role on prostoglandin inflammation [21].

The aim of our study is to evaluate the effects perforatum extract, which is shown to have antiinflammatory effects, on iNOS, phospholipase A2 and cyclooxygenase-2 enzymes whose levels are increased in lungs by the cigarette smoke exposure.

2. Material and Method

8 week old balb/c albino male mice that are obtained from the Experimental Animal Center in Çukurova University, in Adana are used in study. This study was approved by the Animal Care Committee and Ethics Committee of Çukurova University.

Mice diveded into tree gruops as control, smoke exposured and smoke plus extract administered. Hypericum perforatum extract gived dose of 70mg/kg to mice plant extraction described below.

Cigarette Smoke Exposure

8 weeks old male mice were exposed to the smoke of 20 commercial filtered cigarettes per day, during 8 weeks. The smoke exposure was accomplished by enclosing the animals in a chamber 100 cm long, 60 cm wide, and 80 cm high,. The animals were exposed to the smoke by lighting two cigarettes which are mounted the suction vacuum pump upper of chamber and inhaling the smoke through the chamber the smoke was dispersed throughout the chamber by a ventilotor. Two cigarettes were lit and "smoked" over a period of 10 min and followed by a period of 20 min without cigarette smoking. The cycle was repeated until a total of 20 cigarettes were "smoked" over a period of about 6 h. To confirm that this system led to significant smoke inhalation, we obtained blood measurement of cotinine level by ELISA in another group of animals exposed to cigarette smoke under identical conditions. As control group for the effects of cigarette smoke exposure, we also studied control group placed in a similar chamber for a similar period of time during 8 weeks under the same conditions but without using any cigarette, so that only room air was being aspirated into the chamber.

Plant Extraction

Perforatum plant is grinded vigorously after being dried in an incubator. Then, it is mixed with 80% alcohol in 12:1 (alcohol:plant) ratio and put in shaker for 24 hours at room temperature. After 24 hours, it is filtered then alcohol is evaporated by using an evaporator and plant extract is obtained.

Quantitative Analysis

Tissue Homogenization

3 ml/gram RIPA (Radio-immunoprecipitation Assay) buffer, 30 µl PMSF (phenylmethanesulfonyl fluoride), 30 µl sodyum vanadate, 30 µl protease inhibitor is applied on frozen tissue samples that are stored in Eppendorf tubes then homogenates are obtained by using ultrosonication on those tubes on ice.

Homogenates are then centrifuged at 10.000 RPM for 10 minutes and supernatants are taken and pellets are discarded.

Protein Quantification

Bradford method is used to quantify the protein in homogenized tissues. By using Bovine serum albumin (1µg/ml), 1, 2, 3, 5, 7, 8, 10 (µg/ml) standarts are prepared. 10 µl is taken from every sample and completed to 100 µl by adding distilled water. 1 ml Bradford solution is added to standards and samples, vortexed and absorbances at 595 nanometer are measured manually. Protein quantification (µg/µl) is done according to the standart curve drawn in Prism software.

ELISA (Enzyme Linked Immunosorbent Assay) Test

ELISA test is used to examine the expression and activity of cyclooxygenase-2, cytosolic phospholipase A2 and iNOS enzymes. All kits were purchased from CUSABIO, Inc

Statistic analyzes

Results were expressed as means ± S.E.M.., and *n* refers to the number of animals used for each experiments. Differences in results between tissues were tested by analysis of variance (ANOVA) corrected for multiple comparisons (Bonferroni corrections). P values less than 0.05 were considered to be significant.

3. Results

ELISA Phospholipase A2 Enzyme Quantification

While CS exposure increased phospholipase A2 enzyme expression. Extract treatment decreased CS exposure induced increase of cPLA2 (figure 1).

ELISA Cyclooxygenase-2 Enzyme Quantification

While CS exposure increased COX-2 enzyme expression. Extract treatment decreased CS exposure induced increase of COX-2. (figure 2).

ELISA iNOS Enzyme Quantification

While CS exposure increased iNOS expressions. Extract administration decreased this increase significantly(figure 3).

4. Discussion

In our study we evaluated cigarette smoke induced $cPLA_{2,}$ COX-2 and iNOS expression which have role on inflammation. Various studies shown that cigarette smoke enhances $cPLA_{2,}$ COX-2 and iNOS [17, 18]. In our study we observed same results while hypericum perforatum extract treatment decreased the expression of these mediators. CS increases $cPLA_2$ by enhancing NADPH oxydase activity induced production of superoxide radicals [19]. It is known that increase of intracellular superoxide production activates proinflamatory gene transcriptions induced by MAPK, NF-κB, ve AP-1 [19]. In a study it has been showed that cigarette smoke causes increase of COX-2 by enhancing expression of NF-κB ve p300 which are proinflammation factors induced by activating PKC_/c-Src/EGFR and PDGFR/PI3K/Akt signalization pathways [22] .

Nicotine is a main content of cigarette smoke. Some studies showed that nicotine nicotine induces VEGF, PGE2 AND COX-2 expression [23]. Furtheremore a study showed also Nicotine Induces TNF-α and iNOS Expression in Peritoneal Macrophages [24]

Hypericum perforatum has antioxidant and antiinflamatory integridents [16-18]. Extract induced decrease of $cPLA_2$ may due to its antioxidant integridents. Antioxidant integridents of hypericum perforatum may synergistically affected its antiinflammatory response. Extract also decreased expression of COX-2 and iNOS. $cPLA_2$ is head of inflammatory cascades. cPLA2 hydrolyzes membrane phospholipids and produces AA [4, 5]. Blocking $cPLA_2$ induced decrease of AA production by decreasing superoxide production may also effect COX-2 expression. Some studies report that nitric oxide upregulates COX-2 activity [22]. Although Quercetin and amentoflavone which are other integridents of hypericum perforatum downregulates iNOS expression [25]. Decrease of NO production by downregulation of iNOS might downregulate COX-2.

Main integrients of hypericum perforatum are hypericin and hyperforin. It was shown that hypericin inhibit hydrolyze of membrane phospholipids, 5- and 12-lypoxygenase, IL-1a and IL-12 formations. Hypericin also inhibits NF-kB which is prinflamatory mediator [26, 27]. Hyperforin is dual inhibitor of COX-1 and 5-lypoxygenase [28].

To decrease inflammation will cause to attenuate migration of macrophage and monocite and prevent lung damage. Furthermore it will also prevent bronchospasm by decreasing thromboxanes and prostoglandins.

In conclusion cigarette smoke is a risk factor for development of COPD. Cigarette smoke causes inflammation by upregulating inflammatory mediators such as $cPLA_2$, COX-2 and iNOS. Our study showed that diet of hypericum perforatum extract has beneficial effect on cigarette smoke induced lung damage.

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Figure Legends

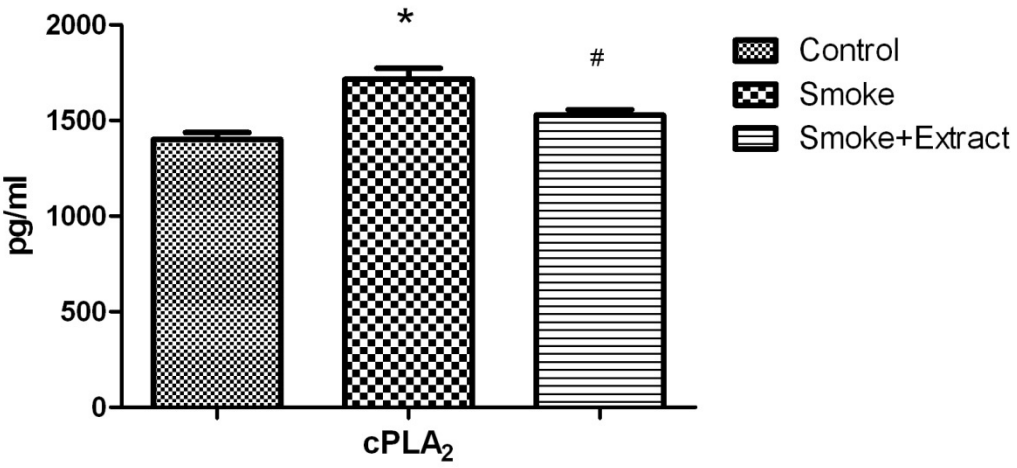

Figure 1. Effects of CS exposure on cPLA2 expression (*n*=6-9). Statistical analysis: ANOVA. Post hoc: Bonferroni. (* : For control $P < 0.05$. # For Smoke Control $P < 0.05$)

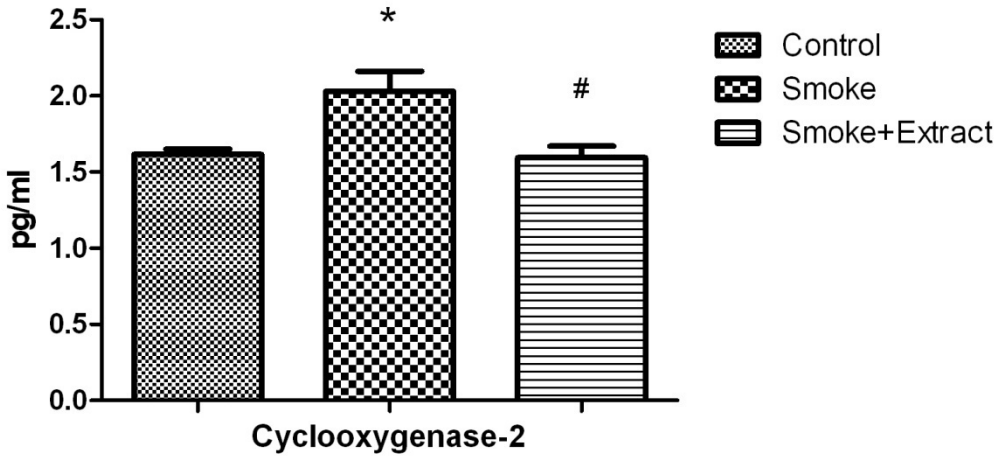

Figure 2. Effects of CS exposure on COX-2 expression (*n*=6-9). Statistical analysis: ANOVA. Post hoc: Bonferroni. (* : For control P < 0.05. # For Smoke Control P < 0.05)

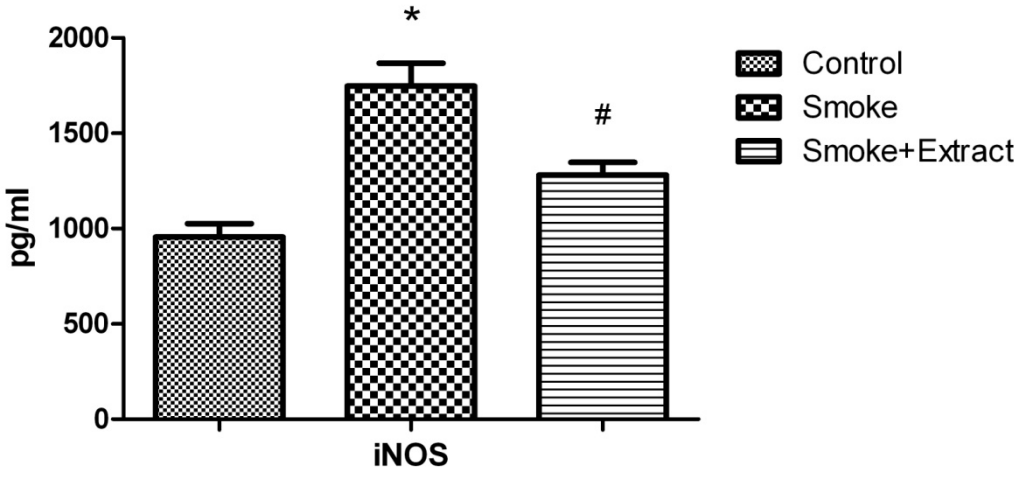

Figure 3. Effects of CS exposure on iNOS expression (*n*=6-9). Statistical analysis: ANOVA. Post hoc: Bonferroni. (* : For control P < 0.05. # For Smoke Control P < 0.05)