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ORIGINAL ARTICLE

Effect of acetone extract from stem bark of *Acacia* species (*A. dealbata*, *A. ferruginea* and *A. leucophloea*) on antioxidant enzymes status in hydrogen peroxide-induced HepG2 cells

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KEYWORDS

Antioxidant enzyme;
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Superoxide dismutase

Abstract *Acacia* species are multipurpose trees, widely used in the traditional systems of medicine to treat various ailments. The major objective of the present study was to determine the gene expression of enzymatic antioxidants by acetone extract from the stem bark of three *Acacia* species (*Acacia dealbata*, *Acacia ferruginea* and *Acacia leucophloea*) in hydrogen peroxide (H_2O_2)-induced human hepatoma (HepG2) cells. The expression of antioxidant enzymes such as superoxide dismutase containing copper–zinc (CuZnSOD)/manganese (MnSOD), catalase (CAT) and glutathione peroxidase (GPx) in HepG2 cells was evaluated by real-time PCR. The results of antioxidant enzyme expression in real-time PCR study revealed that the H_2O_2 (200 μM) challenged HepG2 cells reduced the expression of enzymes such as SOD, GPx and CAT. However, the cells pre-treated with acetone extracts of all the three *Acacia* species significantly ($P > 0.05$) up-regulated the expression of antioxidant enzymes in a concentration dependent manner (25, 50 and 75 μg/mL). In conclusion, the findings of our study demonstrated that the acetone extract of *Acacia* species effectively inhibited H_2O_2 mediated oxidative stress and may be useful as a therapeutic agent in preventing oxidative stress mediated diseases.
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1. Introduction

Reactive oxygen species (ROS) are the endogenous free radicals of normal cellular metabolism. In living organisms, moderate concentrations of ROS benefit physiological functions such as intra-cellular signaling, cellular defense against

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infective agents, and induction of a mitogenic response. However, the excessive productions of free radicals can cause cellular lipid peroxidation, protein degradation, and DNA mutation, resulting in several degenerative diseases, including inflammation, cardiovascular diseases, cancer, diabetes, and neurological disorders (Valko et al., 2007; Carvalho et al., 2014). Generally, all the organisms are well protected against free radical damage by endogenous oxidative enzymes, such as superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione reductase (GR) and catalase (CAT). However, these enzymes are commonly insufficient when it comes to completely preventing degenerative diseases and other health problems (Borneo et al., 2009; Soares et al., 2009). In addition, several non-enzymatic antioxidant compounds such as phenolics, ascorbic acid, tocopherol, glutathione and other dietary compounds play an important role in defending the body against free radical damage by scavenging or neutralizing the oxidizing molecules and maintaining redox balance (Tachakittirungrod et al., 2007). Recent studies have reported that the plant kingdom offers a wide range of natural antioxidant molecules including phenolic acids, flavonoids, and other secondary metabolites and they can also be used for the treatment of various human disorders (Slusarczyk et al., 2009).

Acacia trees (Family – Mimosaceae) are known as a versatile source of bioactive components. The genus *Acacia* comprises about 1350 species widely distributed from tropics and to some extent in the temperature regions, especially in Australia, Africa, India and America. A number of biologically active metabolites have been reported from various *Acacia* species including phenolics, flavonoids, terpenes, tannins, amines, and alkaloids, (Seigler, 2003). The different plant parts of *Acacia* species are widely used as indigenous drugs to treat various ailments. Especially, the bark is traditionally used for the treatment of several diseases in ayurvedic system of medicine (Parrotta, 2001; Kirtikar and Basu, 2003). *Acacia ferruginea* DC., *Acacia dealbata* Link. and *Acacia leucophloea* (Roxb.) Willd. are important medicinal plants with various therapeutic properties. Acetone and methanol extracts of *A. leucophloea*, *A. ferruginea*, *A. dealbata*, and *Acacia pennata* barks registered very strong antioxidant properties under different *in vitro* chemical assays (Sowndhararajan et al., 2013). In Indian traditional medicine, the stem bark of *A. leucophloea* is used to treat inflammation, bronchitis, cough, biliousness, skin diseases, leucoderma, pruritus, erysipelas, vomiting, wounds, ulcers, diarrhea, dysentery, stomatitis, intermittent fevers, leprosy, and toothache. *A. ferruginea* bark is used for the treatments of itching, leucoderma, ulcers, stomatitis, and diseases of the blood (Parrotta, 2001). Ethyl acetate fraction from acetone extract of *A. ferruginea* stem bark exhibited antiulcer activity against ethanol-induced gastric ulcer in rats (Sowndhararajan and Kang, 2013). *A. dealbata* yields a gum, resembling gum arabic, used in bronchial troubles (Prajapati, 2005). Recently, the human hepatoma cell line (HepG2) has been extensively used for examining *in vitro* oxidative damage and xenobiotic metabolism (Lee et al., 2015). Based on the highly acclaimed properties of *Acacia* species, the present study was carried out to investigate the effect of acetone extracts of *A. leucophloea*, *A. ferruginea* and *A. dealbata* stem barks on the expression of antioxidant enzymes (SOD, GPx and CAT) in H_2O_2-induced HepG2 cells.

2. Materials and methods

2.1. Chemicals and materials

3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), trypan blue, hydrogen peroxide (H_2O_2) and penicillin–streptomycin solution were purchased from Sigma–Aldrich Chemical Co. (St. Louis, MO, USA). Dulbecco's modified eagle medium (DMEM) and fetal bovine serum (FBS) were purchased from HyClone Laboratories, Inc. (Utah, USA). RNeasy Mini kit and SYBR green master mix were purchased from Qiagen-GmbH (Hilden, Germany). SuperScript III First-Strand synthesis system was purchased from Invitrogen (Carlsbad, CA, USA). All other reagents were of the highest available purity analytical grade.

2.2. Preparation of extracts

Fresh barks of *A. dealbata, A. ferruginea* and *A. leucophloea* were collected from Coimbatore, Tamil Nadu state, India. The plants were authenticated and herbarium specimens deposited in the Botany Herbarium, Bharathiar University with voucher numbers: BUBH-6140, BUBH-6141, and BUBH-6142, respectively.

The plant materials were washed thoroughly in tap water, shade dried at room temperature (25 °C), powdered, and used for solvent extraction. The plant samples were extracted with petroleum ether (for disposing lipid and pigments), followed by acetone using soxhlet apparatus. The solvents were evaporated using a rotary vacuum-evaporator (RE300, Yamato, Tokyo, Japan) at 50 °C and the remaining water was removed by lyophilization (4KBTXL-75, VirTis Benchtop K, NY, USA). The dried extracts were kept at −20 °C prior to use in the cell culture experiments.

2.3. Quantification of target gene expression by real-time PCR

2.3.1. Cell culture

Human hepatoma cell line (HepG2) was obtained from American Type Culture Collection (ATCC, Manassas, VA, USA). Cells were cultured and maintained in DMEM supplemented with 10% FBS, 50 units/mL penicillin and 50 μg/mL streptomycin. The cells were incubated at 37 °C in a 5% CO_2 incubator (HERAcell 150, Thermo Electron Corp., Waltham, MA, USA).

2.3.2. MTT cell viability assay

The mitochondrial-dependent reduction of MTT to formazan was used to measure cell respiration as an indicator of cell viability. Briefly, HepG2 cells were seeded in 96-well plates at the density of 5×10^4 cells/well. After 24 h of incubation, the cells were treated with various concentrations of the extracts (6.25, 25, 50, 100 and 200 μg/mL, eight wells per concentration). Twenty-four hours later, after changing the medium, MTT was added to a final concentration of 0.5 mg/mL, and the cells were incubated for 4 h at 37 °C and 5% CO_2. The medium was then removed and the formazan precipitate was solubilized in DMSO. The absorbance was measured at 550 nm on a microplate reader (Biotek, Winooski, VT, USA). The results were

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