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Cytotoxic and apoptogenic effect of *Swietenia mahagoni* (L.) Jacq. leaf extract in human leukemic cell lines U937, K562 and HL-60

S. Roy[a], B. Banerjee[b], J.R. Vedasiromoni[a,*]

[a] Drug Development Department, Indian Institute of Chemical Biology (A Unit of Council of Scientific & Industrial Research, India), 4 Raja S.C. Mullick Road, Kolkata 700032, India
[b] Department of Chemistry, K.N. College, Berhampur 742101, India

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ABSTRACT

The apoptogenic activity of *Swietenia mahagoni* leaf extract (SMLE) was investigated against three human leukemic cell lines – U937, K562 and HL-60. SMLE inhibited cell growth and metabolic activity of the leukemic cells and showed characteristic features of apoptosis. Flow-cytometric analysis showed that SMLE arrested U937 and K562 cell populations in the G2-M phase and the HL-60 cell population in the G1 phase of cell cycle. SMLE induced apoptosis was found to be mediated through mitochondrial intrinsic pathway involving the release of cytochrome c into the cytosol and activation of caspase-9 and caspase-3. Two flavonoids, catechin and quercetin-3-O-glucoside, isolated from SMLE, were found to inhibit the growth and metabolic activity of U937, K562 and HL-60 cells at much lower concentrations thus indicating that these two flavonoids might be the active ingredients responsible for the anti-leukemic activity of SMLE.

1. Introduction

It is now well established that there is a close relationship between inflammation and cancer (DuBois, 1995; DuBois et al., 1996; Thun et al., 1991). Tumor promoters recruit inflammatory cells to the application site and cancer development may act by aggravating inflammation in the tissue and vice versa (Rosin et al., 1994). It is also reported that inflammatory cells are capable of inducing genotoxic effects (Rosin et al., 1994). Moreover, some anti-inflammatory chemopreventive agents have been found to suppress growth and proliferation of transformed or malignant cells through induction of programmed cell death or apoptosis (Bellosillo et al., 1998; Smaha et al., 1997). So it is likely that anti-inflammatory agents may possess anti-cancer activity and vice versa. Since, the anti-inflammatory activity of *Swietenia mahagoni* leaf extract has already been reported (Roy et al., 2009), the purpose of the present study was to validate the cytotoxic and apoptogenic activities of *Swietenia mahagoni* leaf extract (SMLE) in human leukemic cell lines – U937, K562 and HL-60 and to make efforts to identify the possible mechanism of action involved in the anti-leukemic activity. An attempt was also made to isolate and purify the active ingredients from SMLE which are responsible for the anti-leukemic activity.

* *Corresponding author*. Tel.: +91 33 24292422; fax: +91 33 24292422.
E-mail addresses: vedasiromoni@gmail.com, vedasiromonijoseph@yahoo.com (J.R. Vedasiromoni).
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2. Materials and methods

2.1. Materials

2.1.1. Plant materials

The leaves of *Swietenia mahagoni* (L.) Jacq. were collected by local supplier from sites around Kolkata, India, during June 2006. The plant was identified by Indian Botanical Garden, Howrah, India, where a voucher specimen (SR-001) has been kept.

2.1.2. Extraction and preparation of test sample

One kilogram of *Swietenia mahagoni* (L.) Jacq. leaves was sun-dried, ground and soaked separately in 3 l of 50% aqueous methanol for one week at room temperature (28–34 °C) with occasional shaking. The supernatant was filtered and to the residue 1 l of 50% aqueous methanol was added and kept for another 1 week. The methanol portion in the whole supernatant was evaporated using a rotary evaporator and then it was lyophilized to remove the water. The solid dark brown residue (30 g) thus obtained from the leaves of *Swietenia mahagoni* was designated as SMLE (*Swietenia mahagoni* leaf extract). SMLE was kept at 4 °C. From 1 mg/ml stock solution of SMLE in phosphate buffer saline (PBS), desired concentrations (50, 100 and 150 μg/ml) were used for in vitro experiments.

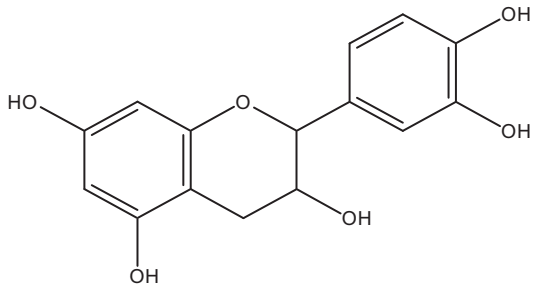
Repeated chromatographic purification of the *n*-BuOH soluble fractions of *Swietenia mahagoni* (L.) Jacq. leaves and preparative thin layer chromatography led to the isolation of two flavonoids, catechin and quercetine-3-O-glucoside (Fig. 1). These flavonoids were also tested for anti-leukemic activity. Stock solution (1 mg/ml) of each compound was made in PBS from where desired concentrations were prepared for different experiments.

2.1.3. Chemicals

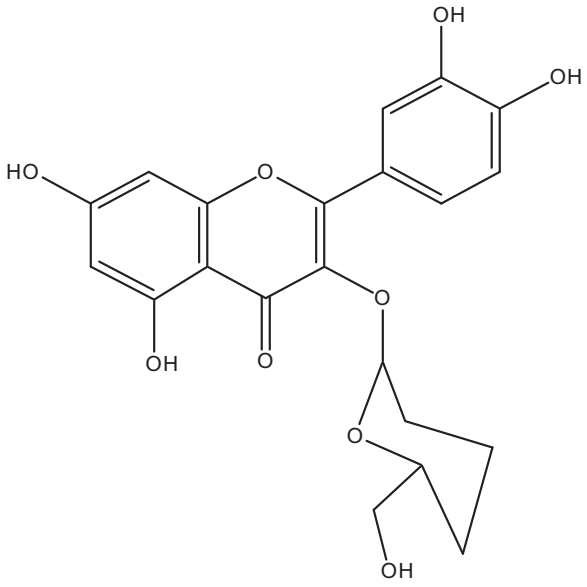
RPMI 1640 was purchased from Gibco, USA, Caspase-9 assay kit from US Biological, fetal bovine serum, streptomycin penicillin, L-glutamine, HEPES, Trypan blue, MTT [3-(4,5-dimethylthiozol-2-il)-2,5-2,5-dipheniltetrazoliumbromide], cytosine-arabinoside (Ara C), acridine orange, ethidium bromide, agarose, ethylene diamine tetra acetic acid (EDTA), proteinase K, propidium iodide (PI), Hoescht 33342, annexin-V FITC, RNase A, Caspase-3 assay kit and Cytochrome c oxidase assay kit were purchased from Sigma–Aldrich, USA. All other chemicals and reagents were of analytical grade and purchased locally.

2.1.4. Cell cultures and normal WBC

U937 (human leukemic monocyte lymphoma cell line), K562 (human myelogenous leukemia cell line) and HL-60 (human promyelocytic leukemia cell line) were obtained from National Facility for Animal Tissue and Cell Culture, Pune, India. For normal WBC, the peripheral blood of four normal individuals was collected and the mononuclear cells were separated by Ficoll-Hypaque technique . The cells were cultured and routinely maintained in RPMI 1640 medium supplemented with 10% heat inactivated fetal bovine serum and were incubated at 37 °C in a humidified atmosphere containing 5% CO_2 inside a CO_2 incubator.



A: Structure of Catechin



B: Structure of Quercetin-3-*O*-glucoside

Fig. 1 – Structures of flavonoids isolated from SMLE: (A) structure of catechin and (B) structure of quercetin-3-O-glucoside.

2.2. Methods

2.2.1. Cell growth inhibition study and cytotoxicity study

U937, K562 and HL-60 cells and normal white blood cells (WBC) (1×10^6) were seeded in 96-well sterile plates and were treated with different concentrations (50, 100 and 150 μg/ml) of SMLE for 24, 48 and 72 h. The cell growth inhibition studies were done by Trypan blue exclusion method (Sur et al., 1995) and the cytotoxicity studies were performed by MTT assay (Cao and Li, 2002). The U937, K562 and HL-60 cells (1×10^6) were also treated with catechin and quercetin-3-O-glucoside at various concentrations (2.5–100 μg/ml) and their effects on cell growth were observed after 24 h of treatment by Trypan blue exclusion method and MTT assay. IC_{50} values were calculated.

2.2.2. Morphological studies for detection of apoptosis

2.2.2.1. *Fluorescence microscopy studies.* U937, K562 and HL-60 cells (1×10^6) treated with SMLE (100 μg/ml) for 24 h were