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Antiproliferative Action of *Moringa oleifera* Lam. Root Extracts in Acute Myeloid Leukemia (AML) Cell Line

Eltayb Abdellatef[1*], Hussein M. Daffalla[1], Amr A. Nassrallah[2], Khalid M. Aboul-Enein[3], Hany A. El-Shemy[2] and Mutasim M. Khalafalla[1]

[1]Commission for Biotechnology and Genetic Engineering, National Center for Research, Khartoum, Sudan; [2]Faculty of Agriculture Research Park (FARP) and Department of Biochemistry, Faculty of Agriculture, Cairo University, 12613 Giza, Egypt; [3]Department of Clinical Pathology, National Cancer Institute, Cairo University, Cairo, Egypt

ABSTRACT: *Moringa oleifera* is a multipurpose plant, the fresh leaves may be cooked and eaten. The roots when mixed with water are used to treat malaria, hypertension, stomach disorders, to expel a retained placenta and to treat other health problems such as asthma and diabetes. To assess the antileukemia potency of different extract of *Moringa oleifera* roots (hot water, cold water and ethanolic extracts) were added to acute myeloid leukemia cell lines that harvested from adult patients to assess it is antiproliferative action using MTT assay. Among the different used extracts, ethanolic extract killed 51% of abnormal cells among primary cells harvested from 3 patients with Acute Myeloid Leukemia. In conclusion roots of *Moringa oleifera* contain active ingredients that were easily dissolved in ethanol and could be used as natural antitumor medicines.

Key words: *Moringa oleifera*, Roots, Acute Myeloid Leukemia

Introduction

Cancer is the largest single cause of death in both men and women (Russo et al., 2005). Acute myeloid leukemia (AML), also known as acute myelogenous leukemia, is a cancer of the myeloid line of blood cells, characterized by the rapid growth of abnormal white blood cells that accumulate in the bone marrow and interfere with the production of normal blood cells. AML is the most common acute leukemia affecting adults, and its incidence increases with age. AML is, accounting for approximately 1.2% of cancer deaths in the United States (Jemal et al., 2002).

Many substances derived from dietary or medicinal plants are known to be effective and versatile chemopreventive and antitumoral agents in a number of experimental models of carcinogenesis. Some foods, such as dietary fibre, vegetables, fruit and soy, have been shown to induce a chemopreventive action on the gastrointestinal tract (Borrelli et al., 2004). There is increasing evidence for an association between a high consumption of fruit and vegetables and the reduced risk of oral cancer (La Vecchia et al., 1997; Morse et al., 2000).

Moringa oleifera is a multipurpose tree. The fresh leaves may be cooked and eaten. The leaves or the roots, when mixed with water, are used to treat malaria, hypertension, stomach disorders, to expel a retained placenta and to treat other health problems such as asthma and diabetes. (Mekonnen et al., 1999). An examination of the phytochemicals of Moringa species affords the opportunity to examine a range of fairly unique compounds. In particular, this plant family is rich in compounds containing anticancer activity include 4-(4'-O-acetyl--L-rhamnopyranosyloxy) benzyl isothiocy-anate (Abrams, 1993), and 4-(-L-rhamnopyranosyloxy) benzyl glucosinolate (Asres, 1995).

Materials and Methods

Plant material

Moringa olifera seeds were obtained from Botanical garden of Botany and Agricultural Biotechnology Department, faculty of Agriculture, University of Khartoum, Sudan. Seeds were germinated in plastic bag containing mixture of soil and sand under green house conditions for 3 months. The aerial parts of root were isolated, dried and chopped finely using a blender.

Samples preparation

The extraction used 1 g of freeze-dried, powdered roots suspended in 1ml of hot water, cold water, or 80% (v/v) ethanol. Extracts were stirred mechanically for 12 h at room temperature (25° C) except the hot water extract (80° C) that was made in 3 min. Solids were removed by centrifugation (4,000, 1min) and the supernatant collected. The resulting extracts were completely dried in a rotary evaporator at 40° C and the lyophilized extracts stored at 4° C for further process.

Viability of tumor cells

The study was performed on cells harvested from adult leukemia patients or healthy relatives admitted to the National Cancer Institute, Cairo University. International protocols governing the ethical treatment of patient were followed.

The viability of AML cells were calculated according to MTT assay (Selvakumaran et al., 2003). The MTT assay is a test of metabolic competence based upon assessment of mitochondrial performance relying on the conversion of yellow MTT to the purple formazan derivative by mitochondrial succinate dehydrogenase in viable cells. Mononuclear cells were separated from other blood cells by Ficoll hypaque density gradient (Pharmacia, Uppsala, Sweden). According to Harbeck, et al. (1982). AML were diagnosed by peripheral blood and bone marrow examination, cytochemistry (and immunological markers in some cases). Mononuclear cells were separated from other blood cells by Ficoll hypaque density gradient (Pharmacia, Uppsala, Sweden). The cells were then washed with three changes of PBS. The cell counts were adjusted to 3 × 103 cell /well and plated in100 µl of medium/well in 96-well plates (Costar Corning. Rochester, NY). After overnight incubation, extracts were in various concentrations (20, 40, and 60 µg/ml) with cytotoxicity to human normal Myeloid cell line (reported in elsewhere); 3 wells were included in each concentration. After treatment with extracts for one day, 20 µl of 5 mg/ml MTT (pH 4.7) were added per well and cultivated for another 4 h, the supernatant fluid was removed, and then 100 µl DMSO were added per well and shaken for 15 min. The absorbance at 570 nm was measured with a microplate reader (Bio-Rad, Richmond, CA), using wells without cells as blanks. All experiments were performed in triplicate.

Calculation:-

The effect of extracts on the proliferation of human AML cells was expressed as the % cytoviability, using the following formula:

cytoviability % = A57of treated cells / A57of control cells 100.

Results and Discussion

After 24 h incubation of the mononuclear AML cells with root extracts, ethanolic extract at 60 µg /ml score the highest cell death (51%) compared to cold water extract and hot water extract at the same concentration which gave 10% and 3% cell death respectively (Table 1). From this observation, it is clear that the antitumor activity of the roots was mostly due to compounds that were ethanol soluble. A previous report of plant derived antileukemia treatment showed that allamandin derivatives that were extracted with ethanol from Allamanda catharica (Apocynaceae) had significant activity in vivo against the p-388 leukemia in the mouse (Kupchan et al., 1976). In this study the major destructive effect on AML cells were obtained by ethanol fractions. The phenolic compounds, most

** Corresponding Author, Email: eltayb@myway.com*

glycosides, will dissolve in ethanol solutions (Bravo, 1988). Therefore, these groups of compounds may contain the major active components for the destruction of leukemia and carcinoma cells (El-Shemy et al., 2007; Khalafalla et al., 2009).

Table.1: The effect of Root extracts on AML cells after incubation for 24 h

Extracts	Concentration (µg/ml)		
	20	40	60
	Dead %	Dead%	Dead%
Etanolic 80%	36± 2.1	41 ±3.0	51± 1.0
Hot water	8± 0.2	10± 0.9	3±0.2
Cold water	11±0.9	40 ±1.2	10±1.0
L.S.D. (0.05)	2.4	3.2	1.0

Each value represents the mean ± S.D (Standard Division) and mean of three replicates (P _ 0.05)

In conclusion the active ingredients that were easily dissolved in ethanol from drumstick tree (Moringa oleifera) roots could be used as natural antitumor medicines. They were active against leukemia cells in vitro. The metabolites within the extract will be identified and their role in killer of cancer cells. Also the result substantiates the value of roots as source of high value metabolites.

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