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THE STUDY OF THE ANTIPROLIFERATIVE ACTION OF *MYRMECODIA PENDENS* EXTRACTS ON COLON CANCER CELLS

Aurica Elisabeta MOATAR[1], Octavian BLOJU [2], Molka BAMBIA [2], Roxana POPESCU [3], Mihai MITULETU[3*], Marioara Nicoleta FILIMON [2], Gabi DUMITRESCU [4], Ioan Valeriu CARABA [4], Adrian SINITEAN [2], Doina VERDES [3], Cristian VLAD [1], Victor DUMITRASCU [1]

[1]Department of Pharmacology and Biochemistry, Faculty of Medicine, "Victor Babes" University of Medicine and Pharmacy, 2 Eftimie Murgu Square, 300041, Timisoara, Romania
[2]Department of Biology and Chemistry, Faculty of Chemistry, Biology and Geography, West University Timisoara, 16 Pestalozzi Street, 300315, Timisoara, Romania
[3]Department of Morphologic Microscopy, Faculty of Medicine, "Victor Babes" University of Medicine and Pharmacy, 2 Eftimie Murgu Square, 300041, Timisoara, Romania
[4]Faculty of Bioengineering of Animal Resources, Banat University of Agricultural Sciences and Veterinary
Medicine "King Mihai I of Romania" from Timisoara, Calea Aradului 119, 300645 Timisoara, Romania
**Corresponding author's e-mail: mihai.mituletu@umft.ro*
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ABSTRACT

The aim of this study is to evaluate the anticancer activity of different extracts of Myrmecodia pendens (the name in the local language is Sarang semut), in the following solvents: water, methanol and N-hexane. The effect of those extracts was tested in four concentrations, which vary between 50µg/ml and 600 µg/ml, and were applied on colon adenocarcinoma cells, cell line CaCo2. The methanolic and N-hexane extracts showed a reduced cytotoxic activity and minor cell lesions, in comparison to the water extracts. We conclude that Myrmecodia pendens extracts can be considered as potential adjuvant in the complementary therapy of the colon cancer, with the mention that the extracts in polar solvent (water) manifest more important anticarcinogenetic properties.

KEY WORDS *Myrmecodia pendens, Caco2 cell line, antiproliferative effect, colorectal cancer, complementary therapy*

INTRODUCTION

The colorectal neoplasia represents a major problem of the public health systems all over the world. According to GLOBOCAN 2020 the colorectal cancer occupies the 3rd place, after breast and lungs cancer, for both genders. The incidence of the colorectal cancer in men is on the 3rd place after prostate and lung cancer, and in women is on the 2nd place after breast cancer (1). Similar to other cancers the therapy consists of operation, chemotherapy and biological drugs. Although in the last time the cancer therapies had importante scientific and technologic progress, many

patients, especially the ones with advanced forms of cancer do not respond to the standard anticancer therapyas it is wished.

The biologic active products which derivate from animal, vegetal or microbial source, were used since ancient times in different therapies on humans or animals, while the therapy products obtained from vegetal sources represented a frequent used therapy alternative, having a leading position in the human Pharmacopoeia over the time (2,3). In oncology, the patients life quality and cancer survival rate increased in the last years, so that the medical practice developed an integrative approach of the oncologic disease, called integrative oncology (2). Nowadays, the alternative of holistic approach of the oncologic patient is accepted. For patients safety reasons and for the patients benefit, all the conventional therapeutical strategies or phytotherapies (plants, algae or the derivate compounds), not depending on their origin or producing technology, need preclinic and/or clinic rigorous tests concerning the safety and efficiency standards before being commercialized.

Myrmecodia belongs to genus of epiphytic myrmecophytes or ant-plant, being known under the native name of Sarang Semut. This genus is native to south-east Asia, but can be found also in Myanmar, Indochina and nord Australia. The plants of the Myrmecodia species are used in the traditional medicine to treat disease like ulcer, tumours, cancer, hepatitis and coronaries disease. Were identified and quantified five types of flavonoids at *Myrmecodia pendans*: apigenin, kaempferol, rutin, quercetin and luteolin. Some of those compunds have anticancer properties. Although *Myrmecodia platytyrea Becc*. is used as a routine therapy of cancer treatment by the natives in south-east Asia, there were performed few scientific researches in what concerns the pharmacologic properties. The bioactive compounds such as flavonoids, morindolide and stigmasterol were found in the plants tubercle (4,5). The research done by Soeksmantoon on rats showed that the toxicity of *M. pendens* extract at a doze of 375 mg/kg induced hepatic degeneration, while a dose of 3750 mg/kg caused cell death though necrosis (6).

The aim of this study is to investigate the anticancer activity through evaluation of the antiproliferative potential of *Myrmecodia pendens* extracts obtained through different ways in different solvents: water, methanol and N-hexane.

MATERIALS AND METHODS

In this study we used *Myrmecodya pendens* obtained from the Papua region. The extraction was performed from the dried plant material. The cells of the Caco2 line were seeded in 96-well plates, at a concentration of $1.5x10^4$ cells / ml, in 100 µl medium. After 24 h, the culture medium was changed and the cells were cultured in a specific medium (DMSO and antifungal-antimicotic solution), with or without test substances, in an incubator at 37°C and 5% CO_2. Cells were exposed to 4 different concentrations of extracts (50 µg/ml, 150 µg/ml, 300 µg/ml and 600 µg/ml,

respectively) for 48 and 72 hours. For each solvent (water, methanol and n-hexane), after extraction and evaporation of the solvents, stock solutions of 1000 µg/ml were prepared in DMSO. Starting from the stock solutions, the final test solutions were subsequently prepared, by diluting the stock solutions in culture medium. In parallel, a control culture was used, without added extracts. All samples were analyzed in triplicate. Cell phenotype analysis was performed under a Nikon TE2000U phase contrast inverted microscope, and images were taken with a Nikon D200 digital camera. For the analysis of cell proliferation, we used the MTT test (3- (4,5-dimethylthiazol-2-yl) -2,5-diphenyltetrazolium bromide) (Vybrant Cell proliferation assay).

RESULTS AND DISCUSSIONS

The cell morphology evaluation was performed under an inverted phase contrast microscope (PCM), at 48h, and respectively 72 hours of incubation with extracts. We found changes in the phenotype of Caco2 cells, especially on cells exposed to aqueous extracts. Cellular lesions ranged from minimal lesions (cytoplasmic vacuolations) to cell death lesions (necrotic and apoptotic). Variations in cell damage were proportional to the dose of extract and the time of exposure. On cells exposed to methanolic and n-hexane extracts, lesions were minimal. Cellular bloating and reduced apoptotic processes were observed, with a slight dependence on dose and exposure time. At low doses, the phenotype was similar to that of control cells (fig. 1).

The rate of cell proliferation inhibition was calculated as a percentage of the values obtained in the control group. We emphasize a dose and time dependent inhibition of the cell multiplication (fig. 2,3).

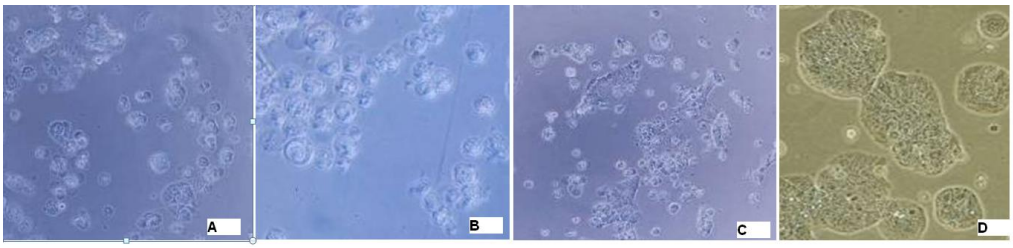

Figure 1. Cellular injuries after 72h of incubation with extracts: necrotic and apoptotic lesions (A) – methanol 600 µg/ml; vacuolations and cell death lesions (B) – n-hexan 600 µg/ml; necrotic and apoptotic lesions (C) - wather 600 µg/ml; (D) – control group

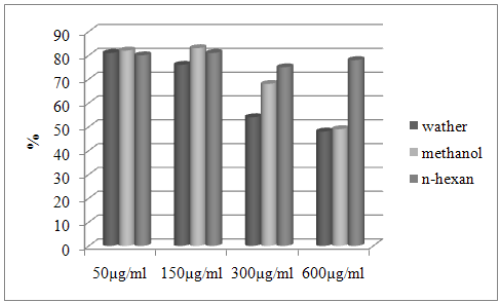

Figure 2. Cell proliferation at 48h

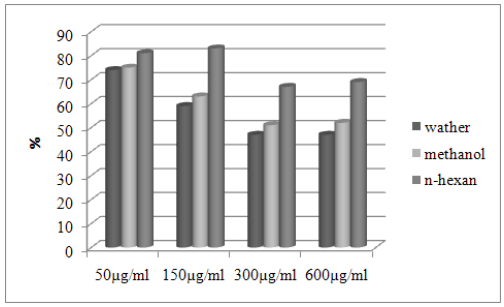

Figure 3. Cell proliferation at 72h

Due to the limitations of synthetic drugs, there has been interest in developing new drugs to combat multidrug-resistant pathogens, as well as various chronic and difficult-to-treat pathologies, such as cancer, diabetes and AIDS. The use of herbal products or other natural sources in cancer is the basis of adjuvant treatments in neoplastic pathology. The molecular mechanisms of action of medicinal plants are variable and incompletely elucidated, from improving the general condition of patients to the concrete action on cancer cells.

Usingt he MTT test method, Sudiono *et al*., reported that *Myrmecodia pendens* extract in 70% ethanol did not show cytotoxicity on healthy cells (human fibroblasts). In addition, higher concentration of extract applied to these cells resulted in an increase in viable cells, as well as a decrease in the percentage of inhibition of cell proliferation. The findings of thisp aper, which demonstrated that the ethanolic extract of *Myrmecodia pendens* has no toxic effect on normal cells, suggest the medicinal potential of this plant, which was previously known to have antibacterial and anticancer potential (7).

Although Sarang Semut is used in some areas for various pathologies, especially chronic diseases, few studies are cited in the literature on the possible therapeutic effects in cancer. In this study, we evaluated the antiproliferative effect of *Myrmecodia pendens* extracts obtained in water, methanol and n-hexane, on neoplastic colon cells of Caco2 cell line. The obtained results revealed cytotoxic effects and altered morphology of Caco2 cells, especially case of in the extracts obtained in the nonpolar solvent.

Bashari*et al.*, assesed the antitumor activity of Sarang Semut extract in n-hexane in colon cancer cell. Their data revealed better results than previous studies, according to which the IC50 of the n-hexane fraction of Sarang Semut in colon cancer cells, Caco-2 and HCT-116, is 24 and 33 ppm, respectively. These data indicate that the n-hexane fraction inhibits the survival of neoplastic cells and has a strong cytotoxic activity on colon cancer cells. Moreover, the trypan blue exclusion test showed that the n-hexane fraction inhibits the proliferation of colon neoplastic cells and prolongs the time of cell division (8).

Our previous study showed that aqueous and ethanolic extracts of *Myrmecodia pendens* showed moderate, dose- and time-dependent antitumor activity on HCT cells incubated with ethanolic extract 24 and 72 h, with an IC50 value of 566.087 µg/ml at 72 hours of incubation. Exposure of cells to aqueous extracts decreased cell proliferation rate, depending on dose and incubation time. The IC50 was 587.95 µg/ml and 512 µg/ml, respectively, for 48 and 72 hours of incubation, respectively. *M. pendens* extracts did not influence IL-8 levels in cellcultures (9).

The anticancer potential of *M. pendens* has also been reported on oral cancer cells derived from the human oral cavity, from the HSC-3 cell line. MTT assay results showed that crude *M. pendens* extract significantly induced cytotoxicity on HSC-3 cell lines after 24 hours (IC50 5 mg/mL) and 48 h (IC50 3 mg/ml). *M. Pendens* - induced apoptotic activity was detected in HSC-3 cell lines by flow cytometry at 24 hours of incubation with plant extract. These results suggest that *M. pendens* may be a potential candidate for the treatment of oral cancer. *M.pendens* extract inhibits the proliferation of HeLa cells and MCM-B2 cells. Several studies have also shown that *M. pendens* extract induces apoptosis in ovarian cancer cell lines (SKOV-3) and inhibits the angiogenesis of cells in the SP-C1 tongue cancer cell line. In addition, Achmad *et al*. reported that ethanol extracts inhibit the Sp-C1 oral cancer cell line at concentrations of 1 mg/ml (10,11,12).

More recent studies have attempted to elucidate the molecular mechanisms involved in the anticancer effect of *M. pendens*. Ju *et al*., investigated the anticancer effects of the methanolic extract of *Myrmecodia platytyrea* Becc. (MMPL) and determined the molecular mechanisms underlying the effects of MMPL on metastases in human hepatocellular carcinoma (HCC) cells. Dependent on the administered dose, MMPL inhibited the migration and invasion of cells in the SK Hep1 and Huh7 lines. In addition, MMPL strongly suppressed the enzymatic activity of matrix metalloproteinases (MMP 2 and MMP 9). MMPL led to the suppression of both telomerase activity and the expression of genes associated with telomerase activity. Urokinase plasminogen activator (uPAR) receptor expression levels as well as phosphorylation levels of the signal transducer and transcription activator factor 3 (STAT3) and extracellular signaling regulatory kinase (ERK) were also reported to be attenuated by MMPL. The above results suggest that MMPL has anticancer effects in human hepatocellular carcinoma and that MMPL may serve as an effective therapeutic agent for the treatment of humanliver cancer (4).

CONCLUSIONS

In conclusion, the results of this study suggest that the cytotoxic effects observed after incubation with *M. pendens* extracts, especially with the aqueous extract, could be useful to further explore the potential of *M. pendens* extracts as adjuvants in cancer. However, further studies are needed on the physicochemical

composition as well as the molecular mechanisms underlying the cytotoxicity and cell death induced by these extracts.

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