

A NUTRIENT MIXTURE REDUCED TUMOR GROWTH OF SK-UT-1 HUMAN LEIOMYOSARCOMA CELLS *IN VIVO* AND *IN VITRO* BY INHIBITING MMPS AND INDUCING APOPTOSIS

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Background: Uterine leiomyosarcoma is a rare malignant smooth muscle tumor originating in the uterine wall that generally responds poorly to chemotherapy and radiation. **Aim:** We investigated the *in vitro* effects of a novel nutrient mixture containing lysine, proline, ascorbic acid, and green tea extract on the human leiomyosarcoma cell line SK-UT-1 by measuring cell proliferation, invasiveness, apoptosis, and expression of matrix metalloproteinases (MMP). We also tested the effects of nutrient mixture *in vivo* using nude mice. **Materials and Methods:** Human leiomyosarcoma SK-UT-1 cells were treated with different concentrations of nutrient mixture. Cell proliferation was determined by MTT assay; MMP expression by gelatinase zymography; invasion by Matrigel assay; migration by scratch test; apoptosis using Live Green caspase kit. *In vivo* studies were conducted on 5–6 weeks old female nude mice inoculated subcutaneously with $3 \cdot 10^6$ SK-UT-1 cells. The mice were fed a regular diet or a diet supplemented with 0.5% nutrient mixture. After four weeks, the mice were sacrificed and the tumors were weighed and processed for histology. **Results:** *In vitro*, nutrient mixture treatment was not toxic to SK-UT-1 cells at 250 µg/ml but exhibited 20% and 40% cytotoxicity at 500 and 1000 µg/ml respectively. Zymography did not show bands for either MMP-2 or MMP-9 in SK-UT-1 cells. However, treatment with phorbol myristate acetate stimulated the expression of MMP-9, both active and inactive forms in equal proportion. Nutrient mixture inhibited the secretion of both active and inactive forms in a dose dependent manner. Invasion through Matrigel and migration by scratch test were inhibited in a dose dependent fashion, with both invasion and migration inhibited at 250 µg/ml. Live Green Caspase apoptosis assay demonstrated slight apoptosis at 100 µg/ml and significant apoptosis at 250 to 1000 µg/ml. The results of *in vitro* studies were further confirmed *in vivo* by showing 50% decrease in tumor weight, 40% reduction in tumor burden compared to the tumors from mice fed regular diet. **Conclusion:** The results suggest a therapeutic potential for nutrient mixture in uterine leiomyosarcoma treatment.

Key Words: MMPS, invasion, SK-UT-1, uterine cancer, leiomyosarcoma, nude mice.

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Leiomyosarcoma (LMS) is a malignant tumor accounting for about 40% of all uterine sarcomas. Sarcomas are a relatively rare type of uterine cancer accounting for 3–7% of all uterine cancers [1]. According to the American Cancer Society, 61,880 new cases of uterine tumors will be diagnosed in 2019. Early detection is vital and the 5-year survival rates achieve 96, 67, and 17% depending on metastasis at the time of diagnosis at the local, regional or distant sites [2]. LMS is an aggressive malignant tumor with poor prognosis. Although the exact cause of LMS is unknown, some studies indicate genetic predisposition and environmental factors such as exposure to ultraviolet rays, and ionizing radiation. Even with early stage diagnosis of tumors that are confined to the uterus, post treatment recurrence is seen in 50–75% of cases [3, 4]. Though survival chances are better with a combination of surgery, chemotherapy and radiation therapy, LMS seems to be less responsive to chemo- and radiation therapy alone without significant improvement in survival rate [5–7]. Therefore, the most efficient intervention could be targeting the molecular mechanisms

of extra cellular matrix and loco-regional spread may potentially impact LMS prognosis.

Matrix metalloproteinases (MMPs) play a significant role in the progression of LMS. MMPs are a family of zinc-dependent proteolytic enzymes which can degrade connective tissue such as basement membrane collagen and are associated with tumor invasion and angiogenesis of many cancers. The involvement of two gelatinases within the MMP family (MMP-2, -9) is seen in the degradation of collagen type IV and gelatin, which are important components of the extracellular matrix (ECM). As such, increased activity of these MMPs may play a role in LMS. MMP-1, -2 overexpression is seen in clinicopathological samples of LMS and is correlated with vascular space involvement and decreased disease free survival [8, 9].

Nutrients like lysine and ascorbic acid have been proposed as natural inhibitors of ECM and consequently, may potentially modulate tumor growth and expansion [10]. Nutrients may work by strengthening connective tissue cells by influencing collagen synthesis and inhibiting MMP expression. Taken together, ECM strengthening and inhibition of MMP expression are important mechanisms of tumor encapsulation. We have developed a nutrient mixture (NM) containing lysine, proline, ascorbic acid and green tea extract which has shown anticancer properties in many cancer cell lines [11, 12]. NM was formulated based on micronutrient synergy principles.

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Abbreviations used: ECM – extracellular matrix; FBS – fetal bovine serum, LMS – leiomyosarcoma; MMP – matrix metalloproteinase; NM – nutrient mixture; PBS – phosphate buffered saline; PMA – phorbol myristate acetate.

We have developed new approaches to prevent cancer development, progression and metastasis using naturally occurring micronutrients that act synergistically to strengthen connective tissue and collagen. We have demonstrated that the NM has anticancer activity *in vivo* and *in vitro* in a number of cancer cell lines derived from different malignancies by inhibiting several hallmarks of cancer including cell proliferation, invasion, metastasis, angiogenesis and induction of apoptosis [13, 14].

In this study we investigated the *in vitro* effect of NM on the proliferation, migration, MMP expression, morphology and induction of apoptosis on human LMS cell line SK-UT-1. We also carried out *in vivo* experiments measuring tumor mass, tumor burden in nude female mice.

MATERIALS AND METHODS

Composition of the NM. The stock solution of the NM (total weight 4.4 g) consists of vitamin C (as ascorbic acid and as magnesium ascorbate, calcium ascorbate and palmitate ascorbate) 700 mg, L-lysine 1000 mg, L-proline 750 mg, L-arginine 500 mg, N-acetyl cysteine 200 mg, Standardized green tea extract 1000 mg (from green tea leaves obtained from US Pharma Lab with total polyphenol 80%, catechins 60%, epigallocatechin gallate 35%, and caffeine 1%), selenium 30 µg, copper 2 mg, manganese 1 mg, 10 mg of NM in 10 ml of the media was prepared.

Cell line and culture. Human LMS cells SK-UT-1 were obtained from American Type Culture Collection (Rockville, MD) and were grown in modified Dulbecco's Eagle medium, supplemented with 10% fetal bovine serum (FBS), 100 U/mL penicillin and 100 µg/ml streptomycin. FBS and the antibiotics were obtained from Gibco, USA. Serum-supplemented media were removed and the cell monolayer was washed once with phosphate buffered saline (PBS) with the recommended serum-free media. The cells were treated with the NM, dissolved in media and tested at 0, 50, 100, 250, 500 and 1000 µg/ml in triplicate at each dose.

MTT cell proliferation assay. The cell suspensions were plated in 24-well tissue culture plates (Nunc, Denmark) at the density of 3×10^4 cells/well. When the cells were near confluence, they were treated with NM for 24 h at 37 °C in a humidified incubator, the cells were treated with the NM at concentrations of 0, 50, 100, 250, 500 and 1000 µg/ml for 24 h and the viability was determined with the MTT assay reagent (Sigma, USA) added to each well followed by 2-h incubation at 37 °C. Following incubation, the solution was carefully aspirated from the wells, the formazan product was dissolved in 1 ml DMSO, and the absorbance (OD) was measured on a microplate reader at a wavelength of 570 nm in a BioSpec 1601 Shimadzu spectrometer. The OD 570 of the DMSO solution in each well

was considered to be proportional to the number of cells.

Gelatinase zymography. Gelatinase zymography was performed in 10% Novex Pre-Cast SDS (Invitrogen Corporation, USA) in the presence of 0.1% gelatin under non-reducing conditions. Culture media (20 µl) were mixed with sample buffer and loaded for SDS-PAGE with Tris-glycine SDS buffer, as suggested by the manufacturer. Samples were not boiled before electrophoresis. Following electrophoresis, the gels were washed twice in 2.5% Triton X-100 for 30 min at room temperature to remove the SDS. The gels were then incubated at 37 °C overnight in a substrate buffer containing 50 mM Tris-HCl and 10 mM CaCl₂ at pH 8.0 and stained with 0.5% Coomassie Blue R250 in 50% methanol and 10% glacial acetic acid for 30 min and destained. Upon renaturation of the enzyme, the gelatinases digested the gelatin in the gel, producing clear bands against an intensely stained background. Protein standards were run concurrently and approximate molecular weights were determined by plotting the relative mobilities of known proteins.

Matrigel invasion. Invasion studies were conducted using Matrigel (Becton Dickinson, USA) inserts in 24 well plates. The SK-UT-1 cells suspended in medium were supplemented with phytonutrients, as specified in the design of the experiment and seeded on the insert in the well. The plates with the inserts were then incubated in a culture incubator equilibrated with 95% air and 5% CO₂ for 24 h. After incubation, the media from the wells were withdrawn. The cells on the upper surface of the inserts were gently scrubbed away with cotton swabs. The cells that had penetrated the Matrigel membrane and migrated onto the lower surface of the Matrigel were stained with hematoxylin and eosin and visually counted under the microscope.

Scratch test. A 2 mm-wide single uninterrupted scratch was made on the culture plates of LMS SK-UT-1 cells grown to confluence. Culture plates were washed with PBS and incubated with NM in tested medium at 0, 50, 100, 250, 500, 1000 µg/ml in triplicate at each dose for 24 h. Cells were washed, fixed and stained with hematoxylin-eosin and photomicrographs were taken.

Morphology and apoptosis. Morphology of cells cultured for 24 h in test concentrations of NM were evaluated by H&E staining, observed by microscopy and photographed. The cells were grown to near confluence and either left in media alone, or challenged with the NM dissolved in media at 0, 50, 100, 250, 500 and 1000 µg/ml, and incubated for 24 h. The cell culture was washed with PBS and treated with the caspase reagent as specified in the manufacturer's protocol (Molecular Probes Image-IT Live Green Caspases Detection Kit 135104, Invitrogen). The cells were photographed under the fluorescence microscope and counted. Green colored cells represent viable cells, while yellow-orange

and red colors represent early and late apoptotic cells, respectively.

In vivo studies. Female nude mice, about 5 weeks of age on arrival were purchased from Simonsen Laboratories (USA) and maintained in microisolator cages under pathogen-free conditions in a 12-h light/dark schedule for a week. All procedures were performed according to human and customary care and use of experimental animals and the protocol was approved by the internal animal safety committee of our Institution.

After housing for a week, the mice ($n = 12$) were inoculated subcutaneously with $3 \cdot 10^6$ SK-UT-1 cells in 0.2 ml PBS and 0.1 ml Matrigel (BD Bioscience, USA). After injection, the mice were randomly divided into 2 groups: group A mice were fed regular Purina mouse chow and group B mice received the regular diet supplemented with 0.5% NM (w/w). The regular diet was Laboratory Rodent Diet 5001 from Purina Mills (USA). The test diet was milled and pressed by Purina Mills, LLS and Generated by VitaTech (USA). During the study, the mice consumed on an average 4 g of their respective diets per day. Thus the supplemented mice received 40 mg of NM per day. After 4 week, the mice were sacrificed and their tumors were excised and processed for histological analysis. Dimensions (length and width) of tumors were measured using a digital caliper, and the tumor burden was calculated using the following formula: $0.5 \times \text{length} \times \text{width}$. The mean weight of the mice at the initiation and termination of the study did not significantly differ between the groups.

Histological analysis. Tissue samples were fixed in 10% buffered formalin. All tissues were embedded in paraffin and cut at 4–5 microns thick. Sections were deparaffinized through xylene and graduated alcohol series to water and stained with H&E for evaluation using light microscopy.

Statistical analysis. The results were expressed as means $\pm$ SD, as indicated in the results, for the groups. Data were analyzed by independent sample “*t*” test using MedCalc Software (Belgium).

RESULTS

Cell proliferation of SK-UT-1 cell line. Fig. 1 shows the cytotoxic effects of each of the 5 doses of NM relative to control of the LMS cell line SK-UT-1. NM was not toxic to LMS cells at 250 $\mu\text{g/ml}$ but exhibited 20%, and 40% toxicity at 500 and 1000 $\mu\text{g/ml}$.

Gelatinase zymography. Zymography did not show bands for either MMP-2 or MMP-9 in SK-UT-1 cells. However, phorbol myristate acetate (PMA) treatment stimulated MMP-9 expression, both active and inactive forms in equal proportions. NM inhibited the secretion of both active and inactive forms of MMP-9 in a dose-dependent fashion. Faint bands were observed at 500 $\mu\text{g/ml}$ with a total inhibition at 1000 $\mu\text{g/ml}$ (Fig. 2, a). Densitometry

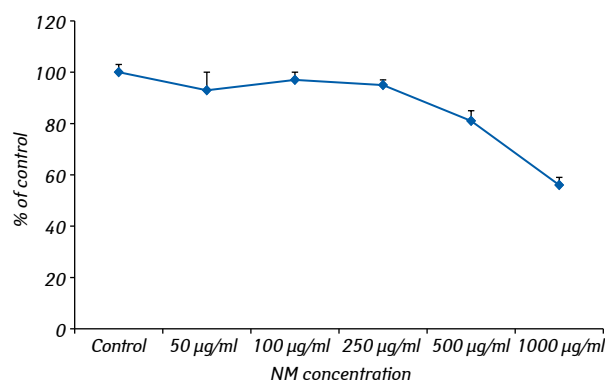


Fig. 1. Effect of NM on SK-UT-1 cell proliferation

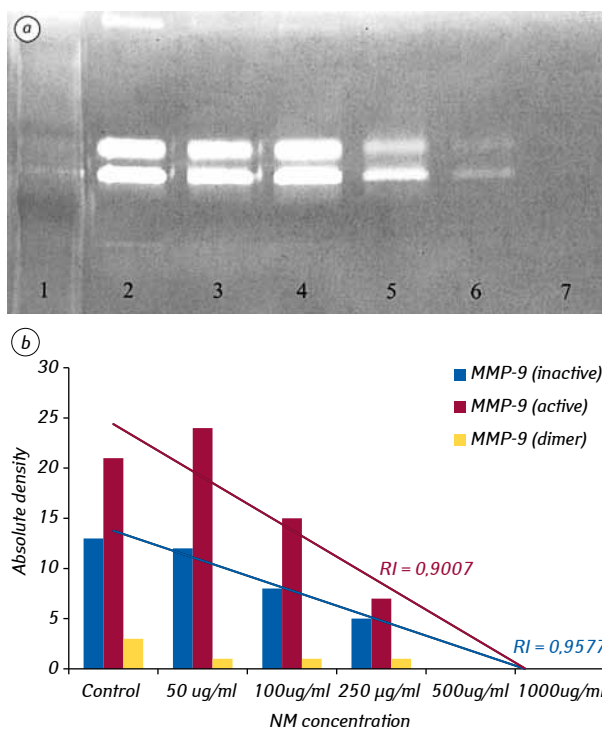


Fig. 2. Effect of NM on MMP-9 secretion by PMA-treated SK-UT-1 cells: a — gelatin zymogram of culture medium samples of PMA-treated SK-UT-1 cells; lanes: 1 — marker of molecular weight; 2 — PMA-treated SK-UT-1 cells; 3–7 — PMA-treated SK-UT-1 cells cultured with NM in doses of 50, 100, 250, 500, 1000 $\mu\text{g/ml}$; b — densitometry of MMP-9 secretion by PMA treated SK-UT-1 cells

analysis showed complete inhibition of active MMP-9 at 1000 $\mu\text{g/ml}$, and an overall $R^2 = 0.88$ (Fig 2, b). Similarly, densitometry analysis of MMP-9 inactive form showed a decrease in expression — 88% at 50 $\mu\text{g/ml}$, 93% at 100 $\mu\text{g/ml}$, 97% at 250 $\mu\text{g/ml}$, and 99% at 500 $\mu\text{g/ml}$, with $R^2 = 0.94$. Band density for MMP dimer was minimal.

Matrigel invasion. Fig. 3, a shows a significant dose dependent inhibition of SK-UT-1 cell invasion through Matrigel membrane. 9% inhibition was observed at 50 $\mu\text{g/ml}$, 35% inhibition at 100 $\mu\text{g/ml}$, 94% at 250 $\mu\text{g/ml}$, and 100% at 500 $\mu\text{g/ml}$. The extent of migration is seen in the invasion photomicrographs as shown in Fig. 3, b–f.

Morphology and apoptosis. H&E staining of SK-UT-1 cells exposed to different concen-

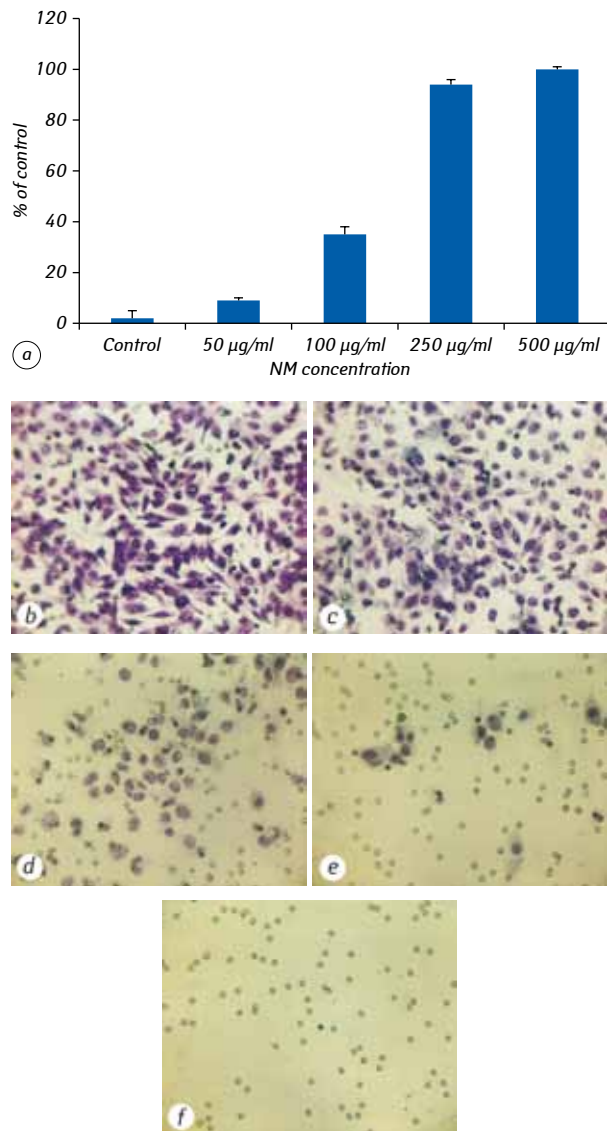


Fig. 3. Effect of NM on invasion of SK-UT-1 through Matrigel: a — percent of invasion; b–f — photomicrographs demonstrating decreased invasion with increasing doses of NM (b — control; c — 50 µg/ml; d — 100 µg/ml; e — 250 µg/ml; f — 500 µg/ml)

trations of NM as shown in Fig. 4, a–f showed no change at the lower doses, however, showed slight changes at 500 and 1000 µg/ml. Using the Live Green Caspase kit, a dose dependent apoptosis of SK-UT-1 cells was evident with increasing doses of NM (Fig. 5, a–f). Fig. 5, a highlights that as the concentration of NM increases the apoptotic events also increase. A quantitative analysis as represented in the Fig. 5, g depicts nearly 82% cells underwent apoptosis at 250 µg/ml (62% in early apoptosis, and 20% in late phases of apoptosis). A similar degree of cells underwent apoptosis at 500 µg/ml (64% in early and 18% in late apoptosis), and nearly 90% of cells were undergoing apoptosis at 1000 µg/ml (18% early and 70% late phase apoptosis).

Migration by scratch test. NM reduced the migration of SK-UT-1 cells in a dose dependent manner. Inhibition is apparent at lower doses with a complete inhibition of migration at 250 µg/ml. Photomicrographs of the scratch test are shown in Fig. 6.

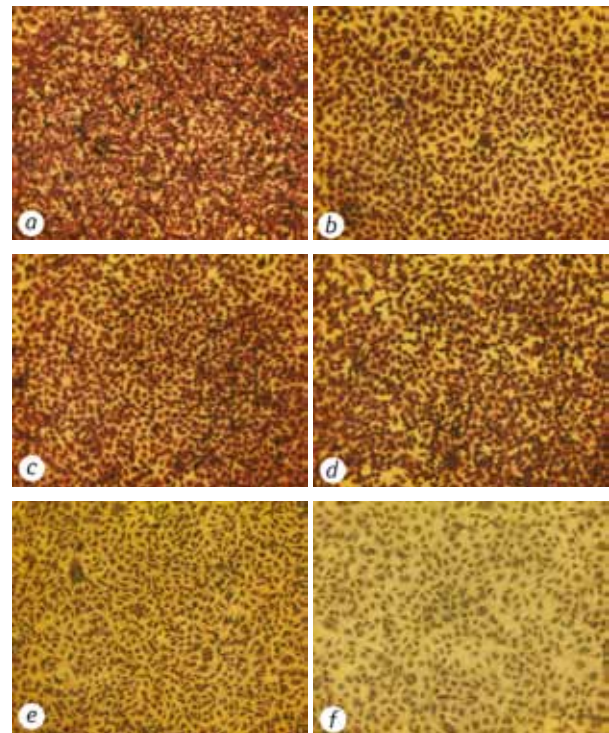


Fig. 4. Changes in SK-UT-1 cell morphology with increasing NM doses as seen on H&E staining (a — control; b — 50 µg/ml; c — 100 µg/ml; d — 250 µg/ml; e — 500 µg/ml; f — 1000 µg/ml)

In vivo studies. NM strongly inhibited the tumor growth of SK-UT-1 cells in female nude mice as evidenced by the tumor size (Fig. 7). Mean tumor weight was reduced by 50% with NM 0.5% dietary supplementation. Mean tumor burden was reduced by 40% ($p < 0.001$).

Histologically, the tumors from the control and the NM supplemented groups showed nests and sheaths of irregularly round-to-spindle shaped cells with necrotic areas. However, the tumors from control group had severe central necrosis involving 60–70% of the tumor mass, as opposed to the tumor sections from the NM group showed the necrosis ranging from 10–45% of the tumor mass (figures not present).

DISCUSSION

The *in vitro* studies of LMS cell line SK-UT-1 demonstrated that NM is effective in inhibiting the cell growth in a dose dependent fashion. The zymography showed that NM inhibited the secretion of both active and inactive forms of MMP-9 in a dose response fashion. Invasion through Matrigel and migration studies further conformed this as 500 µg/ml NM completely inhibited the migration of SK-UT-1 cells. NM also induced dose dependent apoptosis in SK-UT-1 cells and approximately 90% of cells were undergoing some stage of apoptosis at 1000 µg/ml.

It is well known that the MMPs are expressed in multiple cancer types and these proteinases are important for malignant transformation. Liolata *et al.* [15] discovered the ability of tumor cells to denature the surrounding extra cellular ma-

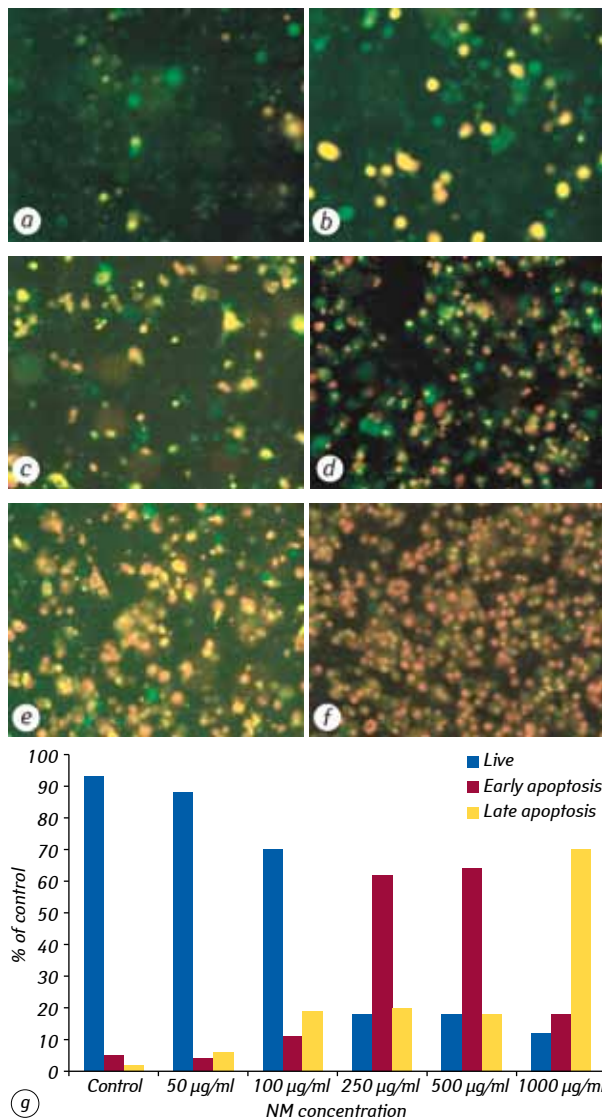


Fig. 5. Apoptosis of SK-UT-1 cells (Live Green Caspase kit). *a–f* — photomicrographs showing apoptosis of SK-UT-1 cells with increasing NM doses (*a* — control; *b* — 50 µg/ml; *c* — 100 µg/ml; *d* — 250 µg/ml; *e* — 500 µg/ml; *f* — 1000 µg/ml); *g* — quantitative analysis of apoptosis

trix and implicated a proteinase secreted by tumor cells and further suggested this as a mechanism for metastasis. This family of enzymes now known as MMPs, has the ability to degrade surrounding stroma. In particular, MMP-2 and MMP-9 are known as gelatinases and have the ability to unwind the triple helix of type IV collagen, as well as the ability to remodel laminin. MMPs are expressed in a variety of tissues including fibroblasts, epithelium and inflammatory cells [16]. Overexpression of MMPs have been discovered in many cancer tissue types. Both *in vitro* [17, 18] and *in vivo* [19, 20] data reveal a correlation between MMP overexpression and malignancy potential. Indeed, Deryugina *et al.* [21] discovered that an increase in MMP expression correlates with tumor progression, metastasis, and a poor prognosis.

Hanahan *et al.* [22] described the invasive metastatic capabilities of tumor cells to be dependent

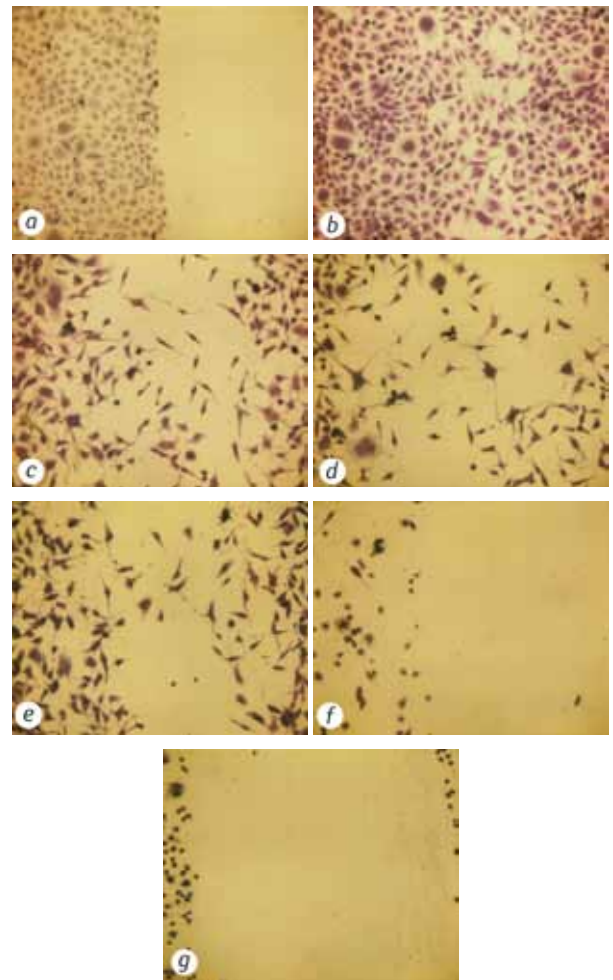


Fig. 6. Photomicrographs showing the effect of increasing doses of NM on migration of SK-UT-1 cells in scratch test (*a* — at zero point; *b* — control without NM; *c* — 50 µg/ml; *d* — 100 µg/ml; *e* — 250 µg/ml; *f* — 500 µg/ml; *g* — 1000 µg/ml)

upon their ability to alter cell-cell interactions via overexpression of extracellular proteases. Furthermore, the tumor must induce angiogenesis to maintain viability. MMPs are implicated in these mechanisms leading to malignant transformation, and MMP-2 in particular has been linked to the “angiogenic switch” in tumor cells [23]. Thus, MMPs are important not only for the enzymatic denaturation of surrounding stroma, but they also play an important role in angiogenesis and suggest a complex interplay between these proteases and their environment.

NM was formulated by defining critical physiological targets in cancer progression and metastasis, such as ECM integrity and MMP activity. Adequate supplies of ascorbic acid and the amino acids lysine and proline ensure proper synthesis and hydroxylation of collagen fibers for optimal ECM structure. Manganese and copper are also essential for collagen formation. Lysine, a natural inhibitor of plasmin-induced proteolysis, plays an important role in ECM stability [10, 24]. Green tea extract has been shown to modulate cancer cell growth, metastasis, angiogenesis, and other aspects of cancer progression [25–29]. N-acetyl cysteine has been

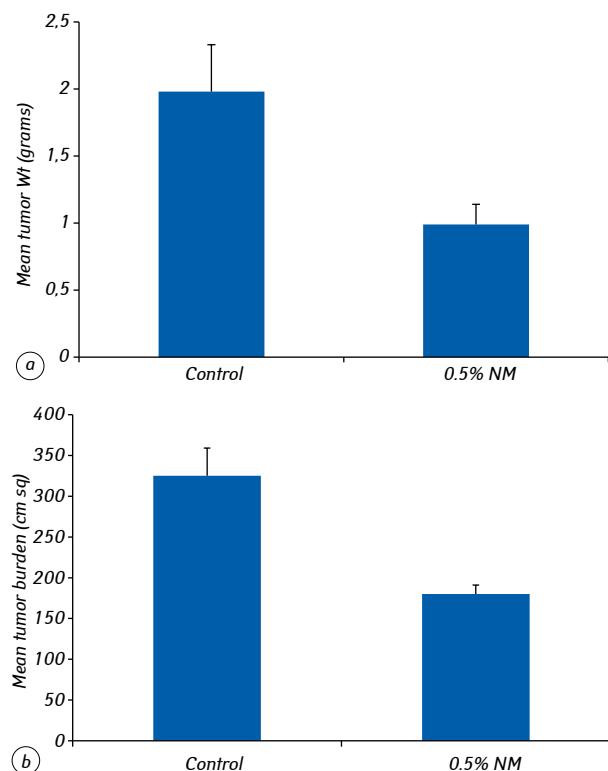


Fig. 7. Effect of NM on growth of tumors in nude mice induced by SK-UT-1 cells (a — tumor weight; b — tumor burden). $p < 0.001$

shown to modulate MMP-9 and invasive activities of tumor cells [30, 31]. Selenium has been shown to inhibit MMP secretion, tumor invasion, and migration of endothelial cells through ECM [32]. Ascorbic acid demonstrates cytotoxic and antimetastatic actions on multiple malignant cell lines [33–36], and cancer patients have been found to have low levels of ascorbic acid [37, 38]. Low levels of arginine, a precursor of nitric oxide, can limit the production of NO, which has been shown to predominantly act as an inducer of apoptosis [39].

To our knowledge, ours is the first study to explore the down-regulation of MMPs, especially, MMP-9 expression using NM in LMS cells. Earlier, several studies by other authors explored the role of MMPs in human LMS. Bodner-Adler *et al.* [9] analyzed the expression of MMP-1 and -2 and the clinicopathological features of human LMS and revealed a statistically significant relationship between MMP-2 expression and vascular space involvement. In a review with meta-analysis of clinical trials showed that MMP-2 was expressed in high percentage of endometrial cancers and its expression may be associated closely with clinical stage, tumor invasion and metastasis [40]. In addition, in an analysis of MMP-2 expression across several tissue types (lymphoma, smooth muscle tumors of uncertain malignant potential [STUMP] and LMS, the LMS was shown to have a statistically significant increase in MMP-2 expression, with 48% of tumor tissue being positive [8].

In our study, we have shown a significant inhibition of MMP-9 expression, important mediators

of angiogenesis and metastasis. In addition, we have shown that NM also has a cytotoxic effect of human LMS cells which suggest an important role in NM in cancer therapies.

NM shows activity against the proliferation of SK-UT-1 cells as well as a marked decrease in MMP secretion, invasion, migration, and induction of apoptosis. These data suggest a role for NM in the treatment of LMS, specifically by targeting MMP expression and thereby inhibiting migration of LMS within the ECM as well as stabilizing the ECM and surrounding the encapsulated tumor. In addition, NM maximally inhibits the proliferation of cancer cells by 30% at high doses and induces apoptotic changes at the cellular level. Overall, this NM may offer a therapeutic benefit and play a role in support of LMS patients.

CONFLICT OF INTEREST

The authors have no conflict of interest.

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**ПОЖИВНА СУМІШ УПОВІЛЬНЮЄ РІСТ
КЛІТИН ЛЮДСЬКОЇ ЛЕЙОМІОСАРКОМИ
СК-УТ-1 В КУЛЬТУРІ ТА ПРИ ПЕРЕЩЕПЛЕННІ
БЕЗТИМУСНИМ МИШАМ, ІНГІБУЄ СЕКРЕЦІЮ
МЕТАЛОПРОТЕІНАЗ ТА СПРИЧИНЮЄ АПОПТОЗ**

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Стан питання: Лейоміосаркома матки є відносно рідкісною злоякісною пухлиною, що походить з гладеньких м'язів стінки матки. Вона зазвичай погано реагує на хіміо- або променеву терапію. **Мета:** Досліджували

вплив інноваційної поживної суміші, що містить лізин, пролін, аскорбінову кислоту та екстракт зеленого чаю на ріст клітин SK-UT-1 лейоміосаркоми людини. Визначали проліферацію, інвазивний потенціал клітин, апоптоз та експресію матриксних металопротеїназ. Вплив поживної суміші на ріст клітин SK-UT-1 *in vivo* визначали на безтимусних мишах. **Матеріали та методи:** Клітини SK-UT-1 лейоміосаркоми людини культивували при різних концентраціях досліджуваної поживної суміші. Проліферацію клітин визначали за допомогою МТТ-тесту, експресію ММП — зимографією за желатиназною активністю, інвазивні властивості — за допомогою тесту з Matrigel, міграцію клітин — за допомогою тесту з подряпиною, апоптоз — за допомогою набору для визначення каспазної активності з Live Green. Дослідження *in vivo* проводили на самках безтимусних мишей віком 5–6 тиж. Мишам вводили підшкірно клітини SK-UT-1 у кількості $3 \cdot 10^6$ та утримували їх на стандартному кормі або кормі з додаванням 0,5% поживної суміші. За 4 тиж мишей забивали, а пухлини зважували та проводили їх гістологічне

дослідження. **Результати:** *In vitro* поживна суміш не була токсичною в концентрації 250 мкг/мл, але виявляла цитотоксичність на 20 та 40% в концентраціях, відповідно, 500 та 1000 мкг/мл. Зимографія не виявляла смуг ані ММП-2, ані ММП-9 в клітинах SK-UT-1. Форболміристат ацетат стимулював експресію ММП-9 порівну як в активній, так і в неактивній формі. Поживна суміш дозозалежно інгібувала секрецію як активної, так і неактивної форми, а також інгібувала інвазію клітин через Matrigel та міграцію в тесті з подряпиною. У концентрації 100 мкг/мл суміш індукували незначний апоптоз, який посилювався з підвищенням концентрації. Результати досліджень *in vitro* були підтверджені *in vivo*. Було продемонстровано 50% зменшення маси пухлин та 40% зниження пухлинного навантаження у мишей, яких тримали на раціоні з додаванням поживної суміші. **Висновки:** Результати свідчать про терапевтичний потенціал даної поживної суміші щодо лейоміосаркоми.

Ключові слова: матриксні металопротеїнази, інвазія, SK-UT-1, лейоміосаркома, безтимусні миші.