



Comparative Study on Myelotoxic and Antineoplastic Action of *Synadenium umbellatum*, *Vitis vinifera* and Resveratrol

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Abstract

Cancer is one of the pathologies that most challenge medicine, not only because of the complexity of its development, but also because of the difficulty of treatment that is not always effective. Due to the significant toxicity and adverse effects profile presented by the main chemotherapeutic agents used to treat neoplasm, there is a constant interest in the search for new drugs that may be a more effective alternative. Therefore, the search for new compounds of plant origin becomes an interesting tool for the discovery of drugs with antitumor activity. *Synadenium umbellatum* is a plant native to tropical Africa known as “cola-nota”, “hazel”, “cancerous”, “miraculous”, among others. The plant is used by the Brazilian population as having anti-inflammatory, analgesic, antineoplastic properties, among others. However, the literature is lacking on reports of the toxicity of *Synadenium umbellatum* macerate. Resveratrol is a polyphenolic compound found in different plant species, mainly in grape species (*Vitis*) and their seeds. *Vitis vinifera* is a species of grape easily found in several regions, also containing a high concentrations of phenolic compounds, including resveratrol in its fruits, leaves and seeds. Therefore, this study was aimed to evaluate the myelotoxicity of macerate, extract and resveratrol and their antitumor activity in Balb/c mice with Ehrlich tumor. The hematological evaluation was obtained by flow cytometry and the tumors were measured using a pachymeter and the tumor masses by means of weighing. No statistically significant difference was observed between results from the control group and the treatment groups. It was concluded that macerate, crude extract and resveratrol did not demonstrate a myelotoxic effect and did not cause a decrease in tumor mass.

Keywords: Antineoplastics, Drug Toxicity, Phytotherapy

1. Introduction

In the last centuries the population profile underwent intense changes that interfered directly in the diseases that affected the population. Among these changes, lifestyle and scientific development are directly related to the increase in life expectancy, currently the main pathologies that affect the population in general are no

longer infectious diseases, but are chronic-degenerative diseases such as diabetes and cancer¹.

Several antineoplastic drugs are available, although their side effects lead to the need for new research for alternative therapies like herbal medicines. Some plants have shown satisfactory effects, representing a good source of research for the development of molecules that in the future may be effective as an alternative in

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antitumortherapy. For example, cashew, garlic, green tea, guarana and other dozens of plant species that have already been studied².

Many species of *Synadenium* are known world wide for their use as anti-inflammatory, antineoplastic and analgesic³.

Synadenium umbellatum, also known as “Cola-Nota”, “Milky Amazon” or “Janauba”, in more central regions, belongs to Euphorbiaceae, a family with about 290 genera and approximately 7,500 species⁴. In the Midwest, latex is used empirically in the fight against various diseases such as allergies, cancer, Chagas disease, diabetes, influenza, internal bleeding, sexual impotence, leprosy, obesity, nervousulcer, menstrual cramps and body aches caused by malignant neoplasms⁵. The exact recommendation for the treatment of cancer as follows; “Macerate 3 leaves in 1 L of water and store in the refrigerator. Replace the water with drinking solution several times a day. Always use for healing and prevention”⁶.

Vitis vinifera (Vitaceae) is another plant that has also shown promising results associated with the prevention and treatment of several pathologies, including neoplasms. Also, known to contain high concentrations of phenylpropanol compounds including polyphenols such as flavonoids, vanillic acids, phenolic acids, chalcones and stilbenes, such as resveratrol⁷⁻¹⁰.

Resveratrol is a bioactive phytoalexin commonly found in about 72 different species, among them, grapes (*Vitis sp.*) and their seeds. This compound can also be found in red wines, reported to exert neuroprotective, antifungal and anti-inflammatory action. It can also be found in cis and trans form¹¹.

Some studies have already evaluated and demonstrated the cytotoxicity and mechanisms of induction of apoptosis of *S. umbellatum* and resveratrol on tumor cells such as the Ehrlich Tumor as well as the myelotoxic potential of plants. It is more specific for tumor cells than for normal hematopoietic progenitors, broadening horizons for its ethnopharmacological and potential use as a herbal adjuvant for cancer therapy^{11,12}.

Currently, studies are available for the evaluation of the effects of these plants, such as antiangiogenic potencies, anti-inflammatory mechanisms, anesthetics, but the literature is lacking about a comparison on the cytotoxic actions of the ways in which the population

has easy access and a simplified manipulation, such as the maceration of *S. umbellatum*, the crude extract of *V. vinifera* and resveratrol, and the latter can be easily found due to its comprehensive commercialization^{11,12}.

Thus, preliminary toxicological testing, independent of pharmacodynamic results, is essential because of ethnopharmacological motifs and existing myelotoxic information. Hence, the present work was carried out to compare the evaluation of the myelotoxicity and antitumor action of the macerate of *S. umbellatum*, of the gross sample of *V. vinifera* and of the resveratrol in mice.

2. Materials and Methods

2.1 Botanical Material

Samples of *S. umbellatum* were collected from a home-grown specimen owned by one of the study's collaborators, who lives in the city of São Bernardo do Campo, in the state of São Paulo, with a latitude of 23.739558741972587 and a longitude of - 46.537785283436634 in a region of temperate climate. The leaves of *Synadenium umbellatum* were desiccated at 40 °C in an air circulation oven for 48 hours. The leaves were then ground in a knife mill until the fine powder was obtained, which was suspended in 0.9% physiological solution at a concentration of 10mg/kg, resulting in a plant product used “therapeutically” by the population in concentrations similar to those used popularly.

The dry extract produced from aqueous extract of *V. vinifera* seeds and resveratrol were kindly provided by the company Naturex Produtos Naturais, Franca. The batch numbers and dates of manufacture and validity of the dry extract of *V. vinifera* and resveratrol seeds are respectively: Extract *Vitis*: lot 0105/2014, Manufacture: May 2014, Validity: May 2016. Resveratrol: lot 0112/2013, Manufactured: Dec 2013, Validity: Dec 2015.

2.2 Animals Studies

All the experimental procedures described in this study were approved by the Animal Experimentation Ethics Committee of the Faculdade de Medicina do ABC in accordance with Law 11794/2008 (Lei Arouca) filed in 04/2013.

This experimental study consisted of 24 male albino mice, Balb/c line, with a mean weight of 30 ± 5 grams, 6 from the Animal Hospital of the Faculdade de Medicina

do ABC. During the experiment, the animals were housed in polypropylene boxes (49x34x16), lined with weekly mahogany and maintained in a photoperiodic cycle of 12 hours light/12 hours dark with ventilation (20 air changes per hour), temperature and relative humidity of the controlled air, being fed with natural water and ration Nuvilab® CR-1 (Nuvital) offered *ad libitum*. The animals were handled quickly and carefully through the base of the tail following the best practices protocol of the FMABC Vivarium.

2.3 Experimental Design

To obtain the solid tumor, tumor cells obtained from the TAE with seven days of evolution were used. The number of total cells that were later implanted was determined by counting in a common optical microscope with the aid of a Neubauer chamber. The ascites fluid was diluted in times with saline solution and the aliquot was made. The Blue Tripan dye exclusion test was performed, considering only suspensions with cell viability greater than 95% according to the protocol of the Laboratory of Clinical Analysis of the FMABC.

Tricotomy was performed with a tricotomy apparatus in the dorsal region of the mice, and the cell suspension was injected with a 24 gauge needle, inoculated with a total of 2.10^6 cells/mL in the lateral region of the back of each animal¹³.

After the development of the solid tumor, the 7th day was classified as day zero of the experiment. The animals were randomly divided into 4 groups:

- G1: 6 mice received saline solution daily for 28 days.
- G2: 6 mice received by gavage macerated "Cola-Nota" at the dose of 10mg/Kg for 28 days.
- G3: 6 mice received by steeping gavage resveratrol at a dose of 10mg/kg for 28 days.
- G4: 6 mice received by gavage macerated *V. vinifera* extract at a dose of 10mg/kg for 28 days.

On the 28th day, the animals were anesthetized with ketamine (100mg/kg/ip) and xylazine (10mg/kg/ip).

A laparotomy was performed to puncture the abdominal aorta for removal of blood. A dorsal-back longitudinal incision was then made for dissection of the tumor, and the latter was preserved with 10% formaldehyde.

The animals were euthanized in continuations in the CO₂ chamber.

2.4 Hematologic Evaluation

It was performed by flow cytometry by ABX Pentra DF 120[™], following good clinical practice.

2.5 Statistical Analysis

The results were submitted to analysis of variance ANOVA and subsequently analyzed by the Dunnett multiple comparison test (95% confidence), Bartlett's test using GraphPad Prism® software. Statistical significance was considered only in the results that presented P value < 0,05.

3. Results

There was no significant difference in the hematological evaluation results, after evaluation of the red series, as shown in the table below (Table 1), with erythrocyte count (million/mm³), hemoglobin (g/dL) (10^3 /mm³) hematocrit (%), when compared to the control, a significant difference was observed in all the components of the series (p = 0.0445), in the comparison between treatments with the Bartlett test. hemoglobin (p = 0.0041), platelet count (p = 0.0064) and hematocrit analysis (p = 0.0431).

The white blood cell count (10^3 /mm³), neutrophil count (10^3 /mm³), monocyte count) and lymphocyte counts (10^3 /mm³) were recorded in the table below (Table 1) completing the verification of the absence of myelotoxicity of the macerate, extract and compound.

Also, no significant variation was found in the evaluation between the initial and final weights of the mice (Figure 1) and a balance was observed in relation to the animals' body mass.

In this way, it was verified that none of the animals of the two groups presented weight loss and, in addition, with the results of the hematological evaluation, it was verified that the animals that received the macerate, the extract and the resveratrol were evaluated, was not found malnourished and weakened by a possible anemia, reinforcing the absence of the myelotoxic action of the treatments.

Table 1.

Parameter	SF		<i>Vitis vinifera</i>		Resveratrol		<i>S. umbellatum</i>	
	median	IC95%	median	IC95%	median	IC95%	mean	IC95%
Erythrocytes (10 ⁶ /mm ³)	9.46	6.93;10.74	9.31	8.91;9.86	9.60	8.62;10.27	9.22	8.30;10.08
Hemoglobin (g/dL)	15.35	12.37;17.30	15.20	14.82;15.74	15.50	14.61;16.14	15.40	13.28;16.68
Hematocrit (%)	44.75	30.34;49.99	43.60	42.16;45.76	44.65	40.38;47.65	45.40	38.05;49.67
Leucocytes (10 ³ /mm ³)	2.65	1.33;4.70	2.60	1.76;2.99	2.25	0.78;3.96	2.60	1.66;3.66
Neutrophils (cells/mm ³)	0.32	0.07;0.55	0.13	0.05;0.25	0.10	0.03;0.29	0.17	0.06;0.33
Lymphocytes (cells/mm ³)	2.29	1.24;3.93	2.31	1.61;2.76	2.12	0.85;3.56	2.51	1.71;3.53
Monocytes (cells/mm ³)	0.025	0.01;0.10	0.01	0.00;0.02	0.1	0.07;0.17	0.01	0.00;0.03
Platelets (10 ³ /mm ³)	644.5	581.2;721.1	672.0	629.9;710.0	691.0	649.0;730.0	617.0	545.1;711.7

Median values of asterisks (*) differ significantly from the control in the same column by the ANOVA and *Post Hoc* Dunnett's test ($p < 0.05$).

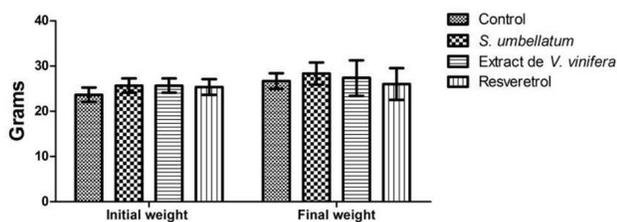


Figure 1. Evaluation of the weight of the mice (g) (n=24)* $p < 0.05$.

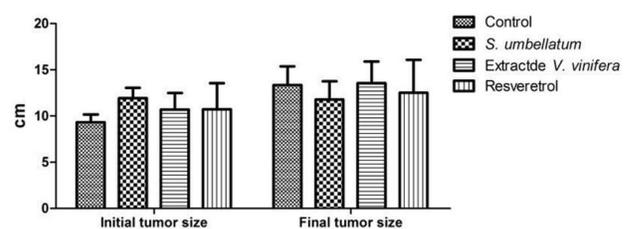


Figure 3. Evaluation of tumor sizes (cm) (n=24)* $p < 0.05$.

Macerated, extract and resveratrol did not cause an effective antitumor action, due to the absence of significant difference in the comparison between the tumor weights of the two groups and also in the evaluation of the initial and final sizes of the tumors (Figures 2 and 3).



Figure 2. Evaluation of tumor weight (g) (n=24)* $p < 0.05$.

4. Discussion

The preclinical study of toxicity and pharmacodynamics is of crucial importance when one observes its clinical applicability, that is, scientific validation of popularly used medicinal preparations ends up leading not only to the confirmation of new therapies for the treatment of cancer, but to form parallel action leads us to understand the action of the plant stratum on tumor cells.

Experimental toxicology develops studies to elucidate the mechanisms of action of toxic agents on biological systems and the evaluation of the effects resulting from its action. Toxicological studies, applied to laboratory animals and under pre-established conditions, determine the possible effects of substances on humans or animals exposed to them. In Brazil, National Health Council resolution 1/78 (OD 17/10/78) establishes several types of toxicological tests, such as acute, subacute,

chronic toxicity, embryotoxicity, and special studies, carcinogenic, mutagenic and neurotoxic studies¹⁴. Once, a preliminary study, demonstrates efficiency and is commonly used in the preliminary screening of plants to detect pharmacological and toxicological activities¹⁵.

The option for a study with the use of macerated and not only with latex was made by the current lack of studies regarding the supposed pharmacological actions of macerated, not finding scientific evidence to prove them. Since latex presents several recent studies on its properties, allowing the definition of its cytotoxic potential and its effects, from its isolation. As other studies have shown, when isolating a lectin from latex and proving its great immunomodulatory potential¹⁶⁻¹⁸.

Although the isolation and identification of plant chemical compounds are extremely useful, as in the case of resveratrol, it is necessary to remember that often in the case of phytotherapeutic drugs, it is not necessarily that isolated substance that is most useful for the treatment of the disease, since not always the isolated molecules, present activity when compared to the vegetal drug extract. An example of this is the case reported by a group of researchers, in which the research group isolated and identified two chemical species from the vegetable *Croton urucurara*, neither of which individually had the same performance of the extract in the "integral" form, or either as it is used by the population^{18,19}.

From the no significant difference observed in the hematological analysis in the comparison between the control and the treatments, the absence of myelotoxicity was demonstrated by the concentration of the administered dose, broadening horizons for studies with new doses, in order to reach the therapeutic dose.

Based on the significant difference found in the evaluation of the components of the red series in the comparison between the treatments, it can be observed that they cause different effects on the hematopoietic tissue, however for more details about what these effects are and how they occur if new tests are necessary and in-depth and specific studies.

It is worth mentioning that toxicity tests should prioritize the path that resembles the maximum of popular use. Even though they are different species, it is notorious that the response of the animals to gavage administration was not the same as that observed

when using other routes, such as the intraperitoneal route, due to the differences between the stomach and intestinal absorption profiles as pH, absorption surface, intestinal motility, blood irrigation^{19,20}. For this reason, the evaluation of myelotoxicity was done using the oral route since there was no report of the use of *S. umbellatum*, *V. vinefera* and resveratrol by another route of administration.

The administered dose of 10mg/kg of the treatments were calculated to reproduce the dose used by the population. Even though the characteristics of the latex were known and the 2000 mg/kg dose of both latex and leaf extract of *S. umbellatum* did not cause any lethal effect²¹.

In a study by Da Silva *et al.*²², similar results were observed for the administration of the extract from the Cola-nota. The same concentration was used, but anti-cachectic action was due to the action of *S. umbellatum* on the metabolic pathways of transforming growth factor protein-b1(TGF β 1). This study noted that no toxicity was observed with *S. umbellatum* nor other compounds.

The main problems of chemotherapy for the treatment of cancer are myelosuppression and anemia²³. The anemia that occurs in the tumor bearing animals is mainly due to the reduction of the number of red blood cells or the production of hemoglobin, and this can occur due to iron deficiency, hemolysis or other myelotoxic conditions²⁴.

In this study, the groups that received the treatments had serum hemoglobin and hematocrit values close to the levels found in the control group, since no significant differences were observed in the hematological evaluation, the non-entailment of the myelotoxicity of the treatments.

Also, in other studies using *S. umbellatum* leaf extract, there were no significant changes in haematological laboratory parameters, in addition to presenting a balance of animal weight²¹. The same was also observed in the treatment with macerate of the present study.

Positive results are those that increase the rate or duration of survival, reduce tumor growth, and decrease the number and leukocytes in the blood²⁴⁻²⁶.

However, the results of this study showed that the macerate, the extract and the resveratrol were not able to

reduce the Ehrlich tumor, and to verify the survival, as previously discussed.

Assuming then that the macerated extract and resveratrol are popularly used by the population, they do not show efficacy in the animal model and concentration used as treatment in those studies.

Contrary to expectations, the same results of tumor growth retardation observed in other studies with resveratrol administered at concentrations, periods and similar experimental models could not be verified^{10,27}.

The low solubility of extract macerate and resveratrol in water described in the literature and the rapid *in vivo* metabolism by phase II reaction of liver biotransformation and high plasma protein binding of resveratrol may have contributed to the observed outcome^{28,29}.

Studies involving extracts and macerates of plant origin, for example, *Euphorbia tirucalli*, demonstrated that the preservation of the integrity of hematopoiesis is closely associated with the drug's ability to increase the survival rate^{26,30}.

The same was also observed in this study, regarding the integrity of hematopoiesis, by the hematological evaluation after the use of the treatments analyzed.

The finding of non-myelotoxicity provides interesting possibilities for future studies, with the application of new doses, to verify or even verify the popular reports about the antineoplastic effect of macerate, extract and resveratrol use.

5. Conclusion

Therefore, it was concluded that the macerated *S. Umbellatum*, the crude extract of *V. vinefera* and resveratrol did not cause myelotoxicity in the administered concentration, and they did not demonstrate antitumor activity, opening perspectives for new research with the administration of new doses and concentrations.

6. Acknowledgements

There are no conflicts of interest.

7. References

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