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Chemical properties and protective effect of *Rosmarinus officinalis*: mitigation of lipid peroxidation and DNA-damage from arsenic exposure

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Summary

Recent studies have implicated dietary factors in the cause and prevention of important diseases, with strong evidence that plant's compounds can protect against these diseases. Moreover, food security and environmental contamination are topics in focus at the moment. In this view, contamination by arsenic (As) has received much attention as well as some spices with medicinal properties. Among these plants, the use of *Rosmarinus officinalis* L. has demonstrated antioxidant properties besides being used for circulatory disorders. Therefore, we measured the mitotic index of *Allium cepa* L. and characterized the antioxidant effects to determine the capacity of *R. officinalis* to ameliorate arsenic-induced DNA damages. *R. officinalis* extract showed no mutagenic effects and exhibited antimutagenic potential, reducing the DNA damages, anaphase-telophase bridges and micronuclei chromosome aberrations that result from treatment with the arsenic. Additionally, reduction in arsenic-induced lipid peroxidation was also observed.

Key words: arsenate, cytogenetic, heavy metal, medicinal plant, rosemary.

Introduction

Rosmarinus officinalis L. is a species of the Lamiaceae family native from the Mediterranean Region, popularly known as rosemary (Ferrari et al., 2011). According to ANVISA (2010), the extracts of rosemary leaves can be used for combating circulatory disorders, and is recommended as an antiseptic and for cicatrization.

Among the chemical constituents of rosemary, the flavonoids and phenolic acids are the two major groups of phenolic compounds (Cunha et al., 2012). These substances are known for their anti-oxidant, anti-inflammatory, antitumor and estrogenic properties, suggesting the role of certain phenolic compounds in the prevention of coronary heart diseases and cancer (Espín, 2001).

Chronic arsenic (As) toxicity from ingestion of contaminated drinking water and food has been reported in many countries and is an environmental problem of colossal proportions with a wide range of deleterious health impacts, including hyperpigmentation, keratosis, skin and internal cancers, and vascular diseases (National Research Council, 2001; Meharg and Zhao, 2012).

The present study aimed to evaluate the concentration of phenolic compounds of *R. officinalis* extracts grown off the ground and its effects on arsenic-induced DNA damage and oxidative stress through *Allium cepa* test and biochemical analysis.

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Materials and methods

Plant cultivation

Onion cultivation

Onion plantlets (*Allium cepa* L.) were obtained from Universidade Federal de Santa Maria (UFSM), RS, Brazil. The plantlets were transplanted to plastic trays containing substrate and cultivated in a greenhouse with shading (50%), at a spacing of 10 cm and a density of 100 per m of surface seedlings. These plants were administered with three daily irrigations (15 min each) with complete nutrient solution containing (mg L^{-1}): 155.90 N; 46.40 P; 5271.00 S; 123.00 Ca; 30.00 Mg; 253.60 K; (mmol L^{-1}) 2622.00 B; 133.00 Na; 277.00 Mo; 2274.00 Zn; 636.00 Cu; 6501.00 Mn and (mg L^{-1}) 1.20 Fe.

At the end of the cycle, the plants were collected and the produced bulbs were subsequently used for the *Allium cepa* test and biochemical analyses.

Rosemary cultivation

The rosemary plants were grown in a 115 m^2 polyethylene shelter, covered with polyethylene additive anti-UV 200 mm thick, at UFSM, Rio Grande do Sul, Brazil. Polypropylene vessels (2.8 dm^3) filled with sand with one plant per pot were used for this experiment. During the cultivation four daily irrigations with nutrient solution were performed to keep the water level in the vessels.

The nutrient solution used had the following ionic composition in mmol L^{-1}: 11.02 NO_3^-; 2.32 NH_4^+; 0.8 $H_2PO_4^-$; 6.0 K^+; 1.75 Ca^{2+}; 1.25 Mg^{2+} and 1.25 SO_4^{2-}. Micronutrients were supplied in concentrations in mg L^{-1}, 0.03 Mo; 0.26 B; 0.06 Cu; 0.50 Mn; 0.22 Zn; through a stock solution Fe was separately provided at a concentration of 1.0 mg L^{-1} in the chelate form. The pH was maintained between 5.5 and 6.0.

The leaves of 10 plants were harvested at 160 days after planting (DAP). These leaves were dried under the shade during 5 days at room temperature before the preparation of extracts and essential oil extraction.

Preparation of aqueous extracts

The extracts were prepared in the Laboratory of Cytogenetic and Plant Genotoxicity of UFSM, by infusion of the leaves at 5 and 20 g L^{-1}, using distilled water as liquid extractor.

The infusions were obtained by boiling distilled water at 100 °C and pouring water on whether the chopped plant material to facilitate the action of water. After mixing, the container remained capped for 15 minutes.

These extracts were filtered and, after reaching room temperature, were analyzed by high performance liquid chromatography with diode arrangements Detection (HPLC-DAD) for identification and quantification of the phenolic compounds, and used for the *A. cepa* test.

Extraction of volatile oil of rosemary

For the extraction of essential oil composite samples of 30 g were prepared from plants collected in four replications. The extraction of the essential oil of the leaves was carried out by hydrodistillation method in a Clevenger apparatus for 3 hours, and stored at -4 °C until the chromatographic analysis and evaluation of the effect on the cell cycle through *A. cepa* test.

High Performance Liquid Chromatography

The analysis by High Performance Liquid Chromatography (HPLC-DAD) was performed on HPLC system (Shimadzu, Kyoto, Japan) with auto injector (sIL 20A) equipped with reciprocating pumps (Shimadzu LC-20AT) connected to the degasser (DGU 20A5) with integrator (CBM 20A) detector UV / VIS diode array (SPD-M20A) and LC software solution 1.22 SP1. The analysis were performed in reversed phase under gradient conditions using C18 column (4.6 mm × 150 mm) packed with 5μm diameter particles.

The mobile phase was composed of: water containing acetic acid 2% (A) and methanol (B), and composition gradient was: 5% B for 2 min, 25% B to 10 min, 40, 50, 60, 70 and 80% (B) every 10 min, following the method described by Coelho et al. (2013), with slight modifications. The mobile phase extracts were filtered through a 0.45 μM membrane filter (Millipore) and then degassed in a sonication bath. The flow rate was 0.9 ml min^{-1}, injection volume of 50 L and wavelengths were 285 nm for carnosic acid, 325 nm for caffeic, chlorogenic and rosmarinic acid, and 365 nm for quercetin, rutin and kaempferol.

Chromatographic analysis of the volatile oil of Rosmarinus officinalis
Gas chromatography (GC-FID)

The gas chromatography (GC) analyses were carried out using an Agilent Technologies 6890N GC-FID system, equipped with DB-5 capillary column (30 m × 0.25 mm; film thickness 0.25 mm) and connected to an FID detector. The injector and detector temperatures were set to 280 °C. The carrier gas was helium, at a flow rate of 1.3 ml min^{-1}. The thermal programmer was 50-300 °C at a rate of 5°C/min. Two replicates of samples were processed in the same way. Component relative concentrations were calculated based on GC peak areas without using correction factors. The injection volume of the oil was 1 μL (Verma et al., 2010).

Gas chromatography-mass spectrometry (GC-MS)

GC-MS analyses were performed on an Agilent Technologies AutoSystem XL GC-MS operating in the EI mode at 70 eV, equipped with a split/splitless injector (250 °C). The transfer line temperature was 280 °C. Helium was used as carrier gas (1.3 ml min^{-1}) and the capillary columns used were an HP 5MS (30 m × 0.25 mm; film thickness 0.25 mm) and an HP Innowax (30 m × 0.32 mm i.d., film thickness 0.50 mm). The temperature program was the same as that used for the GC analyses. The injected volume was 1 μL of the volatile oil.

Identification of the components

Identification of the constituents was performed on the basis of retention index (RI), determined with reference of the homologous series of *n*-alkanes, C_7-C_{30}, under identical experimental conditions, comparing with the mass spectra library search (Nist and Wiley), and with the mass spectra literature date Adams (1995). The relative amounts of individual components were calculated based on the CG peak area (FID response).

Cytogenetic analysis

Cytogenetic analysis of meristematic cells obtained from *A. cepa* radicles were used to evaluate morphological and structural cell modifications and determine the mitotic index.

Nineteen groups of five bulbs were placed in distilled water, constituting 19 treatments with 5 replicates each. The following treatments were used: to check the individual effect of each solution; T1: distilled water, as a negative control; T2: *R. officinalis* oil 0.8%, T3: *R. officinalis* oil 3%, T4: *R. officinalis* extract 5 g L^{-1}, T5: *R. officinalis* extract 20 g L^{-1}, T6: arsenic (100 μM), to check the effect of *R. officinalis* prior to arsenic exposure; T7: *R. officinalis* oil 0.8% (24 h) + arsenic (24 h), T8: *R. officinalis* oil 3% (24 h) + arsenic (24 h), T9: *R. officinalis* extract 5 g L^{-1} (24 h) + arsenic (24 h), T10: *R. officinalis* extract 20 g L^{-1} (24 h) + arsenic (24 h), T11: arsenic (24 h) + *R. officinalis* oil 0.8% (24 h), to check the effect of *R. officinalis* after arsenic exposure; T12: arsenic (24 h) + *R. officinalis* oil 3% (24 h), T13: arsenic (24 h) + *R. officinalis* extract 5 g L^{-1} (24 h), T14: arsenic (24 h) + *R. officinalis* extract 20 g L^{-1} (24 h), to check the effect of *R. officinalis* use concomitantly with arsenic exposure;T15: arsenic with *R. officinalis* oil 0.8%, T16: arsenic with *R. officinalis* oil 3%, T17: arsenic with *R. officinalis* extract 5 g L^{-1}, T18: arsenic with *R. officinalis* extract 20 g L^{-1}, T19: ethanol to check alcohol effect.

For the analysis of cell division, approximately 2 cm of onion radicles were collected and hydrolyzed in 1 N HCl for 5 min, followed by staining with 2% acetic orcein in accordance with Guerra and Souza (2002). The meristematic region was crushed using a small glass rod, and the material was placed onto a coverslip. The slides were observed under the microscope at 40 × magnification. Subsequently, the total cell count (interphase and cell division) was obtained, and the mitotic index (MI) was calculated based on the percentage of dividing cells. A total of 1000 cells were counted per bulb, totaling 4000 cells per group for each treatment and each species studied.

Biochemical parameters

For the biochemical assays, the root samples were collected, immediately placed in liquid nitrogen and pulverized to a fine powder using a porcelain mortar. The level of lipid peroxidation was estimated using a TBARS Assay (thiobarbituric acid reactive substances assay) according to the method of Beleid El-Moshaty et al. (1993).

The H_2O_2 concentration in the roots was determined according to Loreto and Velikova (2001). The activity of peroxidase (POD, EC 1.11.1.7) nonspecific present in the extract was determined according Zeraik et al. (2008), using guaiacol as substrate. The oxidation of guaiacol (a tetraguaiacol) was measured by the increase in absorbance at 470 nm (Chance and Maehly, 1955).

Statistical analysis

The experiments were performed using a randomized design. The analyses of variance were computed on statistically significant differences determined based on appropriate F-tests. The results are presented as the means ± SD of at least three independent replicates. The mean differences were compared using Scott-Knott ($P<0.05$).

Results and discussion

Chromatography and cytogenetic analysis

R. officinalis is an aromatic plant popularly known as rosemary which has important biological properties, especially due the phenolic and the volatile constituents, such as carnosol, carnosic acid and rosmarinic acid present in the extract of rosemary and α-pinene, bornylacetate, camphor and eucalyptol present in the essential oil of this species (Ribeiro-Santos et al., 2015; Arranz et al., 2015).

Also, minor constituents in rosemary may have biological functions, mainly because possible synergistic effects among their compounds (Ribeiro-Santos et al., 2015).

However, studies have demonstrated that there are significant variations in rosmarinic and carnosic acid content according to the harvest season and site of rosemary cultivation (Yesil-Celiktas et al., 2007; Frescura et al., 2013). The compound found in highest amounts in aqueous extracts of *R. officinalis* was rosmarinic acid (Tab. 1). The antioxidant activity has been attributed to this acid in the literature, reducing numerous events deleterious to the organism, such as formation of reactive oxygen species, lipid peroxidation and DNA fragmentation (Simões et al., 2001; Izzo and Capasso, 2007). Besides, Makino et al. (2000), suggest that rosmarinic acid in Lamiaceae herbs is a promising agent to prevent mesangioproliferative glomerular diseases.

There are many studies demonstrating the *in vitro* activity of rosemary diterpenes, such as carnosic acid, carnosol, and rosmanol, in anticancer activity (reviewed by Petiwala and Johnson, 2015). Furthermore, carnosic acid is described as the active ingredient responsible for antimicrobial activity exhibited by the extract from the leaves of *R. officinalis* (Bernardes et al., 2009) and has clinical application for diseases affecting the outer retina, including age-related degeneration, in which oxidative stress is probably one factor that contributes to disease progression (Rezaie et al., 2012).

The rutin has been cited having beneficial effects in reducing symptoms of impaired lymphatic and venous vessels (Pathak et al., 1991). The quercetin offers many benefits of health promotion, including improved cardiovascular health, reducing the risk of cancer and many others (Lakhanpal and Rai, 2007). Still, kaempferol and quercetin have protective action against pancreatic hypertrophy and hyperplasia (Rawel et al., 2002).

The major compounds of the volatile oil were monoterpenes corresponding to 64.6% of 91.4% (Tab. 2) in agreement with published literature (Ribeiro et al., 2012). Interestingly, the two most abundant compounds, 1,8-cineole and camphor in rosemary oil were described as insecticidal constituents against the cabbage looper, *Trichoplusia ni,* showing synergistic interactions and more effective control when used together (Tak and Isman, 2015).

The results demonstrate that both volatile oil in concentrations of 0.8 and 3% and aqueous extracts of *R. officinalis* in concentrations of 5 and 20 g L^{-1}, have no mutagenic effect as compared to arsenic treatments (Tab. 3), and show slightly different effects from control treatment, consistent with the results of other studies using different techniques (Amin and Hamza, 2005). This is an important foundation for future studies including human cell lines to ensure safety of volatile oil and aqueous extracts of *R. officinalis* as a phytomedicine.

Genotoxicity of arsenic compounds has been observed in a variety of cultured human cells (Yih and Lee, 1999; Yih et al., 2005), animals (Patlolla and Tchounwou, 2005) and arsenic-exposed human populations (Horvathova et al., 2003). In the present study, *R. officinalis* (both volatile oil and aqueous extract) reduced arsenic-mediated chromosome aberrations, especially the occurrence of micronucleus in *Allium cepa* (Tab. 3; Fig. 1). When added after 24 h exposure to As, the aqueous extract was able to completely reverse the damage caused by exposure to As. When used concomitant, there was no occurrence of chromosomal aberrations (Tab. 3; Fig. 1).

Some studies have reported the antimutagenic effects of *R. officinalis* in plants (Horvathova et al., 2014), and human cell lines (Wang et al., 2012); but to our knowledge, there were no studies evaluating the effect of *R officinalis* in to repair the DNA damage caused by exposure to As. Higher plants present characteristics that make them excellent genetic models to assess environmental pollutants, being frequently

Tab. 1: Concentration of phenolic compounds, mg g^{-1}, in extracts of *R. officinalis*, prepared with two concentrations (5 g of leaves L^{-1} and 20 g of leaves L^{-1}).

Phenolic compounds	*R. officinalis* extract	
	5 g L^{-1}	20 g L^{-1}
rosmarinic acid (mg ml^{-1})	5.99	26.30
chlorogenic acid (mg ml^{-1})	2.30	8.45
carnosic acid (mg ml^{-1})	1.83	5.09
caffeic acid (mg ml^{-1})	1.33	4.87
canpferol (mg ml^{-1})	0.61	2.40
quercetin (mg ml^{-1})	1.45	5.21
rutin (mg ml^{-1})	0.73	5.71

Tab. 2: Chemical composition of *Rosmarinus officinalis* volatile oil.

	Compounds	RI[a]	RI[b]	Concentration %
M	α-pinene	936	939	11.15
	α-camphene	954	953	4.52
	β-pinene	980	980	3.29
	β-myrcene	991	991	8.18
	α-phellandrene	1003	1005	0.25
	p-cymene	1026	1026	0.44
	1.8 cineol	1037	1033	16.78
	Camphor	1143	1143	21.33
	δ-terpinene	1065	1062	1.28
	Linalool	1098	1098	2.54
	Isoboneol	1156	1156	2.74
	Borneol	1165	1166	1.82
	Menthol	1173	1173	0.94
	Terpin-4-ol	1178	1177	0.51
	Pinocarvone	1160	1162	1.11
	Naphthalene	1178	1179	0.34
	α-terpineol	1189	1189	1.70
	piperitol <cis->	1195	1193	0.93
	Verbenone	1205	1204	7.20
	Pulegone	1237	1237	0.15
	Eugenol	1356	1356	0.73
S	α-copaene	1376	1376	0.01
	Caryophyllene	1417	1418	1.34
	Aromadendrene	1440	1439	0.48
	α-muurolene	1498	1499	0.07
	α-bisabolene	1504	1504	0.46
	α-cadidene	1538	1538	0.25
	Caryophyllene oxide	1580	1581	0.83
	Total identified (%)			91.37

Relative proportions of the essential oil constituents were expressed as percentages. [a]Retention indices experimental (based on homologous series of *n*-alkane C_7-C_{30}). [b]Retention indices from literature (Adams, 1995); M = monoterpenes; S = sesquiterpenes.

used in monitoring studies. However, this feature is not only due to the sensitivity to detect mutagens in different environments, but also to the possibility of assessing several genetic endpoints, which range from point mutations to chromosome aberrations in cells of different organs and tissues (Grant, 1994).

The most common abnormality caused by the arsenic exposure in this study was the occurrence of micronuclei, which was already reported by other studies (Yi and Si, 2007; Banerjee et al., 2013). Banerjee et al. (2013) reported the association between micronuclei frequency in urothelial cells from men, women, smoker group and non-smokers group, and arsenic content in cooked rice. They indicated a strong positive correlation of mean urinary arsenic with mean cooked rice arsenic content among the tested groups.

The detection of micronuclei has been considered by many authors as an efficient and simple way to analyze the mutagenic effect promoted by chemicals (Mekki, 2013). Micronuclei results from damage in the parental cells as acentric fragments or lagging chromosomes that fail to incorporate into either of the daughter nuclei during telophase in cell mitosis (Fernandes et al., 2007) being easily observed in daughter cells as a similar structure to the main nucleus, but in a reduced size. Thus, micronuclei arise from the development of some CA, for instance, chromosome breaks and losses. Moreover, micronuclei may still derive from other processes as polyploidization, in which they originate from the elimination of exceeding DNA of the main nucleus in an attempt to restore the normal conditions of ploidy (Fernandes et al., 2007).

Oxidative stress

R. officinalis, as others Lamiaceae species, contains flavonoids and phenolic acids (Coelho et al., 2013). Phenolic compounds can slow down oxidative reactions in biological systems. The study of phenolic compounds is also associated with important antioxidant activity of these compounds, suggesting that diseases caused by oxidative reactions in biological systems can be delayed by the intake of natural antioxidants found in plants such as *R. officinalis* (Simões et al., 2001).

The exposure to As causes oxidative stress, as can clearly be evidenced by TBARS formation, increased content of H_2O_2 and POD activity. In the present study, the application of *R. officinalis* extract reduced the TBARS concentration in cells exposed to arsenic, as well as the H_2O_2 concentration regardless the moment of exposure (Tab. 4). On the other hand, the volatile oil did not alter TBARS or H_2O_2 concentration under the 0.8% dose when used only 24hs before arsenic exposure (Tab. 4).

Wang et al. (2012) assessed the comparative antibacterial and anticancer activities of *R. officinalis* essential oil as well as its compounds, being the three main components 1,8-cineole, α-pinene and β-pinene. Even though *R. officinalis* essential oil exhibited the strongest antibacterial and cytotoxic activities towards SK-OV-3, HO-8910 and Bel-7402 human tumor cell lines. It is important to point that even though in this study the *R. officinalis* essential oil didn't show high antioxidant activity, it is well known its beneficial proprieties to human health (Posadas et al., 2009).

The POD activity was enhanced by As exposure as compared to the others treatments (Tab. 4). Among the treatments with *R. officinalis*, the concomitant exposure to As and *R. officinalis* resulted in a higher activity as compared to *R. officinalis* prior or after As exposure (Tab. 3).

However, in general terms this activity of the *R. officinalis* extracts was not enough to prevent DNA damage in a higher level as compared to treatments with *R. officinalis* extracts being used after As exposure (Tab. 4).

Tab. 3: Effect of *Rosmarinus officinalis* oil and extract on mitotic index and chromosomal aberrations in root tip cells of *A. cepa* exposed to arsenic.

Treatments	Mitotic index (%)	Total abnormalities (%)	Micronuclei	Chromosomal breakage and lost chromosomes	Anaphasic telophasic bridges
T1 distilled water*	5.37 a	0.25 c	0	0	1
T2 *R. officinalis* oil 0.8%	5.00 b	0.50 c	1	0	1
T3 *R. officinalis* oil 3%	3.15 d	0.75 c	3	0	0
T4 *R. officinalis* extract 5 g L^{-1}	1.37 f	0.50 c	1	0	1
T5 *R. officinalis* extract 20 g L^{-1}	0.80 g	0.75 c	2	0	1
T6 arsenic	4.27 c	3.50 a	10	0	4
T7 *R. officinalis* oil 0.8% (24 hs) + arsenic (24 hs)	1.15 f	2.00 b	7	0	1
T8 *R. officinalis* oil 3% (24 hs) + arsenic (24 hs)	0.20 h	1.50 b	5	0	1
T9 *R. officinalis* extract 5 g L^{-1} (24 hs) + arsenic (24 hs)	0.20 h	1.00 c	3	0	1
T10 *R. officinalis* extract 20 g L^{-1} (24 hs) + arsenic (24 hs)	0.10 h	0.75 c	1	0	2
T11 arsenic (24 hs) + *R. officinalis* oil 0.8% (24 hs)	3.95 c	2.00 b	6	1	1
T12 arsenic (24 hs) + *R. officinalis* oil 3% (24 hs)	1.00 g	0.25 c	1	0	0
T13 arsenic (24 hs) + *R. officinalis* extract 5 g L^{-1} (24 hs)	1.00 g	0.50 c	1	0	1
T14 arsenic (24 hs) + *R. officinalis* extract 20 g L^{-1} (24 hs)	0.95 g	0.00 c	0	0	0
T15 arsenic with *R. officinalis* oil 0.8%	4.10 c	2.00 b	5	1	2
T16 arsenic with *R. officinalis* oil 3%	2.35 e	0.35 c	1	0	0
T17 arsenic with *R. officinalis* extract 5 g L^{-1}	0.75 g	0.50 c	2	0	0
T18 arsenic with *R. officinalis* extract 20 g L^{-1}	0.20 h	0.00 c	0	0	0
T19 ethanol	4.25 c	0.50 c	2	0	0

Different low letters show significant differences among the treatments. *negative control

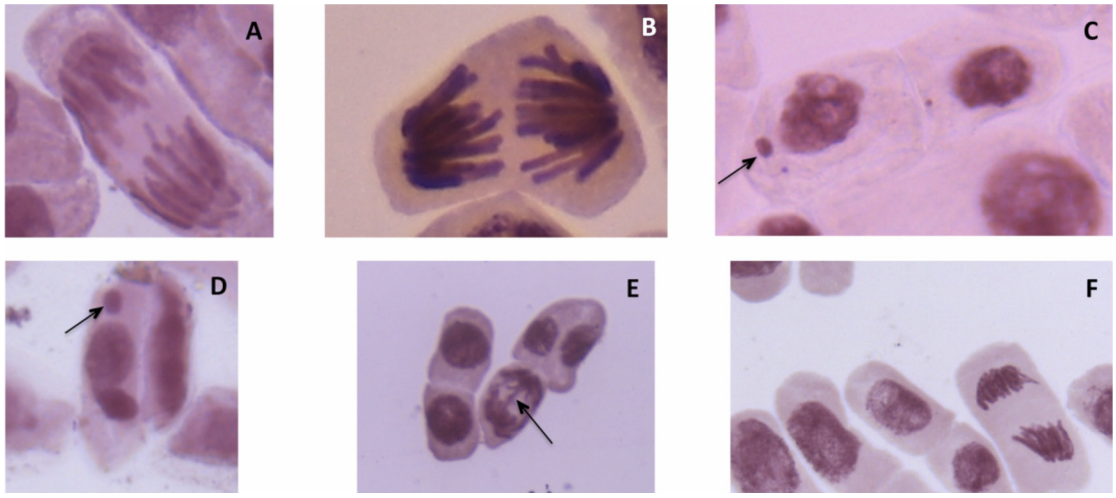

Fig. 1: **A B:** *Allium cepa* cells during normal anaphase (T1 distilled water A and T5 *Rosmarinus officinalis* extract B); **C D** *Allium cepa* cells during interphase exhibiting micronuclei (T6 treated with Arsenic (24 h); and T7 *R. officinalis* oil 0.8% (24 h) + arsenic (24 h)); **E:** *Allium cepa* cells exhibiting anaphase chromosome bridges (T6 treated with arsenic); **F:** *Allium cepa* cells during normal anaphase (T18 arsenic with *R. officinalis* extract 20 g L^{-1}).

Tab. 4: Levels of H_2O_2, TBARS and POD in cells obtained from *Allium cepa* radicles exposed to arsenic and *Rosmarinus officinalis* oil and extract.

Treatments	TBARS (MDA nM g^{-1} FW)	H_2O_2 (uM g^{-1} FW)	POD (umol min^{-1} mg FW)
T1 distilled water*	0.567 d	1.602 c	22.217 d
T2 *R.officinalis* oil 0.8%	0.587 d	2.203 c	26.728 d
T3 *R. officinalis* oil 3%	0.564 d	1.456 c	28.984 c
T4 *R. officinalis* extract 5 g L^{-1}	0.498 d	1.467 c	26.728 d
T5 *R. officinalis* extract 20 g L^{-1}	0.476 d	2.202 c	19.961 d
T6 arsenic	1.876 a	10.267 a	55.173 a
T7 *R. officinalis* oil 0.8% (24 hs) + arsenic (24 hs)	1.654 a	8.467 a	36.428 c
T8 *R. officinalis* oil 3% (24 hs) + arsenic (24 hs)	1.132 c	7.233 a	29.661 c
T9 *R. officinalis* extract 5 g L^{-1} (24 hs) + arsenic (24 hs)	1.109 c	5.533 b	31.917 c
T10 *R. officinalis* extract 20 g L^{-1} (24 hs) + arsenic (24 hs)	1.187 c	4.822 b	36.428 c
T11 arsenic (24 hs) + *R. officinalis* oil 0.8% (24 hs)	1.543 b	8.467 a	38.684 b
T12 arsenic (24 hs) + *R. officinalis* oil 3% (24 hs)	0.765 d	6.875 a	31.917 c
T13 arsenic (24 hs) + *R. officinalis* extract 5 g L^{-1} (24 hs)	0.708 d	4.076 b	29.661 c
T14 arsenic (24 hs) + *R. officinalis* extract 20 g L^{-1} (24 hs)	0.698 d	4.897 b	29.554 c
T15 arsenic with *R. officinalis* oil 0.8%	1.106 c	8.467 a	34.172 c
T16 arsenic with *R. officinalis* oil 3%	0.706 d	5.876 b	43.195 b
T17 arsenic with *R. officinalis* extract 5 g L^{-1}	0.654 d	4.035 b	43.194 b
T18 arsenic with *R. officinalis* extract 20 g L^{-1}	0.698 d	4.876 b	40.706 b

Different low letters show significant differences among the treatments. *negative control.

Conclusion

This study demonstrated that the *R. officinalis* extract exerted no mutagenic effects and showed antimutagenic potential, reducing the DNA damage and lipid peroxidation resulting from treatment with As exposure.

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