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Evaluation of Mutagenic Effects of Monosodium Glutamate Using *Allium cepa* and Antimutagenic Action of *Origanum majorana* L. and *Ruta chalepensis* Medical Plants

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

At the present study, *Allium cepa* root tips were collected during 2,6 and 24 hrs after treated with selected concentrations (1.0,3.0,5.0 and 7.0 g/L) of Mono-Sodium Glutamate (MSG) that used as flavour enhancer in foods. MSG induced mitodepression chromosomal aberrations such as bridges, fragments, disturbance, sticky chromosomes and other morphological abnormalities like enlargement cells. In this study we amid to determine the inhibitory effect of oil extract (1.25 µl/ml) from two medical plant species *Origanium majorana L.* and *Ruta chalepensis* on this food additive. Our results demonstrated that these extracts have the potency to suppress MSG by increasing of mitotic index and reduction of the chromosomal aberration and thus could be a promising antimutagenic and antigenotoxic potential.

Keywords: Mutagenic; monosodium glutamate; antimutagenic; medical plants.

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1. INTRODUCTION

Monosodium glutamate (MSG) has been used as food additives (Egbuonu and Osakwe 2011).

MSG is extensively used as flavour enhancer in food products throughout plenty of countries mainly in South East Asia. MSG was added to meats, poultry, seafood, snacks, soups and stews and other kind of food, subsequently, humans are daily exposed to these chemical substances. Monosodium glutamate is originally extracted from molasses by microbial fermentation of beet sugar, sugar cane, starch and corn sugar [1]. The majority of natural protein foods and some vegetables are containing Glutamate as a major component that plays an essential role in human metabolism [2-4]. Yang et al. [5] determined the optimal concentration of MSG to be between 0.2 and 0.8% for humans being about 60 mg/kg body weight [5]. Monosodium glutamate provides a flavouring role similar to naturally occurring free glutamate in foods [6]. However, the harmful health effects of MSG is seriously discussed, since 1960s, a great disagreement was received against using MSG as a flavour enhancer. There are different reports related to the safety of adding MSG in food, many studies reported that MSG-induced toxicity in different organisms [7-10]. Burde et al. [11] reveal that damage in nerve cells of both immature rats and mice was induced after applying of MSG by oral administration and subcutaneous injection. The effects of oral dosage of monosodium glutamate applied for short- and long-terms were induced severe damage on the histology and ultra structure of testes of the adult rats [12]. MSG inhibited growth of *A. cepa* root tips also reduced the number of roots growing, colour of root tips was changed and a range of chromosomal aberration was induced [13,14]. However, the toxicity or safety of MSG is remain in arguments also, there is no general acceptance of that MSG could be toxic to the humans. In this project we used *Allium cepa* assay, firstly to investigate the genotoxic effects of MSG food additive. Secondly, to reduce its harm effects by exposure root tips to fixed oils of two medical plants. Plants were chosen are *Ruta chalepensis* known as rue and *Origanium majorana* L. (its common name is oregano) from Rutaceae and Lamiacae respectively which are having a wide medical and traditional use in Arabic and Europe countries. Many studies were demonstrated that the extractions of both *R. chalepensis* and *O. majorana* are having as antioxidant, antibacterial, antimicrobial, antiparasitic, antimutagenic, antifungal and anticarcinogenic properties [15-20]. In recent years, using herbal drugs to treat different diseases has been renewed interest and also The World Health Organization (WHO) has recommended evaluation of the efficiency of medical plants in conditions when require or lack safe modern drugs [21]. Because of high cost and the range of side effects may have caused by taking synthetic drugs, alternative medicine became necessary [22]. In general, plants including fruits and vegetables are rich in antioxidants such flavonoids and phenolic compounds [23]. These phytochermicals were proven to be effective against various mutagens either through scavenging free radicals or by their reducing power [24].

2. MATERIALS AND METHODS

2.1 Plant Extraction

Fresh and healthy leaves of *Ruta chalepensis* and *Origanium majorana* L. were collected weighted, washed and then placed in Aceton inside sealed jars for 24 hrs. Solvent was removed from samples by evaporation using (Buchi R 215, P G Vertical 230V) and then oils were collected [25].

2.2 Growing Plants and Treatments

Dry healthy onion bulbs of 1.5 to 2.0 cm in diameter and weighing 20-30 g were purchased from local market. Bulbs were washed by using tab water, peeled and then the old roots were removed. They were then placed on top of small jars containing distilled water that have been changed with fresh water every morning to allow root to germinate for three days at room temperature. *Allium* root were then divided to three groups, the first group was treated with only MSG that dissolved in distilled water at concentrations used in foods of 1.0, 3.0, 5.0 and 7.0 g/L. The second and third groups were specified to run the interaction between MSG and medical plant extraction. 1.25 µl of fixed oil of *O. majorana* and *R. chalepensis* were added to *Allium* root at the same time with MSG to the second and third group respectively. Samples were collected after 2,6 and 24 hours, three replicates for each treatment and controls were included. The root tips were fixed in fixative (ethanol: chloroform: acetic acid, 6:3:1 v/v) for 24 hrs at room temperature, hydrolyzed in drops of

1 N HCL at 60°C for 12 min, moved to clean tubes for staining in 1% aceto-carmain stain for at least 1hr. The tips were then and squashed on microscope slides, two slides were made for each treatment and counting was randomly done on five fields to determine the mitotic index (MI) and chromosomal aberrations. The mitotic index was calculated for each treatment as the number of cells in mitosis/total number of cells counted (1000 cell) and expressed as percentage [26]. Images were taken under Olympus microscope attached with digital camera.

2.3 Statistical Analysis

Un-paired t-tests were used to compare the mitotic index values of both control and treated samples. For each treatment, root tips were taken from four different bulbs (4 replicates). Data were performed using SPSS computing software, results with $P<0.05$ were considered to be statistically significant.

3. RESULTS

3.1 MSG Treatment

Exposure *Allium cepa* root tips to different concentrations of MSG (1.0, 3.0, 5.0 and 7.0 g/L) lead to inducing different types of chromosomal aberrations and morphological abnormalities. These include bridges, breaks, fragments, sticky chromosomes, lagging chromosomes *etc* (Fig. 1). The most common chromosomal aberrations noticed were bridges and sticky chromosomes. Two morphological abnormalities, enlargement and elongated cells were found in samples treated with 5 and 7g/L of MSG (Fig. 1). In addition, declines in mitotic index values were generally caused after exposure to higher concentrations of MSG (Fig. 2). In our experiments, root tips were collected after 2, 6 and 24 hours duration time. The reduction in MI was increased with increasing incubation time, as it's starting with 6.9% and ending with 1.2% at 2 hr and 24 hr incubation time respectively (Table 1). There were significant differences ($P>0.05$, $P>0.01$ and $P>0.001$) in mitotic index values as compared to the control (6.7, 12.1 and 17.2%) at 2, 6, and 24hr, respectively. The lowest values were obtained when the highest MSG concentration 7 g/L were applied are 4, 1.7 and 1.2 at 2, 6 and 24 hours duration time in that order. Whereas the highest values of MI were obtained with the smallest concentration (1 g/L) and the same duration times are 6.9, 5.3 and 2.4.

3.1.1 MSG and medical plants treatment

The Tables 2 and 3 show the number of dividing cells in each phase of mitotic stage and MI recorded from tips that exposed to both food additive and oils extracted from selected medical plants. Generally, less chromosomal aberrations frequents were noticed and higher MI values were obtained toward the controls. Table 2 illustrates the interaction between MSG and fixed oil that extracted from *O. Majorana.* Applying 1.25µl of oregano's oil: resulting in decreased MI as the concentration of MSG increased and incubation time were recorded but significantly higher than the values that obtained after treating with only MSG (Fig. 3). The values of mitotic index that recorded subsequent to adding *R. Chalepensis* oil and MSG as indicated in Table 3 were very significantly higher than data achieved from adding mono-sodium glutamate (Fig. 4). The lowest and the highest MI values were obtained after treating with MSG and *oregano* oil are 2.6 and 6.4% whereas 4.7 and 7% are the values that achieved after adding *rue* oil to MSG. The comparison between MI values subsequent to adding oil extracts were statically significant ($P< 0.001$) and *rue* seemed to be more effective than *oregano* in recovering MI particularly at 24 h (Fig. 5).

4. DISCUSSION

Recently, many different aspects of plant biology have been studied in *Allium cepa* roots, such as chromosomal abnormalities, mitotic index, cells and root morphology. *A. cepa* root tips assay provide a relatively tractable system to detect cytotoxicity and genotoxicity of many chemical substances and is important for better understanding of the action of chemicals that may affect cell division [13,27,28].

The cytotoxicity level of test chemicals can be monitored based on the increase or decrease in the mitotic index (MI). According to [29] Smaka-Kincl et al. 1997 the decreased in mitotic index of meristematic cells of *A. cepa* may be considered a reliable method to determine the presence of cytotoxic compounds in the environment, and thus be a reliable test for monitoring toxicity levels. Whereas chromosomal aberration was reported to be a good indicator to access the mutagenicity of chemical *in vivo* [24,30]. Results that indicate the tested substances are affecting the normal conditions in the model was used should be taken as a warning and may represent a possible health risk. MSG has been used

widely as a food additive and consumed in large amount in restaurants of many countries. In this study we aimed to, firstly, investigate the genotoxic effect of MSG using *A. cepa* root tips assay. Secondly, inhibit that using oil extract of *Origanium majorana* L. and *Ruta chalepensis* traditional medical plants. The genotoxic potential of MSG flavour enhancer is clearly proved by results that demonstrated in present study. Many food additives including MSG have been reported to be genotoxic [14,27,31,32]. In both short (2 hr) and long term (24 hr) exposure to MSG lead to a wide range of chromosomal abnormalities (Fig 1). Sticky and clumbing chromosomes were observed and highly frequented which are metaphase's abnormalities. Previous aberrations suggested that MSG may inhibit spindle fiber correct formation or may influence the mechanism of chromosome separation in anaphase [33,34]. Disturbance in chromosomal proteins adhesion or DNA and RNA metabolism may cause sticky chromosome. It has been reported that stickiness reflects a highly toxic that probably leads to cell death [31,35]. Moreover, many abnormalities such as lagging chromosomes, bridges, fragmentations, granular prophase and disturbance chromosomes were observed indicating a direct effect of MSG on the chromosomes. Most of indicated abnormalities have previously been reported in different model plants including *A. cepa* [13,14,27,31,32,].

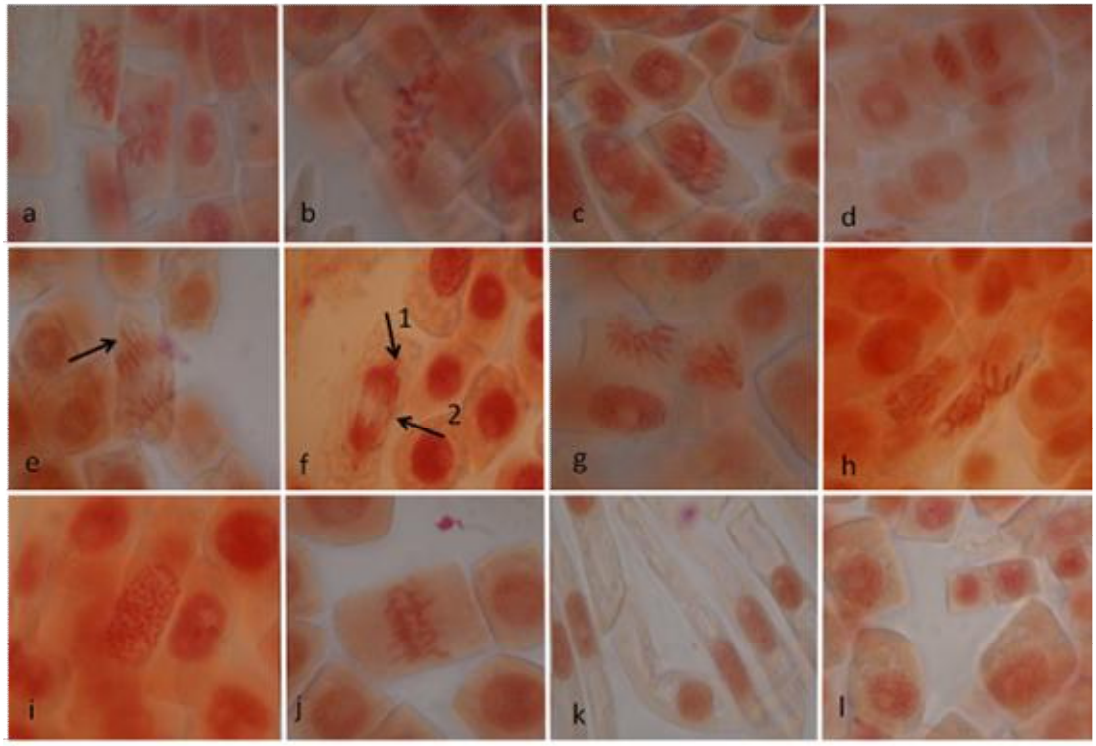

Fig. 1. Chromosomal aberrations induced by MSG in *Allium cepa* root tips

a) Sticky metaphase at 1g/L,24hr, b) Thickness chromosome metaphase at 3g/L,2hr, c) Clumping metaphase chromosomes at 3g/L,6hr, d) Sticky anaphase at 1g/L,6hr, e) lagging chromosome at 3g/L,2hr, f) 1. Lagging chromosome and 2. Bridge at 5g/L,2hr, g and h) Centromic attraction at anaphase at 3g/L,24hr and 5g/L,6hr respectively, i) Granular prophase at 7g/L,2hr, j) Disturbance metaphase 5g/L,2hr, k) Elongated cells at 5g/L,24hr, and 7g/L,6hr, l) Enlargement cells at 7g/L,2hr

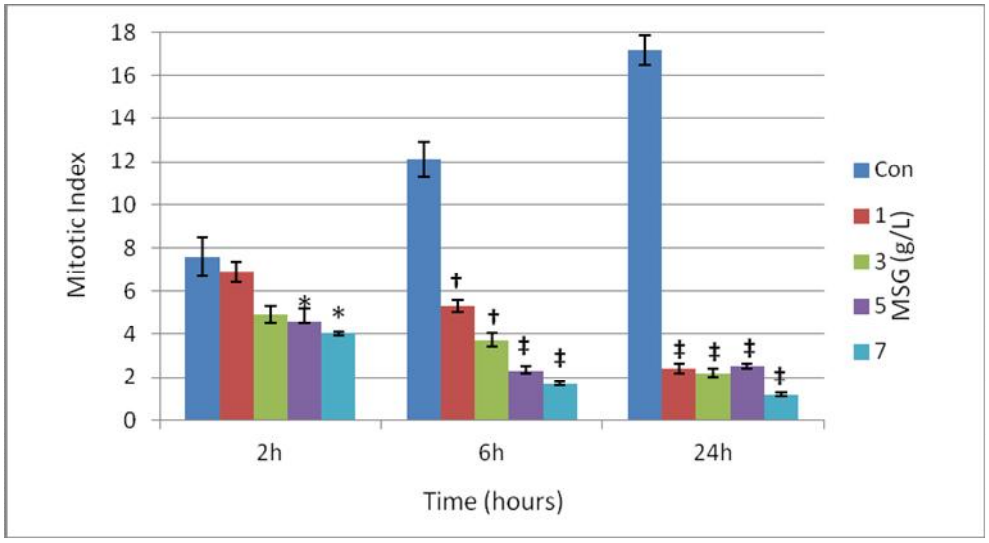

Fig. 2. Mitotic index in *Allium cepa* root tips treated with increasing MSG concentrations at 2, 6 and 24 hours

Values plotted are taken from 4 readings of four bulbs. Error bars represent the standard errors. Values were statistically tested using unpaired t-tests. Symbols represent differences that were significant (indicates: P>0.05, † indicates: P>0.01 and ‡ indicates: P>0.001)*

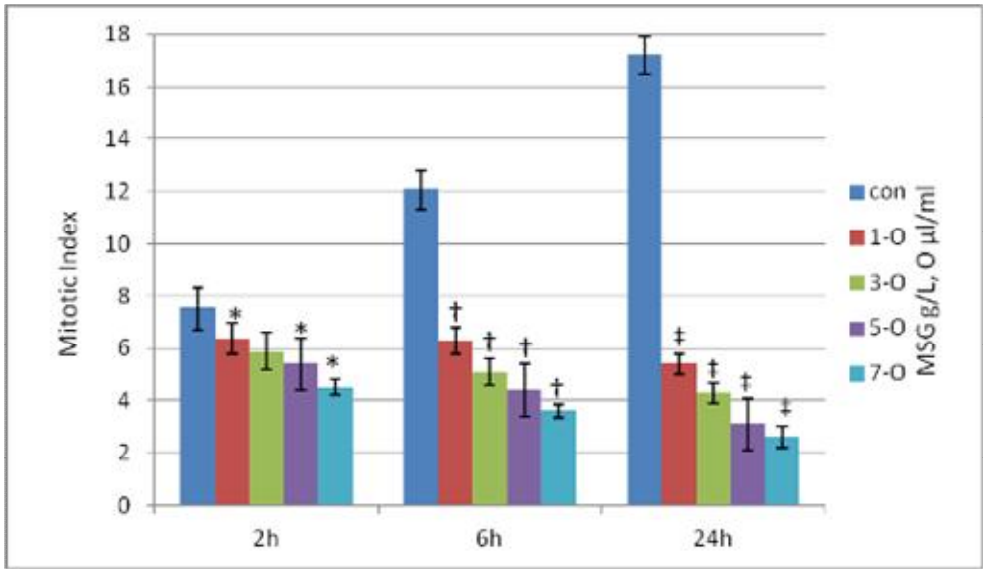

Fig 3. Mitotic index in *Allium cepa* root tips treated with increasing MSG concentrations and oil extracted from *Origanium majorana* at 2, 6 and 24 hours

Values plotted are taken from 4 readings of four bulbs. Error bars represent the standard errors. Values were statistically tested using unpaired t-tests. Symbols represent differences that were significant (indicates: P>0.05, † indicates: P>0.01 and ‡ indicates: P>0.001)*

The observed anaphase abnormalities might be resulting from the inhibition of microtubules activity in spindle fiber that affecting spindle formation [36]. Reduction in MI was gradually noticed with higher concentrations of MSG and longer incubation time (Table 1) demonstrating that both concentration and time are evidently affecting the MI. These results suggested that MSG may has a mitodepressive effect by arresting cells in one or more of mitotic phases such as arrest in pre-prophase or prophase [13,37]. Also remaining cells in G2 phase in cell

cycle where cells prevented from entering mitotic stage [38] or the inhibition of DNA synthesis could be a reason of lowering MI [39]. In addition to that, longer S phase or damage and weakness in G1phase resulting in less entering cells to S phase subsequently to mitotic stage [40]. Chauhan and Sundararaman (1990) have reported that chemicals could reduce the mitotic index by affecting DNA, RNA or protein synthesis [41]. The high intake of MSG may affect the cell division and growth, chromosome behaviour and may lead to mutations and subsequent to cancer. Our positive results in *Allium cepa* root tips may reflect a potential biological hazard that basically cause a wide range of abnormalities should be taken as mutagenic potential of MSG food additive.

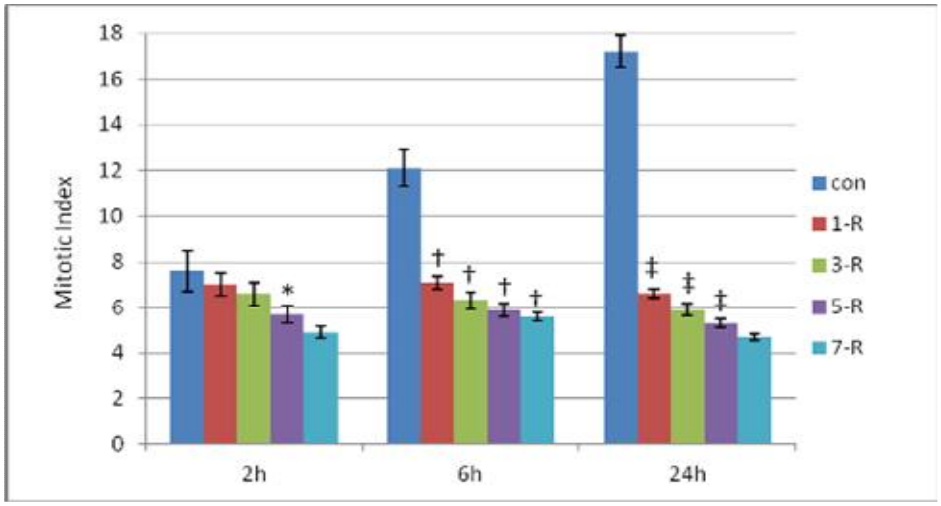

Fig 4. Mitotic index in *Allium cepa* root tips treated with increasing MSG concentrations and oil extracted from *Ruta chalepensis* at 2, 6 and 24 hours

Values plotted are taken from 4 readings of four bulbs. Error bars represent the standard errors. Values were statistically tested using unpaired t-tests. Symbols represent differences that were significant (indicates: P>0.05, † indicates: P>0.01 and ‡ indicates: P>0.001)*

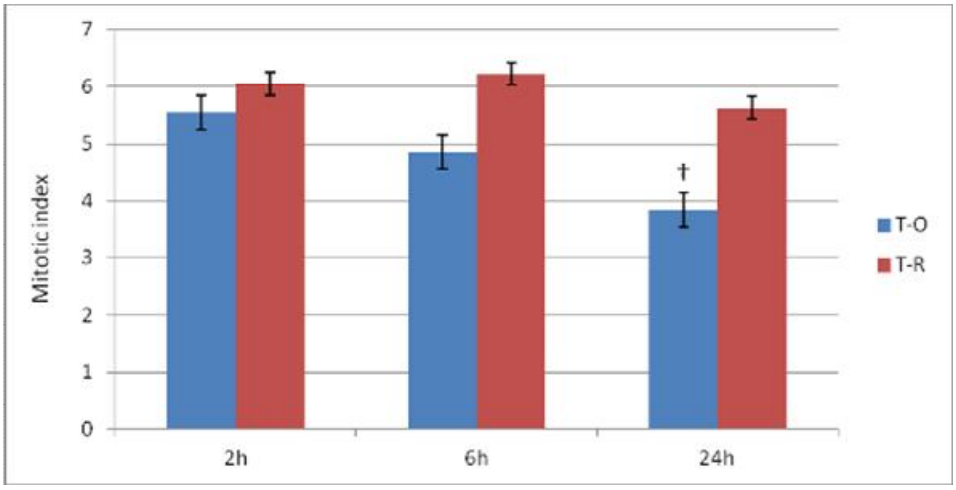

Fig. 5. A comparison between the mean of mitotic index of root tips treated with MSG and both oils extracted from *Origanium majorana* and *Ruta chalepensis* at 2, 6 and 24 hours

Values plotted are taken from 4 readings of four bulbs. Error bars represent the standard errors. Values were statistically tested using unpaired t-tests. Symbols represent differences that were significant (indicates: P>0.05 and † indicates: P>0.01)*

Table 1. Number of dividing cells and mitotic index in *Allium cepa* root tips exposed to different concentrations of MSG

Duration of collection	Cons of MSG (g/L)	No and sum of dividing cells in mitotic stage					
		Prophase	Meta phase	Ana phase	Telo phase	sum	Mitotic index %
2h	0	60	5	5	6		7.6
	1	18	12	21	18		6.9
	3	11	10	15	13		4.9
	5	15	7	12	12		4.6
	7	20	2	8	10		4
6h	0	85	10	14	12		12.1
	1	20	9	13	11		5.3
	3	25	3	5	4		3.7
	5	13	3	2	5		2.3
	7	10	2	2	3		1.7
24h	0	120	15	20	17		17.2
	1	15	2	3	4		2.4
	3	13	2	3	4		2.2
	5	16	4	3	2		2.5
	7	8	2	1	1		1.2

Table 2. Number of dividing cells and mitotic index in *Allium cepa* roots exposed to different concentrations of MSG and oil extracted from *Origanium majorana*

Duration of collection	MSG(g/L), O (1,25 µl/ml)	No and sum of dividing cells in mitotic stage					
		Prophase	Meta phase	Ana phase	Telo phase	sum	Mitotic index %
2h	0 60	5	5	6		7.6	
	1,O 40	10	8	6		6.4	
	3,O 24	14	11	10		5.9	
	5,O 29	5	9	11		5.4	
	7,O 15	13	9	8		4.5	
6h	0 85	10	14	12		12.1	
	1,O 44	5	9	5		6.3	
	3,O 35	4	3	9		5.1	
	5,O 22	5	7	10		4.4	
	7,O 30	2	3	1		3.6	
24h	0 120	15	20	17		17.2	
	1,O 44	4	4	2		5.4	
	3,O 27	4	5	7		4.3	
	5,O 19	3	5	4		3.1	
	7,O 9	5	7	5		2.6	

The present study also evaluates the antimutagenic affect of *Origanium majorana* and *Ruta chalepensis* traditional plants. Less chromosomal aberrations were noticed in samples that treated with 1.25 µl of fixed oil of selected plants. Higher MIs were recorded at all point time tested 2, 6 and 24hr as indicated in Tables 2 and 3. The lowest and highest mitotic index values of MSG samples are 1.2 and 6.9% whereas MI values reached to 2.6, 6.4% and 4.7, 7% of *oregano* and *rue* treated roots respectively. Results indicated that both selected plants have the ability to increase the mitotic index reflecting their anti-mitodepression action against the MSG enhancer. The higher MI in oil-treatment roots probably enable us to classify both *O. majorana* and *R. chalepensis* firstly as antimutagenic factors acting outside the cell (desmutagenicity). Secondly as inside antimutagenic factors (antimutagenicity) according to the medical plant extractions classification [42]. The essential components of fixed oil might be able to block the DNA sensitive regions thus preventing the binding of MSG. Enhancing the DNA repair system to recover the damage that caused by MSG is also proposed.

Another possibility is inducing enzymes in some organs that able to eliminate the toxicity and assist the cell in DNA replication reducing DNA damage and mutations [43-45].

The antimutagenic potential of fixed oil of both *O. majorana* and *R. chalepensis* perhaps prevent the MSG or its metabolites from entering the cell or by forming harmless compounds following the reaction between oils and MSG. Moreover, oils may discontinue reactions that activate MSG's metabolite and thus prevent its formation. All above suggested mechanisms are lead to the same biological termination which is inhibition of the mutation and protecting cell from cancer [43-45]. The antimutagenic ability of both *oregano* and *rue* perhaps due to their content of antimutigen and antioxidant. The inhibitory effects detected in current study of chosen plant can be attributed with different studied plants such as chlorophyll and many phytochemicals including phenols, flavonoids, carotenoids and ascorbic acid [46]. The HPLC analysis of *oregano* extraction demonstrate that it contains many antioxidant compounds such as flavonone, flavone apigenin, eridictyol, dihydroflavonols, dihydroquercetin and dihydrokaempferol [17]. According to Quiroga et al. [47], c-terpinene, a-terpinene, p-cymene and thymol were the major compounds found in oregano essential oil, gallic acid and DPPH scavenging activity. It has been reported that rue contains various active compounds like flavonoids, coumarin, furoquinolines, volatile oils, undecanone and others [48]. Furthermore, it has proved that *Ruta* has pharmacological functions including anti-inflammatory, analgesic, antiandrogenic, antihyperglycemia, antihyperlipidemia, anti-gout, antimutagenic and anticancer activities, among others [49]. Rutin and quercetin are two of the most common flavonoids in *Ruta*, it has been reported that rutin exhibits multiple pharmacological activities including antibacterial, antimutagenic, antitumor, antiinflammatory, and more other [50,51]. The data presented in figure 5 illustrate the significant differences between of oregano and rue oils in recovering MIs. Higher significant differences (P<0.001) were obtained at 24 h and relatively less differences at 6hr (P<0.01) and then 2 hr incubation time. The observed differences could be partially explained by the levels of total phenolic compounds and total antioxidant in the investigated oils. Another reason could also be the variation in the family of each plant as a Thin Layer Chromatography analysis (TLC) revealed 7 and 11 bands for oregano and rue respectively (data not shown). The phenolic compounds and many flavonoids were reported to have the capacity to scavenge mutagens or free radicals [52,53]. The antimutagenic activity of some flavonoids was caused by radical scavenging effect [54]. According to [55] Ferguson, 1994 the antioxidants compounds are well known to have inhibitory effects on genotoxic actions of several known mutagens. Therefore, total antioxidant and phenol compounds may be responsible for the inhibitory effects in tested plants of the current study.

Table 3. Number of dividing cells and mitotic index in *Allium cepa* roots exposed to different concentrations of MSG and oil extracted from *Ruta chalepensis*

Duration of collection	MSG (g/L), R (1,25 µl/ml)		No and sum of dividing cells in mitotic stage					
			Prophase	Meta phase	Ana phase	Telo phase	sum	Mitotic index %
2h	0	60	5	5	6			7.6
	1,R	52	6	4	8			7
	3,R	39	7	11	19			6.6
	5,R	44	2	4	7			5.7
	7,R	31	3	4	11			4.9
6h	0	85	10	14	12			12.1
	1,R	34	12	10	15			6.3
	3,R	44	3	6	10			5.1
	5,R	46	3	2	8			4.4
	7,R	35	7	5	9			3.6
24h	0	120	15	20	17			17.2
	1,R	43	5	7	11			6.6
	3,R	35	4	11	9			5.9
	5,R	27	9	9	11			3.5
	7,R	33	4	6	5			4.7

5. CONCLUSION

Results presented in current study indicate that MSG has a potential genotoxic and mutagenic effects. MSG enhancer flavour clearly caused a wide range of chromosomal abnormalities and affecting MI in root tips and may lead to similar cytogenic effects in higher organisms. *Oregano* and *rue* oil extracts have a good antimutagenic effects on MSG by decreasing chromosomal abnormalities and increasing MI. Significantly *rue* was more effective than oregano, further investigations are in progress to validate the antimutagenic property of *rue* using *in vivo* different assay. Current medicinal plants can be considered as promising sources of natural antioxidants for medicinal and commercial uses.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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