DOI:10.53539/squjs.Splisspp45-55

Sultan Qaboos University Journal for Science

Journal page: www.squ.edu.om/index.php/squjs/index

Preventive Effects of Dill Oil on Potassium bromate-induced Oxidative DNA Damage on Garlic Root Tips

Hoda, A. Khatab*, Samia Eissa and Nagat, S. Elhaddad

Department of Botany, University of Omar Almukhtar, Al-Baida, Libya.

*Email address: hoda.khatab@omu.edu.ly

ARTICLE HISTORY

Received 11 March 2024
Received revised 27 June 2024
Accepted 29 June 2024

ABSTRACT: In this study, the potential antimutagenic/cytoprotective effect of the oil extract (2.5µg/ml) from *Anethumgraveolens* L. (Dill) on oxidative DNA damage was tested. The experiments were carried out to inhibit mutagenicity induced by potassium bromate ($KBrO_3$-9g/l) in *Allium sativum* assay using mitotic activities and nuclear DNA contents. Results showed a clear reduction in radical length in all $KBrO_3$-treated groups compared to control plants. Mitotic activity, measured by mitotic index (MI) and mitotic stages were significantly decreased in meristematic cells after $KBrO_3$ application. The results showed a decrease in the mitotic index to reach about 0.8% at 24 hours compared to 23.9% in untreated plants. The analysis of potential cyto- and geno protective dill extract on nuclear DNA contents showed a statistically significant increase in the nuclear DNA content compared to $KBrO_3$-treated roots. Clearly, dill oil showed high performance in protecting the mitotic cells against mutagenic $KBrO_3$ effects by increasing the MI and decreasing the abnormal chromosomes. Also, the percentage of damage reduction was observed in the pre- (28.9, 57.9 and 46.4 %), simultaneous (53.0, 70.6 and 35.7 %), and post- (37.3, 61.8 and 50 %) treatment. The results of the qualitative study showed that the dill extracts were rich in chemical compounds belonging to several phytochemical classes. GC-MS analysis detected 31 chemical compounds with different concentrations. The predominant ones were octadecanoic acid (31.75%) and 2-pentanone, 4-hydroxy-4-methyl (16.77). The findings of these experiments will help to understand and uncover the mechanism of action of dill oil against the mutagenic effects of $KBrO_3$ (i.e whether they have a prophylactic or treatment activity).

Keywords: Mitotic index; DNA content; $KBrO_3$; Antimutagenic; *Anethumgraveolens* L. (Dill).

التأثيرات الوقائية لزيت نبات الشبت على تلف الحمض النووي المؤكسد المستحدث ببرومات البوتاسيوم في جذور نبات الثوم

هدى قطب، نجاة الحداد و سامية عيسى

الملخص: في هذه الدراسة، تم اختبار التأثير الوقائي المحتمل المضاد للطفرات لمستخلص الزيت (2.5 ميكروغرام/مل) من *Anethum gravolens* L. (الشبت) على تلف الحمض النووي المؤكسد. أجريت التجارب لتثبيط الطفرات المحدثة ببرومات البوتاسيوم ($KBrO_3$) بتركيز9 جم/لتر باستخدام الأنشطة الانقسامية ومحتويات الحمض النووي. وقد أظهرت النتائج انخفاضا واضحا في الطول الجذري في جميع المجموعات المعالجة بـ $KBrO_3$ مقارنة بمجموعة التحكم. حيث انخفض معامل الانقسام الخلوي، الذي يقاس بمؤشر الانقسام ألفتيلي (MI) والمراحل الانقسامية، بشكل ملحوظ في الخلايا المرستيمية بعد التعرض $KBrO_3$، وأظهرت النتائج أن مؤشر الانقسام الفتيلي (MI) انخفض ليصل إلى حوالي 0.8 عند 24 ساعة مقارنة مع 23.9٪ في النباتات غير المعاملة. أظهر تحليل القدرة الخلوية والحماية الجينية لمستخلص الشبت على محتويات الحمض النووي وجود معنوية في محتوى الحمض النووي مقارنة بالجذور المعالجة بـ $KBrO_3$. من الواضح أن زيت الشبت أظهر أداءً عاليًا في حماية الخلايا الانقسامية ضد تأثيرات $KBrO_3$ المطفرة عن طريق زيادة MI وتقليل الكروموسومات غير الطبيعية كما لوحظت نسبة تقليل الضرر في المعاملة الوقائية (28.9، 57.9 و 46.4٪)

والمعاملة المتزامنة (53.0، 70.6 و 35.7٪) والعلاجية (37.3، 61.8 و 50٪). وأظهرت نتائج دراسة التركيب الكيميائي النباتي GC-MS أن مستخلصات الشبت غنية بالمركبات الكيميائية التي تنتمي إلى عدة فئات كيميائية نباتية. حيث بينت الدراسة وجود 31 مركبًا كيميائيًا بتركيزات مختلفة. وكانت أبرزها حمض الأوكتاديكانويك (31.75%) و2-بنتانون، 4-هيدروكسي-4-ميثيل (16.77). بحيث تساعد هذه التجارب على فهم وكشف آلية عمل زيت الشبت ضد التأثيرات الطفرية لـ $KBrO_3$ (أي ما إذا كان لها نشاط وقائي أو علاجي).

الكلمات المفتاحية: معامل الانقسام، محتوى الحمض النووي، برومات البوتاسيوم، مضاد الطفرة، نبات الشبت.

1. Introduction

For thousands of years, people have used food additives to enhance flavor and to improve the taste of food. This includes food colouring such as tartrazine and flour improver such as potassium bromate-$KBrO_3$). Food and Drug Administration (FDA) published a list of different food additives, including flavorings, colours, baking improvement [1]. However, the use of food additives has changed significantly over time [2] and has become increasingly common in all processed foods from the early 19th century [3]. Studies on the toxicological potential of some food additives have received a great deal of attention as people have expressed concern about the mutagenic and carcinogenic potential of food additives around the world [4]. Over the past century, the use of additives in food has increased significantly. More than 3,000 substances have been approved as food additives by national and international food regulators [2]. $KBrO_3$ is added into flour to improve the activity of gluten. The process aids in the strengthening and softening of the dough and imparts a pleasing white colour to the completed bread. When added in the range of 15-30 ppm, it chemically changes during baking to harmless products. Because potassium bromate is converted to potassium bromide, KBr during baking, the final product should ideally contain no potassium bromate, [5]. However, if the mixture contains a greater proportion of $KBrO_3$, any remaining $KBrO_3$ would be toxic to consume [6,7]. Despite its many benefits, the International Agency for Research on Cancer (IARC) categorized $KBrO_3$ as a possible carcinogenic compound (categorized in a group II-B), with renal tumor risks at high doses. Studies have shown that consumption of $KBrO_3$ is related to cancer in experimental animals [8]. As a result, numerous nations, including the United Kingdom, France, Nigeria, and Canada, have outlawed the use of $KBrO_3$ as a food additive [9,10]. According to [11], $KBrO_3$ is a strong oxidizing compound that causes cells to produce free radicals, known as reactive oxygen species (ROS). One of the main elements that encourages chemical carcinogenesis is ROS, which plays a significant role in the mechanism of bromate toxicity through cell damage [12,13]. Researcher reported that using high level of $KBrO_3$ generates high level of ROS, which can damage all major cellular structure [14]. The ROSs disturb DNA replication, inducing oxidative damage, and consequently, genetic and chromosomal mutation [15]. In Libyan bakeries, despite the ban on using potassium bromate fifteen years ago, it is still currently used. Now, the implementation of the ban is a serious issue in Libya and the authorities must take decisive and drastic measures to limit the use of this food poison [16]. Recent studies have shown that the antioxidant (AO) activity of numerous plant materials may have potential anti-$KBrO_3$ toxicity benefits [17,18]. Since then, there has been a rise in the search for more potent natural antioxidants, particularly those with a plant origin. Currently, the use of natural products in the pharmaceutical and food industries is becoming important. Manufacturers are looking for natural compounds that preserve food and promote perception of taste and aroma [19]. *Anethumgraveolens*L.commonly known as dill, is widely used in food and pharmaceutical industries. It has been widely used in traditional medicine, as well as in the culinary, cosmetic, and nutraceutical industries. Dill has wide ranging biological activities such as antioxidant, antimicrobial, anti-inflammatory, anticancer agent [20]. As a result, using it as natural antimutagens is one of the best strategies to reduce the harmful effects of $KBrO_3$ mutagens. From this point, two strategies are available to stop induced cellular mutagenesis. In the first, "desmutagens" render mutagens inactive so they cannot damage DNA. In the second, "bio-antimutagens" obstruct the mechanisms by which cells mend damage to DNA. These inhibitors of induced mutations are demonstrated in action, and their potential to lessen genetic damage in humans is examined [21]. There are now two methods available to halt induced cellular mutagenesis. Firstly, "desmutagens" make mutagens dormant so they can't harm DNA. In the second, "bio-antimutagens" block the processes that cells use to repair DNA damage. These induced mutation inhibitors are shown to function, and their potential to reduce genetic harm in human is investigated.

The aim of this study was to examine the potential antimutagenic and antigenotoxic properties of *Anethumgraveolens* L. plant extracts against $KBrO_3$ effect on mitotic behaviours and the nuclear DNA content. In this context, we also aimed to extend our knowledge to determine and identify compositions of dill fixed oil using Gas chromatography-mass spectroscopy (GC-MS).

2. Materials and Methods

2.1 Potassium bromate

Potassium bromate (Sigma Aldrich) working solutions were prepared by dissolving 9 grams of potassium bromate in distilled water (ddH_2O) (9% concentration) and stored at 4 °C [22].

2.2 Plant extraction

About100 g of fresh dill leaves were washed carefully, dried and then soaked in acetone for 48 hrs. The solvent was removed by rotary evaporator yielding about 50 ml of dill extract. Dill oil was used at dose of 2.5 µg/ml for 2, 6 and 24 hrs, which represents the overall experimental durations [23].

2.3 Preparation of root tips for microscopic observation

Chromosomal mitotic changes were defined and calculated using root-squash method [24]. The mitotic index was counted after 2, 6, 24 hrs treatment with $KBrO_3$. Total divided and non-divided cells were calculated as percentage of each phase in relation to control. Total of dividing cells as well as Total Chromosomal Aberrations TCA in 1000 counted cells were recorded and calculated using the following formula:

$$\textbf{MI (\%)} = \frac{\textbf{Av. No. o fdividing cells}}{\textbf{Total No. of cells counted}} \textbf{ X 100}$$

Phases index (PI) was recorded and calculated using the following formula:

$$\textbf{PI (\%)} = \frac{\textbf{No. of cell in particular phase}}{\textbf{Total No.of dividing cells}} \textbf{ X 100}$$

2.4 Evaluation of antimutagenic effect

The activity of the dill oil in suppressing the mutagenic action of $KBrO_3$ on the mitotic activity of *Alliumsativum* was tested by adding 2.5µg /ml of dill oil to the solution of the $KBrO_3$ (before, with and after) for 2, 6 and 24 hrs.

Both MI and TCA at each treatment were recorded and calculated. Percentage reduction of chromosomal aberrations was calculated as stated below.

$$\% \text{ R of CA} = \frac{A-C}{A-B} \text{ X } 100$$

where '% R' represents the % reduction of chromosomal aberration (CA); 'A' is the frequency of CA induced by only $KBrO_3$; 'B' is the frequency of CA induced by the combination of dill oil and $KBrO_3$ and 'C' is the frequency of CA induced in control.

2.5 Determination of Nuclear DNA Content

Nuclear DNA content of garlic meristematic cells was measured based on the resulting amount fuchsinstained nuclei [25].The percentage of Nuclear DNA reduction was calculated by the following formulae:

$$2 - \text{DNA content of treated nuclei(pg)} = \frac{\text{measured of treated samples}}{\text{standred prophase means}}$$

2.6 Photochemical Analysis

The phytochemical components of the dill extracts were identified using a qualitative investigation by the GC-MS (Science Way Company-Al Waha, Cairo Egypt). TG-5MS column has a film thickness of 30 m x 0.25 mm x 0.25 µm. The temperature within the column oven was maintained at 35 °C. After that, it was raised to 200 °C for three minutes at a rate of 3 °C per minute. Eventually, the temperature reached 280 °C, a rise of 3 °C/min, and stayed there for ten minutes. Temperatures were maintained at 260 °C for the injector and 250 °C for the MS transfer line. One mL of diluted samples was automatically injected using an autosampler AS1300 connected to a GC in split mode, with a three-minute solvent delay.

2.7 Statistical analysis

Data were combined from four independent biological replicates, and subjected to two way analysis of variance (ANOVA) to analyze the effects of $KBrO_3$ and exposure time on mitotic index and DNA content. The Least Significant Difference (LSD) test was used for the mean separation with significant differences claimed at the different significance levels of 0.05 using CoStat statistical package program (CoStat software). All data were expressed on mean + SE (standard error of the mean).

3. Results

3.1. The genoprotective effect of dill oil on mitotic process

Mitotic and phases indices exhibited a significant decrease when treated with $KBrO_3$ as can be seen from table 1 and figure 1. However, the damages induced by $KBrO_3$ were reverted by th e protective effect of dill oil for all the tested hour periods. For all incubation times, the best effect was recorded following the treatment for 6 hrs reaching 10.4% compared with 7% and 6.4% recorded at 2 and 24 hrs, respectively. Thus, the therapeutic action of dill oil leads to a clear increase in the number and percentage of diving cells.

Our results showed that, even in the presence of $KBrO_3$, dill oil continued to suppress the $KBrO_3$-induced damage by increasing dividing cell. A significant raise of prophase was recorded after 6 hrs incubation with the mixture of dill oil, where the prophase frequency was 265 compared with 12 in $KBrO_3$-treated root. Similarly, there was a marked change in the percentages of recovered metaphase stages with approximately 5-folds was recorded compared to $KBrO_3$ groups.

It was found that dill oil stimulates the increase in MI fat all tested exposure times after treatment with combination of dill oil and $KBrO_3$ (Table 1, Figure 1). The mitotic index recovers faster at 2 and 6 hrs when compared with 24 hrs duration time showing that the maximum value 4.9% at 6 hrs, while minimum mitotic value of 0.8% was recorded at 24 hrs compared to 0.8% at the respective does and duration time of $KBrO_3$ group. Similarly, the therapeutic effect of dill oil increased the percentage of prophase and metaphase when compared to $KBrO_3$-treated roots, after all exposure time. From table1 it can be seen that the dill oil displayed remarkable therapeutic activity against $KBrO_3$, MIs increased to 1, 2.8 and 2% compared with 0.9, 0.8 and 0.4% after 2, 6 and 24 hrs respectively.

Table 1. Mitotic phases index in *A. sativum* meristematic cells after treatment with dill oil against $KBrO_3$ at different exposure times

Treatments (g/l)	Time (hours)	interphase±SE	Met%	Ana %	Telo%	IP%	IM%	IA%	IT%
control	2	2596±10.23	26±0.25	19.3±2.44	3.3±1.1	88.1	6	4.7	0.8
	6	2460±7.9	28±0.2	17±0.53	3±0.25	91.2	5.2	3.1	0.6
	24	2288±5.23	42±1.11	30±2.02	8.3±2.2	88.8	5.9	4.2	1.2
dill oil	2	2501±5.21	42±0.93	12±0.63	7±1.0	87.2	8.8	2.5	1.5
	6	2386±3.85	39±2.03	20±0.92	11±1.4	88.3	6.6	3.3	1.9
	24	2277±6.21	79±0.87	26±1.26	18±1.6	83.1	10.9	3.5	2.5
$KBrO_3$	2	966.0±6.3	7±0.25	2±0.44	2±0.23	38.9	38.9	11.1	11.1
	6	993.0±4.76	10±0.34	2±0.03	2±0.41	46.2	38.5	7.7	7.7
	24	899±10.23	1±0.13	2±0.21	1±0.20	42.9	14.3	28.6	14.3
O→Pb	2	2790±10.23**	28±0.22	1±10.23	0	86.4*	13.2	0.5	0
	6	2687±4.9**	48±0.23	1±10.23	0	84.3*	15.4	0.3	0
	24	2934±11.20**	23±0.43	2±10.23	0	87.6*	11.4	1.0	0
Pb+O	2	2901±3.29	7±0.2	1±0.0	0	92.0*	7.0	1	0
	6	2851±10.23**	14±0.22	2±0.15	0	89.6*	9.1	1.3	0
	24	2975±10.23**	1±10.23	2±0.0	1	85.9	3.5	7.1	3.5
Pb→O	2	2942±5.53**	6.0±1.2	3±0.20	2±0.30	73.8	14.3	7.1	4.8
	6	2915±8.63**	12±0.17	3±0.0	1±0.13	82.7	13.0	3.2	1.1
	24	260±11.45	3±0.03	1±0.11	2±0.28	66.7	16.7	5.6	11.1

SE = standard error ; IP = index of prophase ; IM = index of metaphase ; IA = index of anaphase ; IT = index of telophase; O→ $KBrO_3$= protective ; $KBrO_3$+O=parallel; $KBrO_3$→O = therapeutic; (*) significantly different from control at p <0.05

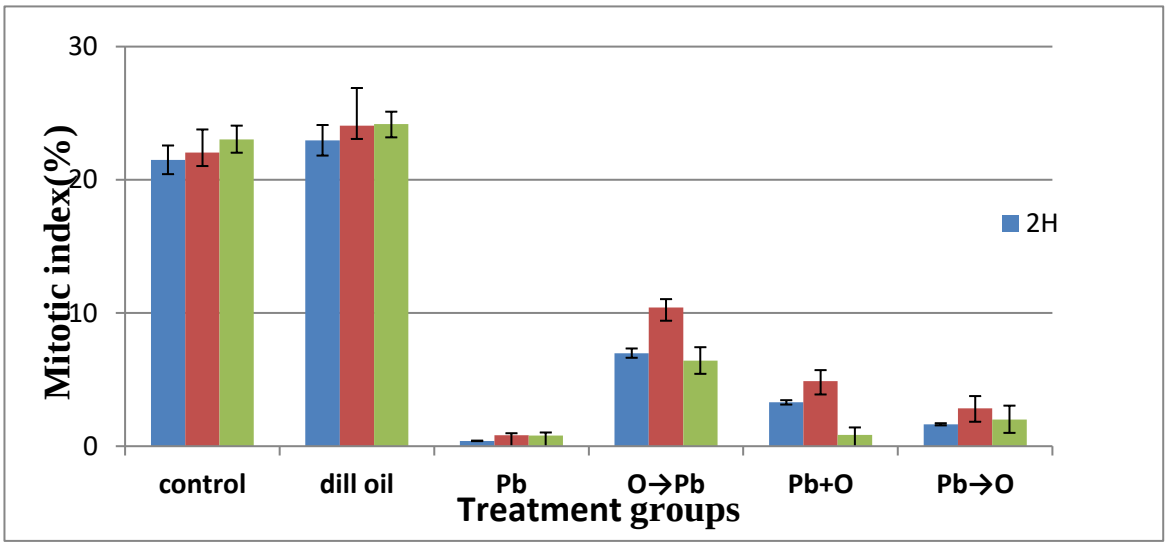

Figure 1. Mitotic index in *A. sativum* root tips treated of $KBrO_3$ T4 concentration and dill oil extracted from *A.graveolens*L. at 2, 6 and 24 hrs. The statistical analysis of the results was carried out using the *two-way ANOVA*and mean values and standard errors were calculated from three independent replicate. Different letters indicate significant differences(p<0.05). Potassium bromate (Pb),protective (o→Pb), parallel (Pb+o),therapeutic (Pb→o).

3.2 The antimutagenic effect of dill oil on total chromosomal aberrations (TCAs):

Compared to the control group, $KBrO_3$ induced a clear rise in chromosomal abnormalities (CA) (Table 2). Binucleate interphase(BC) and micronuclei(MN) were the most abundant abnormality observed in the present study.The other abnormalities included, sticky chromosomes(S), C-metaphase, lagging chromosomes anaphase(L), telophase bridges, multipolar anaphase and telophase (Figure 2). TCA was extremely reduced by the protective effect of dill oil. The recorded value after 2, 6 and 24 hrs was 12.75,17.26 and 14%. These values fell to 9.06, 7.27 and 7.5% after 2, 6 and 24 hrs, respectively. The parallel effect of dill oil on reduction of CA induced by $KBrO_3$ was observed from 12.75 to 5.99% (at 2 hrs after treatment), 17.26to 5.08% (6 hrs after) and 14 to 9% (24 hrs after treatment), respectively (Table 2). A notable decline in the TCAs (28.9, 57.9 and 46.4% when applying before $KBrO_3$), (53, 70.6 and 35.7% when mixed at the same time with $KBrO_3$) and (37.3, 61.8 and 50% when treated after $KBrO_3$) (Figure 3). In general, a significant changes in the meristematic tissue after the treatment with 2.5µg/ml of dill

oil, resulted in reduced mitosis damage induced by $KBrO_3$ as indicated by clear mitotic figures. These treatments ameliorated also the cell division alternations of the meristematic tissue lead to the cell structure of meristematic tissues was integrated (Figure 4).

Table 2. Reduction in total number, types and means of chromosomal abnormalities in *A. sativum*meristematic cells after treatment with different mixture of $KBrO_3$ and dill oil at different exposure times.

Treats (g\l)	Duration time(h)	CM%	MN%	S%	L%	F%	BN%	TCA%	R of TCA%
Con.	2	0	0	0	0	-	0	0	-
	6	0	0	0	0	-	0	0	-
	24	0	0	0.33	0	-	0	0.33	-
Dill oil	2	0	0	0	0.11	-	0	0.11	-
	6	0	0	0	0	-	0	0	-
	24	0	0	0	0	-	0	0	-
Pb	2	2	5	0	2	1.75	2	12.75	-
	6	2.6	8	0.11	0.45	1.5	4.6	17.26	-
	24	0	8	0	0	0	6	14	-
O→Pb	2	0	3	2	1	1	2.06	9.06	28.9
	6	1.23	2	0.36	1	0.17	2.51	7.27	57.9
	24	1.67	2.5	0	0	1	2.33	7.5	46.4
Pb+O	2	1.48	2	0	0.51	0	2	5.99	53.0
	6	0.45	2.96	0	0	0	1.67	5.08	70.6
	24	1	3	1	0	2	2	9	35.7
Pb→O	2	1	4	0	0	0	3	8	37.3
	6	0.77	3	0.48	0	0.35	2	6.6	61.8
	24	2	2	0	0	0	3	7	50

Con. = control; CM = C-mitosis; MN = micronucleus; S = sticky chromosomes; L = laggards chromosomes; F = ragments; BN = binucleated cells; TCA = total chromosomal aberrations; R= Reduction of TCA.

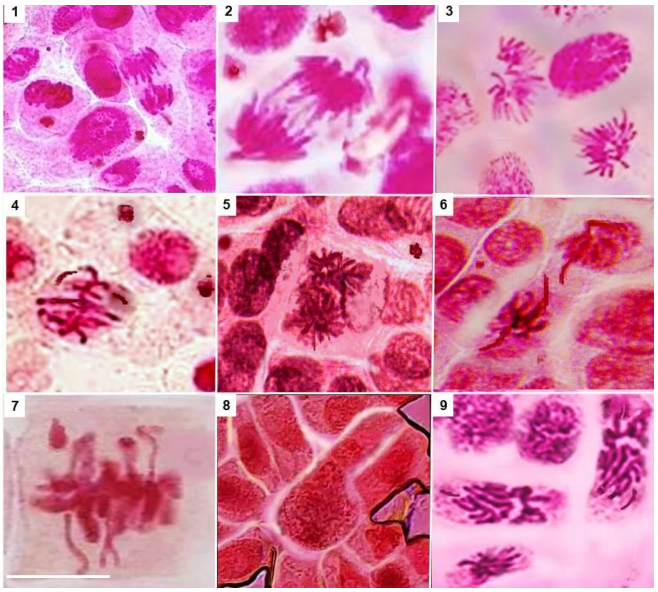

Figure 2. Different Chromosomal Aberrations induced by $KBrO_3$ in mitotic stages of root tip of *A. sativum* L. 1) anaphase with laggard (black arrow head), 2) multiple bridges at anaphase, 3)Star anaphase, 4)metaphase c-banding, 5), star-like morphology to telophase nuclei separated by cytokinesis 6) Anaphase with fragments, 7) Clumped chromosome with micronuclei, 8) Nuclear buds, 9) anaphase with unequal segregation. All stages observed with a 40× objective lens; Scale bar equals 50 µm**.**

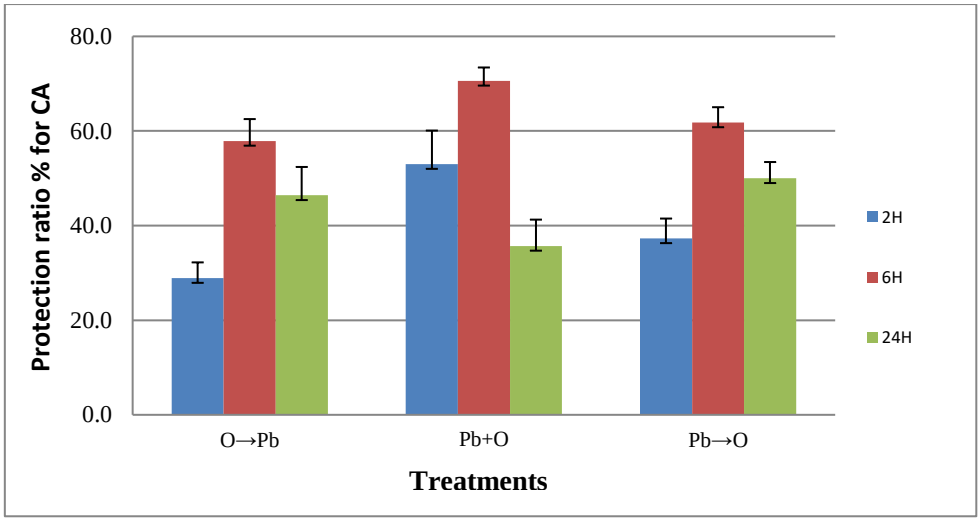

Figure 3. A comparison between the mean of the protection ratios provided by dill oil when given before, with and after $KBrO_3$ treatment at 2, 6, and 24 hrs. CA: chromosomal aberrations. The statistical analysis of the results was carried out using the *two-way ANOVA*and mean values and standard errors were calculated from three independent replicate. Different letters indicate significant differences($p< 0.05$) protective (o→Pb), parallel (Pb+o) ,therapeutic (Pb→o).

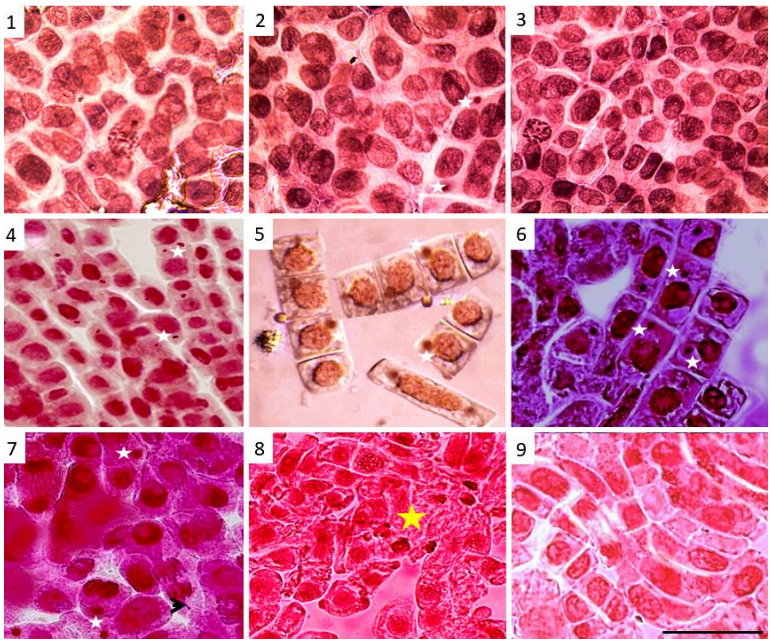

Figure 4. Meristematic cells of *A. sativum*showing resurgence to normalcy after modulatory treatment with dill oil as therapy against $KBrO_3$ induced inhibition.(1-9) shows normal mitotic division appearance except for moderate small degeneration (marked with yellow stars).This improvement was time-dependent since the dill oil was reduced the damaged cells. Micronuclei marked with white stars. All stages observed with a 40× objective lens; Scale bar equals 50 µm**.**

3.3 Dill oil impacts on nuclear DNA repair activity

The results from the current experiment compared with control and $KBrO_3$ group are given in Table 3.When dill oil and $KBrO_3$ were co-incubated for 2, 6, and 24 hours, the dill oil demonstrated significant and moderate genotoxic repair effects. The total mild inhibition rate values for all treatments were greater than the $KBrO_3$ treatment values.The best effect was recorded following the protective dose showing DNA content of 1.86, 1.72, 1.71 % for incubation times of 2, 6, and 24 hrs, respectively (Table 3). The action of dill oil against DNA damage induced by $KBrO_3$ was clearly noticeable in the parallel combination. Showing remarkable DNA recovery after 6 hrs incubation with 1.69 compared with 1.43 in $KBrO_3$-treated cells. In addition, dill oil used as therapeutic resulted in a clear enhancement in nuclear DNA content. This values were

increased to 1.53, 1.6 and 1.59% after 2,6 and 24 hrs incubation times, respectively. Our results highlight the antimutagenic potency of dill oil in DNA protection from mutagens.

3.4 Phytochemical analysis of dill oil with GC-MS

The obtained GC-MS chromatogram of the dill fixed oil is shown in figure 5. More than 10 peaks were obtained, indicating the presence of many important phytochemical constituents. Table 4 shows the probability of some of the identified compounds present in the dill oil from the CG-MS analysis. Detailed phytochemical assessment revealed that the fixed dill oil was mainly composed of octadecanoic acid and tris(2,4-di-tert-butylphenyl) phosphate(31.75 for each), 2-hydroxy-1- (hydroxymethyl) ethylester (16.77%), followed by 2,4-Diphenylimidazole, 2H-1-BENZOPYRAN, 5,7-DIMETHOXY-2,2-DIMET HYL and Coumarin (9.27%), Oxirane(7.66%), 3,3-Diethoxy-1-propyne and Dikegulac acid (4.87%), Tris(2,4-di-tert-butylphenyl) phosphate (2.55), 1,2,4,5-Tetrazine(2.26), CIS-isoapiole (1.79), 3Furanmethanol, à-(3,4-dimethoxyphenyl) tetrahydro-3-hydroxy-4-veratryl (1.28),1,2-Benzenedicarboxylic.

Table 3. Image analysis of the DNA content of the root tips nuclei of *A.sativum.*

Treatment	duration time(h)	No.	Mean(pg) ± SE	C-value
$KBrO_3$ (9g/l)	Prophases	60	85.19 ±6.32[a]	2
	2h	63	64.05 ±10.2[c]	1.51
	6h	65	58.10 ±13.5[c]	1.43
	24h	67	54.28 ±17.35[c]	1.32
O→$KBrO_3$	Prophases	63	83.02 ±4.01[a]	2
	2h	59	78.90 ±10.34[b]	1.86
	6h	60	70.15 ±8.37[b]	1.72
	24h	61	59.99 ±10.36[c]	1.71
$KBrO_3$+O	Prophases	63	83.65 ± 4.11[a]	2
	2h	67	68.96 ±9.37[b]	1.62
	6h	63	68.85 ±8.57[b]	1.69
	24h	61	65.06 ±6.51 [b]	1.58
$KBrO_3$→O	Prophases	63	82.9 ± 4.12[a]	2
	2h	60	65.04 ±12.3[b]	1.53
	6h	62	64.99 ±10.3[b]	1.6
	24h	60	65.07 ±6.16 [b]	1.59

SE=standard error, No= number of nuclei, C= DNA content,pg = picogram,No.=number of examined cells; $KBrO_3$= potassium bromate; O→ $KBrO_3$= protective; $KBrO_3$+O=parallel; $KBrO_3$→O= therapeutic. Different letters indicate significant differences($p < 0.05$).

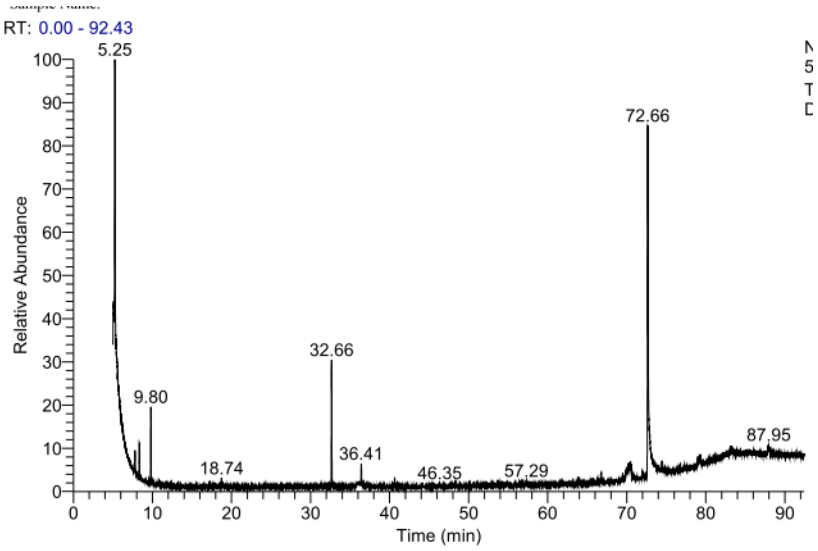

Figure 5. GC-MS total ion chromatogram showing the presence of bioactive compounds of dill fixed oil.

Table 4. The identified chemical compounds of *A. graveolens* (dill) leaves by GC-MS analysis.

No.	RT	Name of compound	formula	MW	Area%
1	5.08	Oxirane, [(hexyloxy)methyl	C7H16O	116	7.66
2	5.25	2-PENTANONE, 4-HYDROXY-4-METHYL	C6H12O2	116	16.77
3	5.53	Oxirane	C9H18O2	158	0.46
5	7.07	2,5-Hexanediol	C6H14O2	118	0.21
6	7.70	4,5,5-D3-TRANS-3,4-DIHYDR OXY-CYCLOPENTENE	C5H5D3O2	103	0.45
7	7.79	3Furanmethanol, à-(3,4-dimethoxyphenyl) tetrahydro-3-hydroxy-4-veratryl	C5H5D3O2	103	1.28
8	8.34	1,2,4,5-Tetrazine	C6H16N4	144	2.26
9	9.55	Hexane, 1,1'-oxybis	C12H26O	186	0.20
10	9.80	3,3-Diethoxy-1-propyne	C7H12O2	128	4. 87
11	9.80	Dikegulac acid	C12H18O7	274	4.87
12	18.74	Dill ether	C10H16O	152	0.76
13	18.74	Camphor	C10H16O	152.2	0.76
14	32.66	2,4-Diphenylimidazole	C15H12N2	220	9.27
15	32.66	2H-1-BENZOPYRAN, 5,7-DIMETHOXY-2,2-DIMET HYL	C13H16O3	220	9.27
16	32.66	Coumarin	C14H20O2	220	9.27
17	35.98	Apiol	C12H14O4	222	0.44
18	36.41	CIS-ISOAPIOLE	C12H14O4	222	1.79
19	40.64	Erucic acid	22H42O2	338	0.72
20	40.64	cis-13-Eicosenoic acid	C20H38O2	310	0.72
21	57.29	Octadecanoic acid	C26H54	366	0.42
22	63.87	DOTRIACONTANE	C32H66	450	0.66
23	66.76	1,2-BENZENEDICARBOXYLIC	C24H38O4	390	1.03
24	69.49	HAHNFETT	C27H30O16	610	0.72
25	69.92	Prostaglandin A1-biotin	C45H58O4	622	0.48
26	69.98	Prostaglandin A1-biotin	C45H58O4	622	0.45
27	70.27	Prostaglandin A1-biotin	C35H60N4	664	1.29
28	70.47	Tris(2,4-di-tert-butylphenyl) phosphate	C42H63O4P	662	2.55
29	72.66	13-Docosenamide, (Z)	C22H43NO	337	31.75
30	72.66	Bis(cis-13-docosenamido)methane	C45H86NO	686	31.75
31	76.11	STEARIC ACID	C39H78O3	594	0.69

4. Discussion

The human body is continuously exposed to high levels of ROS, which requires strong AO defense mechanisms to protect and preserve its biological functions [17]. This is important since redox disturbances are known to negatively impact body systems through the generation of ROS, which can modify proteins, lipids, and DNA. [26]stated that oxidative stress related *in vivo* mutagenicity and genotoxicity exerted by $KBrO_3$ and its mutagenic effects were associated with genetic material damage. This includes different mutations, base changes, chromosomal aberrations, and altering gene expression, leading to cancer and also the production of ROS.

Thus, it is important to identify natural AO sources with strong ability to prevent the detrimental effect of different toxicants-induced ROS and associated with genetic damage. Researchers have strongly recommended using medicinal plants as source of repairing agents in the prevention and treatment of genotoxic effects mutagene therapeutic agents [27-29]. To the best of our knowledge, the determination of the antimutagenic effects of *A. graveolens* (dill) extracts combined with any known mutagenic substance has not been studied. For this reason, we initiated this study to investigate whether dill fixed oil could abrogate the $KBrO_3$-induced oxidative damage to mitotic cell through assessment of DNA content, mitotic index and total mitotic aberrations index (TAI).

The results in the evaluation of the antimutagenic effect of dill oil suggest that the effects of $KBrO_3$ cannot be inhibited, but when dill oil was administered as an adjuvant, not only was the MIs and DNA recovered faster but also the mutagenic effect of $KBrO_3$ modulates was, which reflected in the frequency of total chromosomal aberration (TCA). In the same context, [23] have shown that *A.graveolens*extract exhibits cytotoxic/anticancer activities against mouse leukemia L1210 cells. Our findings also agree with previously published studies [30] which state that dill oil was able to inhibit micronuclei formation induced by benzo(a) pyrene in mouse bone marrow. It also illustrates the protective effects of *A. graveolens* L. against esophageal damage by increasing the levels of cyclooxygenase (COX-2) in effected tissue. MI is a trusted assay that detects cytogenotoxic effects of chemical substances mutagen on the cells, as the increases and decreases of MIs may suggest the degree of cytotoxic inflicted on normal process of cell development. In this study, higher MIs induced by dill oil compared with MIs of individual $KBrO_3$. Our results suggest that dill oil had a protective effect against $KBrO_3$ damage and this is in agreement with the study reported earlier [30]. The genoprotective effect of dill plants against the toxic side effects of $KBrO_3$ increased since the beginning of the treatment period, affecting the rate and inefficiency of normal cell division, A significant increase in the ability of the dill oil to regulate mitotic division was observed, indicating that dill plants have genoprotective properties. Consequently, the AO activities of dill oil might contribute to its antigenotoxicity and anticancer mechanisms [31]. An early study reported that plant extract that contain chemical compounds like limonene, terpenoids, menthone, carvone, dihydrocarvinone, menthol and other compounds belonging to the same class reveal anti-mutagenic activities, showing therapeutic and cancer chemo-preventive activities[32]. Ozliman and his college [33] determined the dill oil total composition of leaves and were found to be in the range of 64.08-84.33% with dill apiole, carvotanacetone, α-phellandrene and limonene being the most abundant compounds constituting around 22.02 to 43.55% of the investigated total concentration of dill oils. However, we found some differences in the composition of the main components of essential oils extracted from dill plants. Our results determined that out of more than 20 recognized compounds, the 13-Docosenamide, (Z), Bis(cis-13-docosenamido) methane (31.75%) and 2-pentanone-4-hydroxy-4-methyl (16.77%) are the most abundant compounds of the investigated total concentration of dill fixed oils. Therefore, we can conclude that the antimutagenic activity of dill oil against $KBrO_3$ mainly originates from 13-Docosenamide, which is present in 32% in the plant oil as a natural antioxidant source. Dill fixed oil also has a trace amount of Cholestan-3-one, cyclic 1,2-ethanediyl aetal, vitamin E, which is clinically approved as a powerful antioxidant with strong capability of preventing lipid peroxidation, which can harm DNA, proteins, and cell membranes in the human body [34]. Another interesting compound found in dil oil 1,2,4,5-Tetrazine, heterocyclic compounds, with the pyridine1 nucleus exhibits a variety of intriguing biological properties, including antioxidant, antiviral, anticancer, and antimicrobial. Moreover, 1,2,4-triazepines have been shown to have a variety of biological and therapeutic effects. Among many constituents detected are 3,3-Diethoxy-1-propyne, which has been reported to exhibit biological action through inhibiting the growth of cancer cells by causing apoptosis. The enzyme dihydrofolatereductase, which is involved in DNA synthesis, is inhibited as part of the mechanism of action. 3,3-Diethoxy-1-propyne targets both normal and cancerous cells biologically [35]. Dill oil, in the present study, produces 61% inhibition when it used as therapeutics. Clearly, genetic repairing agents act at cellular level by enhancing the activities of enzymes involved in desmutagens by suppressing the enzymatic activities involved in formation of mutagens metabolites. The latter can protect the cells though attracting electrophiles, scavenging ROS, inhibiting metabolic activation, or blocking the nucleophile sites of DNA [36]. Present findings are suggestive of the fact that the antimutagenic effect is due to the presence of various phyto-active compounds in dill oil. These polyphenols, act at the cellular levels for scavenging free radicals and also deactivate groups of mutagens or protect the DNA molecules from being attacks by the mutagen [37]. In this study, the mixture of dill oil and $KBrO_3$ had a synergistic effect, which was more effective compared to other antimutagenic treatments. A significant inhibition of 70.6% was observed against the mutation induced by $KBrO_3$ at 6 hrs duration time. Treatment with dill oil before the $KBrO_3$ provided protection for the DNA damage, showing the best results for the nuclear content of DNA. So, dill oil could be classified as “desmutagen” in the first order, and “bioantimutagen” in

the second order. According to Verschaeve and Van Staden [37] to determine the antimutagenic potential of a sample, a value smaller than 25% inhibition of the mutagen action demonstrates a weak or non antimutagenic action, a mild effect when the value is between 25% and 40% and strong antimutagenicity when the value is higher than 40%. Our results are in agreement with previous evaluation, where the used concentration(2.5µg/ml) and period times (2, 6 and 24 hrs) do display a moderate and strong protective effect of dill oil on $KBrO_3$-induced chromosomal damage.

5. Conclusions

Food additives and the residues they leave in food appear to be issues that human will continue to face for a very long time. However, there is growing evidence that plant-based phytochemicals such polyphenols and antioxidants, can effectively stop and repair DNA damage. Our findings, indicate oxidative stress caused by $KBrO_3$-induced chromosomal abnormalities and DNA damage in *A. sativum bioassay*. Our results also demonstrate that the well-known flavoring plants dill significantly reduces the genotoxic damage caused by $KBrO_3$ and recovers the activity of cell division. Our results have shown that cells were protected from $KBrO_3$'s carcinogenic effects by dill plants.

Author contributions: HAK and NSE designed the study and wrote the manuscript. SEY performed the experiments.

Conflict of interest

All authors declare that they have no conflict of interest.

References

1- Andreozzi, L., Giannetti, A., Cipriani, F., Caffarelli, C. and Mastrorilli, C. Hypersensitivity reactions to food and drug additives: Problem or myth?*ActaBioMedica.,* 2019, **90**, 80-90.

2- Wilson, B.G. and Bahna, S.L. Adverse reactions to food additives. *Annals of Allergy, Asthma and Immunology.*, 2005, **95**, 499-507.

3- Jansen, T., Claassen, L., van Kamp, I. and Timmermans, D.R. All chemical substances are harmful. Public appraisal of uncertain risks of food additives and contaminants. *Food and Chemical Toxicology*., 2020, **136**, 110959.

4- Anderson, J.A. Allergic reaction to food.Crit. Rev. Food 2 Sci. Nutr. 1996, **36**, S19-S38.

5- Aldaek, A.M. 2020. ProtectiveEffect of Red Onion on Potassium Bromate-Induced Toxicity in Male Albino Rats. PhD dissertation.Alzawia university. Libya.

6- Oyekunle, J., Adekunle, A., Ogunfowokan, A., Olutona, G. and Omolere, O. Bromate and trace metal levels in bread loaves from outlets within Ile-Ife Metropolis, Southwestern Nigeria. *Toxicology reports.,* 2014, **1**, 224-230.

7- Ben Saad ,H., Driss, D., Ben Amara, I., Boudawara, O. and Boudawara, T. *et al*. Altered hepatic mRNA expression of immune response associated DNA damage in mice liver induced by potassium bromate: Protective role of vanillin. *Environmental Toxicology.*, 2016, **21**, 10-22181.

8- IARC. 1986. Potassium bromate. In: Monographs on the Evaluation of the Carcinogenic Risk.
of Chemicals to Human, International Agency for Research on Cancer, *Vol. 40, World Health Organization, Lyon, France.*

9- Fawell, J. and Walker, M. Approaches to determining regulatory values for carcinogens with particular reference to bromate. *Toxicology.*, 2006, **221**, 149-153.

10- Qin, J., kim, M.S., Chao, K., Dhakal, S. and Cho, K. Non-targeted and targeted raman imaging detection of chemical contaminants in food powders, Proc. *Sensing for Agriculture and Food Ouality and Safety X.,* 2018, **10665**, 57-62.

11- Khale, S., McMahon, B. and Otto-Hitt, S. 2018. Effect of potassium bromate on OXR1 gene expression and cell growth in tetrahymenathermophila. Life and Environmental Sciences Course-based Research Projects, 18.

12- Ahmad MK and Mahmood R. Potassium bromate causes cell lysis and induces oxidative stress in human erythrocytes. *Environmental Toxicology*, 2014, **29**, 138-145.

13- Ahmad, M.K., Naqshbandi, A., Fareed, M. and Mahmood, R. Oral administration of a nephrotoxic dose of potassium bromate, a food additive, alters renal redox and metabolic status and inhibits brush border membrane enzymes in rats. *Food chemistry*, 2012, **134**, 980-985.

14- Murata, M., Bansho Y, Inoue S, Ito K, Ohnishi S., Midorikawa K and Kawanishi S. Requirement of glutathione and cysteine in guanine-specific oxidation of DNA by carcinogenic potassium bromate. *Chemical research in toxicology.*, 2001, **14**, 678-685.

15- Starek A and Starek-Świechowicz B. Toxicological properties of potassium bromate. *Journal of Pharmacological Reports.*, 2016, **1**, 1-9.

16- Alhanash, H.B., Edriss, N.A, Ksheid, S.M. and Issa, R.M. Residual Bromate Assessment in Bread Samples from Tajoura city bakeries, Libya. *Academic Journal of Research and Scientific Publishing Vol*, 2(13), 2020.

17- Khan, N., Sharma, S. and Sultana, S. Nigella Sativa (black cumin) ameliorates potassium bromate-include early events of carcinogenesis: diminution of oxidative stress. Toxicology and Industrial Health l, 2003, **22(4)**, 193-203.

18- Ali, B.H., Al Za'abi, M., Karaca, T., Al Suleimani, Y. and Al Balushi, K.A, *et al*. Potassium bromate-induced kidney damage in rats and the effect of gum acacia thereon. American Journal of Translational Research, 2018, **10**, 126-137.

19- Garcez, J.J., Barros, F., Lucas, AM., Xavier, V.B., Fianco, A.L., Cassel, E. and Vargas, R.F. Evaluation and mathematical modeling of processing variables for a supercritical fluid extraction of aromatic compounds from Anethum graveolens. *Industrial Crops and Products.*, 2017, **95**, 733-741.

20- Grosso, C., Ferraro, V., Figueiredo, A., Barroso, J., Coelho, J. and Palavra, A. Supercritical carbon dioxide extraction of volatile oil from Italian coriander seeds. *Food Chemistry.*, 2008, **111**, 197-203.

21- Malik, M.N., Haq, IU., Fatima, H., Ahmad, M., Naz I, Mirza B., *et al.* Bioprospecting Dodonaea viscosa Jacq; a traditional medicinal plant for antioxidant, cytotoxic, antidiabetic and antimicrobial potential. *Arabian Journal of Chemistry.*, 2022, **15(3)**, 103688.

22- Khatab, H., Hamed, S. and Elhaddad, N. Mitodepressive effect and chromosomal aberrations induced by KBrO3 on *A. sativum* L root tips. *Libyan Journal of Science and Technology.*, 2020, 11(2).

23- Al-sheddi, E.S., Al-zaid, N.A., Al-oqail, M.M., Al-massarani, S.M. and Farshori, N.N. Evaluation of cytotoxicity, cell cycle arrest and apoptosis induced by *Anethum graveolens* L. essential oil in human hepatocellular carcinoma cell line. *Saudi Pharmaceutical Journal*, 2019, **27**, 1053-1060.

24- Grant, W.F. Chromosome aberration assays in Allium: A report of the US Environmental Protection Agency gene-tox program. *Mutation Research/Reviews in Genetic Toxicology*.,1982, **3**, 273-291.

25- Khatab, H., Elhaddad, N. and Eissa, S. Genotoxicity Assessment of Potassium Bromate by Means of DNA Image Analysis on the Root Tip Nuclei of Allium sativum L. *Biotechnology Journal International*., 2020, **24(1)**, 9-14.

26- Al-Anazi, K.M, Al-mareed, A.A., Farah, M.A., Ali, M.A and Al-hemaid, F.M. Protective Effect of Capparis spinosa Extract against Potassium Bromate Induced Oxidative Stress and Genotoxicity in Mice. *Evidence-Based Complementary and Alternative Medicine*, 2021.

27- Akram, M., Riaz, M., Wadood, A.W., Hazrat, A., Mukhtiar, M., Ahmad, Z. and Zainab, R. Medicinal plants with anti-mutagenic potential. *Biotechnology and Biotechnological Equipment*, 2020, **34**, 309-318.

28- Andrijauskaite, K. and Wargovich, M.J. (2022, May). Role of natural products in breast cancer related symptomology: targeting chronic inflammation. In *Seminars in Cancer Biology* (Vol. 80, pp. 370-378). Academic Press.

29- Akash, Z., Rajput, N.A., Atiq, M. and Malik, A.U. Assessment of synthetic fungicides against wilt of chilli caused by Fusarium oxysporum f. sp. capsica. *Pakistan Journal of Agricultural Sciences*, 2022, **59**, 485-492.

30- Nam, H.H., Nan, L. and Choo, B.K. Anti-Inflammation and Protective Effects of Anethum graveolens L.(Dill Seeds) on Esophageal Mucosa Damages in Reflux Esophagitis-Induced Rats. *Foods.*, 2021, **10**, 2500.

31- Mai, T.T., Kayansamruaj, P., Taengphu, S., Senapin, S., Costa, J.Z., Del-pozo, J., Thompson, K.D., Rodkhum, C. and Dong, H.T. Efficacy of heat-killed and formalin-killed vaccines against Tilapia tilapinevirus in juvenile Nile tilapia (Oreochromis niloticus). *Journal of Fish Disease.* 2021, **44**, 2097-2109.

32- Satomi, H., Umemura, K., Ueno, A., Hatano, T., Okuda, T. and Noro, T. Carbonic anhydrase inhibitors from the pericarps of Punica granatum L. *Biological and Pharmaceutical Bulletin.*, 1993,**16**, 787-790.

33- Ozliman, S., Yaldiz, G., Camlica, M. and Ozsoy, N. Chemical components of essential oils and biological activities of the aqueous extract of Anethum graveolens L. grown under inorganic and organic conditions. *Chemical and Biological Technologies in Agriculture.*, 2021, **8**, 1-16.

34- Sharma, A., Singh, S., Tewari, R., Bhatt, V.P., Sharma, J. and Maurya, I.K. Phytochemical analysis and mode of action against *Candida glabrata* of *Paeoniaemodi* extracts. *Journal of Medical Mycology.*, 2018, **28**, 443-451.

35- Hassan, A.Y., Shabaan, S.N., El-Sebaey, S.A. and Abou-Amra, E.S. Synthesis of pyrido-annelated [1, 2, 4, 5] tetrazines, [1, 2, 4] triazepine, and [1, 2, 4, 5] tetrazepines for anticancer, DFT, and molecular docking studies., *Scientific Reports*: 2023, **13**, 5585.

36- Azqueta, A. and Collins, A. Polyphenols and DNA Damage: *A Mixed Blessing. Nutrients.*, 2016, **8(12)**, 785.

37- Verschaeve, L. and Van Staden, J. Mutagenic and antimutagenic properties of extracts from South African traditional medicinal plants. *Journal of Ethnopharmacology*. 28, **119(3)**, 575-87