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Genotoxic impacts of *Teucrium apollinis* medicinal plant via HGPRT gene and DNA damage

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Abstract

We report the applicability of the Hypoxanthine Guanine Phosphoribosyl Transferase (HGPRT) gene to test the potential mutagenic activity of *Teucrium apollinis* (TA). Genotoxicity of low dose (LD) 50µl and high dose (HD) 250µl of fixed oil extracted from TA were examined using asexual cell (oidia) of *Coprinopsis cinerea*. Surprisingly, survival of wild type cells was significantly decreased to 45.8 and 29.3% when oidia incubated with LD and HD respectively illustrating its cytotoxic effect. The mutation rate decreased to 0.33 and 0.4 with selected doses of the plant extract correspondingly which means that both were mutagenic. DNA damage of *Coprinopsis cinerea* was also quantified by spectrophotometer. There were significant differences (LSD= 0.05) between negative control and treatments, since the highest value was 94.33±2.41µg/ml in control, and the lowest amount of DNA (32.53±3.40) was obtained when hypha was treated with the positive control (MSG). Unsurprisingly, LD and HD reduced DNA values to 33.84±3.26 and 31.56±2.70 respectively, indicating the genotoxicity of TA concentrations. Ten components were identified by GC-MS of the oil extract involving Camphor and Furanone. Thus cytotoxic and mutagenic of medicinal plants must be assessed to ensure a relatively save use for our important population.

Keywords: HGPRT gene, mutation, *Teucrium apollinis*, DNA damage, genotoxicity.

1. Introduction

The Hypoxanthine Guanine Phosphoribosyl-Transferase HGPRT gene mutation assay is a remarkable tool for testing genotoxic chemicals and allows the isolation and screening of mutation in different living organisms such as humans, hamsters, bacteria, and fungi. In the current project, the HGPRT assay for gene mutation at the HGPRT locus of *Coprinopsis cinerea* was used to test the mutagenicity of *the Teucrium apollinis* medicinal plant. The genus Jaada (Teucrium L.) is considered one of the most important plants of the Lamiaceae family in Libya. Plants of this genus are stunted shrubs and shrubs, with plant heights ranging from 10 to 100 cm based on [1]. Some plants of this genus are popularly used in Libya to treat stomach and intestinal pain reduce blood sugar levels blood and cure colds, and is used to treat kidney

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stones and blood pressure [1]. Many studies demonstrated that the extractions of a range of medicinal plants have antioxidant, antibacterial, antimicrobial, anti-parasitic, anti-mutagenic, antifungal, and anti-carcinogenic properties. In recent years, using herbal drugs to treat different diseases has renewed interest, and the World Health Organization (WHO) has recommended an evaluation of the efficiency of medical plants in conditions that require or lack safe modern drugs [2]. Because of the high cost and the range of side effects that may have been caused by taking synthetic drugs, alternative medicine became necessary. Traditional medicinal plants are used over long periods for the above reasons, however, this is not always the situation [3, 4]. Acute toxicity due to the use of extracts from medicinal plants is more common than is often thought. It was estimated that annual deaths were between 8000 and 20,000 because of inappropriate use of medicinal plants (*1*). In addition, research has shown that many plants that are either used as food additives or in traditional medicine not only can be toxic drugs [4], but they can also be mutagenic [4, 5, 6, 7] or even carcinogenic [4, 7]. Although plant extracts have been used in the treatment of diseases according to knowledge accumulated over centuries, scientific research has shown some substances present in these medicinal plants to be potentially toxic and carcinogenic [7]. Many plant species commonly considered medicinal can contain potentially dangerous substances [8]. Recent research studies conducted in vitro and in vivo assays have revealed that many plants used in traditional medicine have cytotoxic and genotoxic effects [9, 10, 11, 12]. However, according to [13], rats were injected with different Concentrations of *Teucrium polium L.* or normal saline, following 28 days of *T. Polium* consumption, kidney damage was not increased in comparison with a control group. However, following 28 days, kidney damage including degeneration, destruction, and vacuolization, appeared and they concluded that *T. polium* should not be used or should be consumed with great caution [14]. The aqueous extract of *Teucrium polium* has strong hypoglycemic properties in experimental animals consequently it was concluded that it is not suitable for use in humans as an antidiabetic agent [15]. Moreover, [16] suggested that female rats are more sensitive to higher doses of *Teucrium* and that the liver could serve as a target organ toxicity of this extraction. Moreover, [17] reported the first case of hepatotoxicity was probably caused by *T. viscidum*one of *Teucrium* species indicating that using *T. viscidum* may pose similar to the other *Teucrium* species. Similarly, *Lavandula stoechas* was used to treat various diseases around the world but [18] reported cytotoxic and genotoxic effects of aqueous extracts from *L. stoechas* on *Allium cepa* root tip meristems. Their results showed that aqueous extracts significantly reduced mitotic index, induced chromosome aberrations, and mitotic aberrations in comparison with control [18]. Traditional plants, even though natural,

can be harmful. There is a rare of published data on the safety profile of these herbals. It was recommended to enhance the investigations of the safety of medicinal herbs and the adverse effects of their products and also consuming them in clinical practice [17].

Investigation of traditionally medicinal plants is thus valuable on two levels: firstly, as a source of potential chemotherapeutic drugs, and secondly, as a measure of safety for the continued use of medicinal plants [19]. Mutagenicity studies of traditional medicinal plants are important for risk assessment when applied as a medication against different diseases. Since mutagens are involved in the initiation and/or promotion of cancer, research would focus on the identification of a novel of traditional plants that may act as mutagenesis. HGPRT gene mutation assay is one of the strategies that is quite accessible to test mutagenicity. The objective of this study is to examine the mutagenicity/cytotoxicity of *Teucrium apollinis* extract using HGPRT gene mutation assay and the analysis of DNA damage of *Coprinopsis cinerea*.

2. Materials and Methods

2.1 The fungi

The wild strain of *Coprinopsis cinerea* used in this study is H1(allelic gene pairs of A and B). It has undergone five name changes in the last 30 years, most recently from *Coprinus cinereus* to *Coprinopsis cinerea* [20].

2.1.1 Strain and culture conditions

Strain AmutBmut is a self-compatible homokaryon of *Coprinopsis cinerea* which due to mutations in the A and B mating type genes, produces fruiting bodies without mating to another compatible monokaryon [21]. The hypha was isolated from horse dung and cultivated on YMG agar plates (4 g yeast extract, 10 g malt extract, 4 g glucose, and 10 g agar per l) for 5 days at 28°C until the mycelium fully covered the substrate [22]. Oidial suspensions were prepared by harvesting the hypha that were cultured on about 50 Petri dishes [21].

2.1.2 Complete media

The medium consists of (4 g yeast extract, 10 g malt extract, 4 g glucose, and 15 g agar per L) and it is named Yeast malt-glucose (YMG) medium. A complete medium is used to grow and sustain the wild- types of monokaryons [23].

2.1.3 Selective media

This medium is mainly used for genetic experiments such as isolating spontaneous and induced mutations and for measuring fungal growth rates. The medium contains the following: 4 g yeast

extract, 10 g malt extract, 4 g glucose, and 15 g agar per L, and then 20µl/ml 6-thioguanine was added to 100 ml medium. A 6-thioguanine (6- TG, 20µl/ml) solution was prepared by dissolving 120µg of 6-thioguanine in the freshly prepared 0.1M NaOH solution that made up to 60ml with distilled water and then stored at -20 C° until use (Sigma instructions).

2.1.4 Isolation and identification of fungi

Coprinopsis cinerea was isolated from the horse's dung in sterilized conditions. It was planted on the YMG media and incubated at 25 °C for 2-4 days, where the *C. cinerea* appeared to clear growth several seedlings using a needle sterile vaccination and this process was repeated until pure and good growth of *C. cinerea* was obtained.

The morphology of *Coprinopsis cinerea* was studied and the colony was first grown on the YMG medium and using the standard cover-slip technique with lacto phenol cotton blue staining procedure. The cover of the slide was inserted in the petri plate itself and the culture was allowed to grow for fourteen days. Every two days with the help of forceps, the coverslip was taken and inverted on a slide containing a drop of stain and visualized under a microscope (Lica microsystem 1000 Led) at 10X, 40X, and 100X magnification App. The fungi were identified based on mycelia and spore characteristics.

2.2 Hypoxanthine-guanine phosphoribosyl transferase locus (HGPRT) assay

The HGPRT assay was performed according to [24] Experiment was divided into four groups; the first group is the negative control in which the asexual (oidia) cells were incubated with Distilled Water (DW). Cells in the next two groups were treated with selected concentrations of oil extract of *Teucrium apollinis* (50 and 250µg/l) for one hour at 37°C. The fourth group, Oidia were treated with 7g/ml of mono-sodium glutamate as appositive control. Three replicate tubes were used for each group. After the incubation, cells were washed twice with DW and centrifuged at 1500 rpm. Treated oidia were then divided and cultured on two different culture mediums. Non-selective medium (YMG agar plates) to detect the wild-type phenotype, whereas, the selective medium (YMG agar plates containing 6-TG) to detect the mutant phenotype cells. Plates were incubated at 28°C for 48 hrs. Colonies on both non-selective and selective medium were counted. Three separate experiments (n=3) were carried out for each treatment. Viability was expressed as the number of colonies on the non-selective medium. Mutation frequency was determined phenotypically as the number of mutant colonies in the selective medium and the number of colonies in the non-selective medium.

2.3 Preparation of plant extractions

2.3.1 Plant material

In April aerial parts of *Teucrium apolinis* were collected from natural populations in the regions of Sousa in the AlJabel Alakhdar area. The specimens of *T. apolinis* were confirmed and deposited in the sylphium Herbarium at the Department of Botany, Faculty of Science, University of Omer-Almukhtar. The collected plant material was kept in sterilized plastic bags and immediately transferred to the laboratory.

2.3.1.1 Fixed oil extraction

Plant material was transferred to tightly dark-colored bottles and was soaked in 250ml of acetone and then stored at room temperature. After 48 h, the infusions were filtered through Whatman No. 1 filter paper. After that, the combined supernatants were evaporated under vacuum at 40 °C using a Rotary evaporator according to [25]. The obtained oil extracts were kept in a sterile bottle and stored in a refrigerator at 4°C until needed.

2.3.1.2 Determination of antioxidant

The antioxidant content of *the Teucrium apolinis* plant was determined following the Prussian blue method [26]. One gram of powder was defatted twice with petroleum ether, the defatted powder was then extracted sequentially by stirring with 10ml methanol twice, then with 10 ml 1% hydrochloric acid: methanol (v/v). The three combined extracts were evaporated under vacuum and the residue was dissolved in 10ml methanol. Half ml of the solution was diluted with 3 ml distilled water, 3 ml 0.008 M $K_3Fe(CN)_6$ was added, 3 ml 0.1M HCl, and 1 ml 1% $FeCl_3$. The blue color was allowed to develop for 5 min and the absorbance was measured at 720nm against the blank.

2.3.1.3 Determination of total phenol

Aliquots of the extracts were taken in a 10 ml flask and made up to a volume of 3 ml with distilled water. Then 0.5 ml folin ciocalteau reagent (1:1 with water) and 2 ml Na_2Co_3(20%) were added. The test solutions were warmed for 1 minute, cooled and absorbance was measured at 650 nm against the reagent used as a blank [27].

2.4 Gas Chromatography-Mass Spectrum Analysis

The extraction was kept in a dark bottle and transferred to (the central lab of the faculty of science at Alexandra University-Moharam Bey) to commence to Gas Chromatography- Mass Spectrum (GC-MS) examination.

2.5 Plant extract applications

In this experiment, oidia were treated with monosodium glutamate MSG (7g/l, published data at http://www.scholarsresearchlibrary.com) as a positive control and two different concentrations of the fixed oil (low and high) and then incubated for two hours which were determined based on the preliminary experiments. The experiment of the interaction between MSG and plant extracts was divided into two sup-experiments as follows: in the first treatment: three sterile test tubes containing 2mL of oidial suspension were allocated for all of the negative, and positive control groups and treatment groups.

In the treatment group: 7g/l of MSG was added, cells were incubated for two hours, washed, and then the fixed oil (50 and 250 µl) was added to the oidia. The tube was incubated for another two hours at 37°C, subjected to centrifugation, and then the supernatant was discarded. The washing step was repeated twice using distilled water and then the oidia were re-suspended with 2ml of distilled water to be ready for cultivating. The non-treated oidia tubes represent the negative control group. The rest of the tubes represent a putative mutagenicity of the oil extract. In the second treatment: the steps mentioned above in the first treatment were repeated but this time the oidia were incubated for two hours with oil extract, then the MSG was added and incubated for two more hours. Here the first group represents negative untreated oidia, while the rest of the tubes represent the putative enhancing mutagenicity role of the fixed oil were incubated for two hours with the plant extraction before adding the food enhancer MSG for more two hours.

2.6 Fungal genomic DNA extraction

The genomic DNA was extracted from five to seven day old fungal cultures grown in culture plates. The fungal mass of 10-20 mg fungal mass (20-40 mm^2mycellial tissue) from the culture plate was collected with the help of a fine spatula. The fungal mass obtained from the culture plate placed in a 2ml tube containing a500µl extraction buffer (1M Kcl, 100 mM Tris-Hcl, 10 mM EDTA). Homogenization of fungal mass was done using sterilized sand and vortexed for 30 seconds, 50µl freshly made proteinase K (10mg/ml PBS) was added. The resulting fungal tissue homogenate was centrifuged at 5,000 rpm for 10 min and the supernatant was transferred to an Eppendorf tube containing 300µl of isopropanol and mixed by inverting tube several times, centrifuged at 12,000 rpm for 10 min, and the supernatant discard as much as possible, then dissolved the DNA pellet in 1X TE with vortex at the low speed [28]

2.6.1 Quantitative estimation of genomic DNA:

DNA quantitation of different treatments by Spectrophotometer. The A260/A280 of each sample was read against distilled water as a blank using an Eppendorf Spectrophotometer, fitted with 1000 µl of DNA samples, demonstrating genomic DNA by spectrophotometric analysis.

2.7 Statistical Analysis

One-way ANOVA was used to compare the viability and rate of mutation values of both controls and treated samples. For each treatment, three tubes were included and then from each tube, six plates were divided into two groups and cultured on complete and selective media (n=27). Data were collected using CoStat statistical computing software, and results with The Least Significant Difference (LSD<0. 05) were considered to be statistically significant. Results were presented as mean ± standard deviation (SD). In the estimation of DNA quantities, differences in average values between control and treated samples (n=3) were analyzed using one-way ANOVA. Again, results with (LSD<0.05) were considered to be statistically significant.

3. Results

3.1 Identification of fungi

3.1.1 Cultural characteristics

The fungi were isolated from horse dung and grown on Yeast malt-glucose (YMG) medium, colonies attaining a diameter of 7-8cm after 5 days at 28C, edges and aerial mycelium were white (Figure 1).

Figure 1: *Coprinopsis cinerea* grown on YGM medium

3.1.2 Microscopic examination

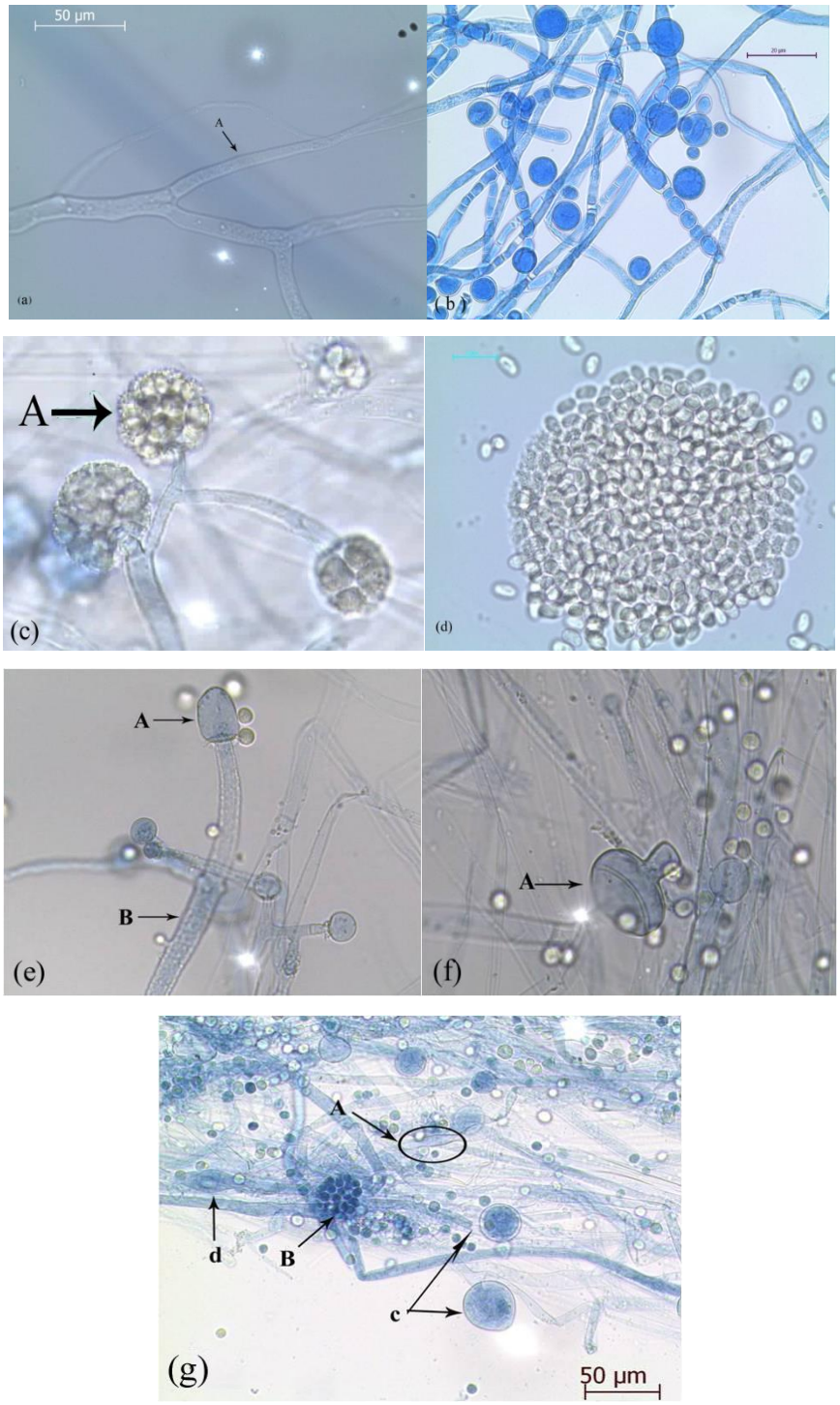

Figure 2 Developmental structures of *Coprinopsis cinerea*.(a-g) a: Mycelium, b: Chlamedospores, c: Oidia with oidiophore, d: Oidia, e: (A.Carpophore. B, Basidiocarp), f: Fruit body (A, young fruit body), g: (A, Basidia. B, Oidia. C: Cheilocystidia. D: Pilealcuticular elements).

Oidia with 44.95µm in diameter (Figure 2), small unicellular haploid asexual spores are produced in abundant numbers in the aerial mycelium of monokaryons by splitting short oidial hyphae produced at the tips of specialized conidiophores, terminal chlamydospores, odioiphores caring oidia, basidiocarp (young fruit body) on the tip of carpophore 68.13µm in height. Septa hyphal foot cells within the conidiophore were found, heteromorphous showing cheilocystidia (27.72µm in diameter), basidia and pilealcuticular elements, and Fused clamp cells at hyphal septa

3.2 The consequence of plant extract applied to the wild-type cells

A fluctuation of the viability percentages was noticed when the interaction between two selected doses of plant extract and the positive control (highest mutagenic concentration of MSG, previously published data at (http://www.scholarsresearchlibrary.com) where applied on *Coprinopsis cinerea* (Figure 3). The lowest viability (27.5%) was remarked when the oidia were treated with 7g/l of mono-sodium glutamate while the control oidia reached the highest viability. Surprisingly, the number of wild type cells decreased to 45.8 and 29.3% after applying low dose (LD) 50 µl and high dose (HD) 250 µl of plant extract respectively. There were four altered treatments of the interactions; a low dose of plant extraction and then 7g/l of MSG (LD-7) was the first, in the second treatment MSG was added to the cell, and then a low dose of plant extraction (7-LD). The addition of a high dose of plant extract before adding 7g/l of MSG (HD-7) was the third treatment and lastly, the reverse (7-HD) was the fourth application. The interaction between plant extract (LD and HD) and the positive control (7g/l) of MSG illustrates an improvement in viability. 59.5% was the highest reached viability when cells were treated with a low dose of plant extraction then 7g/l of mono-sodium glutamate (LD-7). However, the viability decreased to the lowest percentage 35.6% following the application of MSG and then a high dose of plant extraction (7-HD). There was a significant difference at ($p< 0.05$) when comparison was made between the mean of each treatment and the mean of oidia that treated with only MSG. Whereas, there was no significant difference between oidia treated only with a high dose (HD, 29.3%) of plant extraction and the oidia treated only with MSG (7g/l, 27.5%).

3.3 The consequence of the plant extract applied to the mutant cells

In this experiment, cells were treated in the same manner as described above in **the** 3.2 section; negative control group, both of low and high doses of the plant extract, 7g/l of MSG as a positive control, and the interactions. Figure 4 demonstrates that there were no mutations in the

control group whereas the mutation rate reached 0.33 and 0.4 of both selected doses of the plant extract correspondingly which means that both were mutagenic. The highest mutagenic rate attained was 1.68 when cells were treated with 7g/l of mono-sodium glutamate. The mean number of the mutant colonies on the 6-thioguanine plates for the interactions was significantly ($p< 0.05$) less than the means obtained in the MSG group. It was not expected to develop a higher mutagenic rate when cells incubated with plant extract (LD-7 and HD-7) before the MSG than the reverse (7-LD and 7-HD) incubation which were 0.51, 0.55,0.47, and 0.36 respectively.

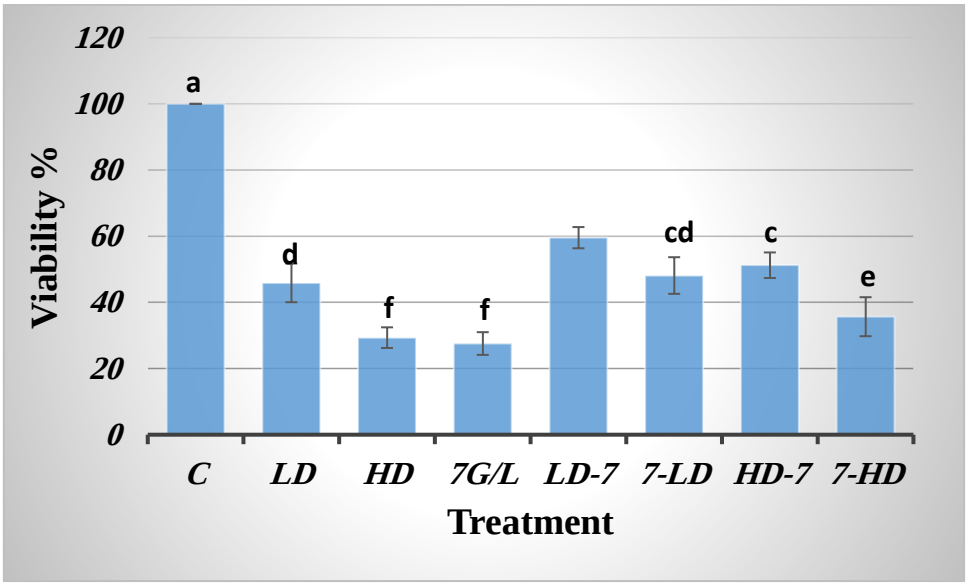

Figure 3. The consequences of the interaction between two selected doses of the plant extract and the highest mutagenic concentration of MSG (7g/l) on the viability. Values plotted are means of 3 readings from 27 plates. Error bars represent the standard deviation. Values were statistically tested using one-way ANOVA (small letters: significant differences at $p< 0.05$). C= control, LD=low dose, HD= high dose.

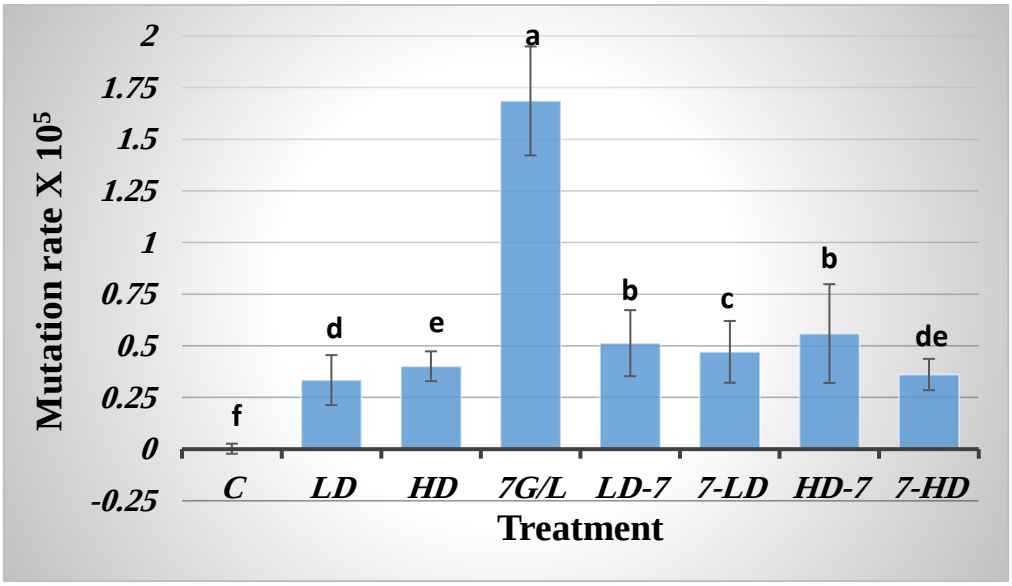

Figure 4. The consequences of the interaction between two selected doses of the plant extract and the highest mutagenic concentration of MSG (7g/l) on mutation rate. Values plotted are means of 3 readings from 27 plates. Error bars represent the standard deviation. Values were statistically tested using one-way ANOVA (small letters: significant differences at $p< 0.05$). C= control, LD=low dose, HD= high dose.

3.4 The analysis of DNA damage

The genomic DNA was extracted from the hypha of *Coprinopsis cinerea* and divided as the following; negative control, mono-sodium glutamate as a positive control, *Teucriuma pollinis* extract, and interaction between plant extract and MSG. This experiment was carried out to determine the in vitro DNA damage that may be caused by exposure to low and high doses of *Teucrium apollinis*.

The DNA concentration for untreated and treated samples was measured by spectrophotometer, three replicates were recorded at 260/230nm of each treatment (Table 1). Unsurprisingly, the negative control had the highest concentration (94.33µg/ml) whereas the concentrations of DNA after MSG (7g/l) application were significantly (P>0.05) decreased to 32.33µg/ml. The significant decline in the amount of DNA was also noted when samples were treated with low (LD; 50µg/ml) and high (HD; 250µg/ml) doses of plant extract. The DNA quantity obtained following the LD treatment (33.84µg/ml) was slightly higher than the DNA quantity that was recorded after HD application (31.56µg/ml). The interaction between two doses of the fixed oil and 7g/l of MSG was also assessed. Unexpected quantities of DNA were achieved especially when hypha incubated with the plant extract before adding MSG. DNA quantities were 30.83, 32.53, 30.73, and 28.03 µg/ml following the treatments of 7g/l of MSG then LD

of plant fixed oil, the reverse application, 7g/l of MSG then HD of plant extract, and the last treatment of the fixed oil first then MSG correspondingly.

Table 1: DNA quantitation of different treatments recorded by Spectrophotometer

Treatments	Amount of DNA (µg/ml)±SE
Control	94.33±2.41a
7g/l(MSG)	32.53±3.40b
LD	33.84±3.26b
HD	31.56±2.70b
LD then7g/l	23.53±2.84b
7g/l then LD	30.83±4.11b
HDthen7g/l	30.73±2.96b
7g/l then HD	28.03±3.99b

MSG: mono-sodium glutamate, LD: low dose of plant extract, HD: high dose of plant extract. Small letters: significate differences at (P>0.05)

3.5 Analysis of plant extract:

3.5.1 Determination of antioxidant and phenolic compounds

The total phenolic compounds and the antioxidant content of *T.apollinis* were measured by spectrophotometer and the values were 11.312%±0.46 and 1.334%±0.005 correspondingly per 1g of dry weight of plant material

3.5.2 Analysis of plant fixed oil by GC–MS technique

The oil extracted from fresh aerial parts of *Teucrium apollinis* was analyzed by gas chromatography–mass spectrometry (GC–MS). Fixed oil components were identified based on their retention index values and also by comparing their mass spectra with known compounds existing in the data system libraries. Table 2 summarizes the composition of *T.apollinis* leaf fixed oils. In total, 10 compounds were identified (Figure 5). The active principles with their retention time (RT), molecular formula, molecular weight, and peak area (%) are presented, and Figure 6 shows: a qualitative analysis of the chemical composition of *Teucrium apollinis*.

Table 2: Chemical composition of the fixed oil of *Teucrium apollinis* fresh leaves identified by (GC–MS) technique

Peak	Compound	molecular formula	MW	RT	Peak area (%)
1	Propanoic acid, 2-methyl-, (dodecahydro-6a-hydroxy-9a-methyl-3-methylene-2,9-dioxoazuleno[4,5-b]furan-6 –yl)methyl ester, [3aS-(3aα,6β,6aα,9aβ,9bα)]-	C19H26O6	350	15.244	100
2	Eucalyptol	C10H18O	154	5.394	42.5
3	1,5,5-Trimethyl-6-methylene-cyclohexene	C10H16	136	8.026	28.96
4	10,12-Tricosadiynoic acid, methyl ester	C24H40O2	360	12.557	25.51
5	6,9-Octadecadiynoic acid, methyl ester	C19H30O2	290	17.163	16.85
6	Spiro[13ricycle[4.4.0.0(5,9)]decane-10,2'-oxirane], 1-methyl-4-isopropyl-7,8-dihydroxy-, (8S)-	C15H24O3	252	16.548	14.92
7	9,10-Secocholesta-5,7,10(19)-triene-3,25,26-triol, (3β,5Z,7E)-	C27H44O3	416	16.308	12.89
8	Androstan-17-one, 3-ethyl-3-hydroxy-, (5α)	C21H34O2	318	16.062	11.05
9	2(3H)-Furanone, dihydro-4,4-dimethyl-5-(2-oxopropyl)	C9H14O3	170	6.679	7.75
10	Camphor	C10H16O	152	7.479	6.94

MW=Molecular weight, **RT**= Retention time

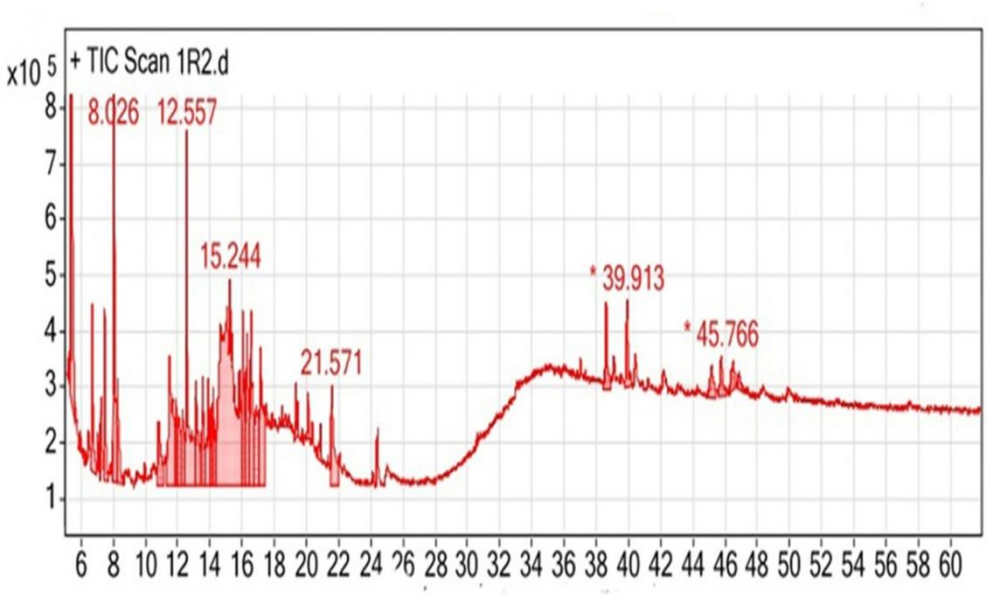

Figure 5. Qualitative analysis of fixed oil of *Teucrium apollinis* fresh leaves by Gas chromatography/Mass spectrometry (GC/MS)

4. Discussion

Fungi display several characteristics that make them suitable objects for a great variety of studies: although they are eukaryotic and, therefore, are related to higher organisms, they are modest regarding cultivation requirements, and the genetics of many strains are well documented. In the current study, we use *Coprinopsis cinerea* fungi for the assessment of genotoxicity of TA extract.

The fungal genera and species were identified based on mycelium and spore characteristics via microscopic examination and referring published data. *Coprinopsis cinerea* (previously called *Coprinus cinereus*) is the developmentally best-understood species of the Agaricomycetes that produces typical fruit bodies with the sexual basidiospores on the dikaryon (Figure 2: e &f). Genetics proved that the tested model has morphological varieties of the same species [29, 30]. Oidiophores can also differ very much in structure, four main types were distinguished by the attendance or non-attendance of an oidiophore stipe, by the presence or absence of a septum that separates oidiophores from their foot cells, by the length of the oidiophores, and by existence or lack of side branches at the oidiophores. About these four types, our strain matches the type I of the oidiophore (Figure 2: c).

4.1 The analysis of HGPRT gene mutation assay

The purpose of the *in vitro* gene mutation test is to detect gene mutations induced by chemicals. This test measures forward mutations in the reporter gene, endogenous hypoxanthine guanine phosphoribosyl-transferase gene (HGPRT). The HGPRT mutation test detects different spectra of genetic events. Base pair substitutions, frameshifts, small deletions, and insertions are mutational events that were detected by the HGPRT test. The assessment of mutations on the HGPRT locus is expected to be an effective tool for various genetics, medical, and agricultural applications [31,32,33]. Organization for Economic Co-operation and Development (OECD) recommended many tests for assessing the genotoxicity of chemicals including HGPRT Gene Mutation [34]. This test is an operative and widely used method in the detection of mutagenicity and genotoxicity of chemicals in laboratory conditions [35, 36, 37, 38, 39].

4.1.1 The consequences of *Teucrium apollinis* extract on survival and mutation cell frequency

Traditional medicine plants are widely worldwide used and have attracted renewed interest in developed countries over the last decades. Despite the positive observation of herbal medicines, cytotoxic cases have been reported in different literature [40, 41, 42]. Some herbal medicines have been shown to contain toxic compounds, these compounds react with cellular macromolecules, including DNA, causing cellular toxicity and/or genotoxicity [43]. The HGPRT mutation assay used in the present study for evaluating the cytotoxic and mutagenic potentials of medicinal plant, *Teucrium apollinis*. Two different ranges of concentrations (low and high) were selected and tested in the current study exhibiting significant cytotoxic influence by decreasing cell viability (Figure 3). The deficiency of chemo-protective effect in current extraction was also evident from the high mutant phenotype in selective medium and thus the high mutation frequencies (Figure 4). It was reported that components of plant extracts can contribute to a variety of effects on cells, and run specified mechanisms of cytotoxicity [44, 45]. Ten compounds were identified by (GC-MS) analysis of the air aerial parts of *Teucrium apollinis* of greenish oil with a strong deterrent odor. Those ten compounds are represented as the main constituents of the oil (Table 2). The chemical composition of *T. apollinis* oil from different parts of the world has been reported by some researchers. The variations in the composition of the oil are likely the result of plant parts used, harvesting time, and geographical location [46]. Our results could be related to the chemical composition of the extract since the GC-MS analysis exhibits Camphor and Furanone compounds that were previously reported as genotoxic phytochemicals [47, 48, 49, 50]. Camphor, a terpenoid

compound, is a common traditional remedy that has been used to cure a wide range of symptoms [51]. However, the main problems with camphor toxicity in humans are connected more to the large availability of camphor secondary products and their diffused perception rather than to the toxicity of camphor [52]. The oral administration of different doses of camphor to rats and rabbits caused pronounced signs of toxicity. Reduced body weight gain and food consumption with a reduction of motility were observed in both rats and rabbits [52]. The chemical composition of the extract, as well as their relationship, may alter the cytotoxicity of plants and could be the reason for their different effects [44]. Following that, the difference in the effects between the different species of the same genus Teucrium may depend on the chemical composition of the extracts or can be caused by many ecological factors. Moreover, plant compounds and selected doses may affect the activity of many cell receptors and enzymes [53]. In general, both doses extract of *Teucrium apollinis*may somehow impaired the set of signaling pathways and metabolic functions that affect the cell cycle and proliferation, protein synthesis, RNA biosynthesis, DNA replication, repair, and membrane biosynthesis. For this reason, in mice, the HGPRT gene regulates multiple developmental and metabolic pathways of embryonic stem cell neuronal differentiation [54, 55, 56].

4.2 The analysis of genomic DNA damage

In this section, the mutagenicity of fixed oil of *Teucrium apollinis* in comparison to negative and positive controls was evaluated by assessing the genomic DNA damage based on spectrophotometer analysis. The quantities of the genomic DNA extracted from treated hypha are illustrated in Table 1. In the current study, it can be said that MSG and extract of *T. apollinis* had a negative effect on genomic DNA, which produced DNA with very low concentrations in all treatments.

4.2.1 *Teucrium apollinis* extract showed DNA damage effect

Much research effort has focused on the identification of phytochemicals in traditional plants, fruits, and vegetables that utilize beneficial effects. However, Assessment of the potential genotoxicity of traditional medicines is indeed an important issue as damage to the genetic material may lead to critical mutations and therefore also to an increased risk of cancer and other diseases. In the current project, we are evaluating the damaging effects of *Teucrium apollinis* plant extract on genomic DNA. Numerous studies provide evidence for mutagenic/antimutagenic or prooxidant /antioxidant activities largely depending on the concentration used of medicinal plants [57, 58, 59]. That is why two different doses (50 and

250µg/ml) of current oil extract were applied. Data in Table 1 reflect the DNA damage when mycelia were treated with low and high doses of plant extract and also when combined with 7g/l of MSG. This damage might have resulted from direct interaction between a DNA-reactive component in *Teucrium apollinis* extract, and DNA since such kind of interaction is one of several pathways that may lead to primary DNA damage [60, 61]. Specific components in the composition of plant extracts can contribute to a variety of effects on cells, and run specified mechanisms of cytotoxicity [62, 47]. Our data may reflect the changes in the redox status of the DNA by increasing the level of superoxide anion radicals following *T.apollinis* oil application [44]. In addition, *T. apollinis* extraction in present research could alter the production of reactive oxygen species (ROS) and reactive nitrogen species (RNS) that can modify signaling pathways in the cell resulting in damage to DNA [63. The total phenolic compounds (11.312%±0.46) and the antioxidant content (1.334%±0.005) per 1g of the dry weight of plant material of *T. apollinis* were measured, both values were relatively high as represented in the 3.5.1 section. The situation of the fairly high content of phenols and antioxidant content is in agreement with Tepe and his colleagues in 2011[64], who examined antioxidant and DNA damage protection activities of *Teucrium* and reported that it was rich in phenolic and flavonoid contents and can be used as an alternative to a synthetic antioxidant source [64]. In contrast, the present study reflects that the oil of *T. apollinis* induced significant DNA damage suggesting that components in our extract might interact directly with the DNA. In plant extract, major components have been identified and evaluated in the present study (Table 2), but the potential genotoxicity of these constituents remains rather unclear. However, there is little data regarding furanone (which was detected in our extract components) which was responsible for DNA-breaking activity [65]. also, the furanone/transition metal–mediated generation of reactive oxygen species was held responsible for DNA strand breaks and the formation of 8-hydroxy-2′- deoxyguanosine [66]. Moreover, the furanone, which are known pro-oxidants in foods [67], can produce superoxide radicals through, the reduction of cupric ions to cuprous ions, resulting in the conversion to hydrogen peroxide and hydroxyl radicals [68]. It is therefore fair to assume that the DNA damaging effect observed at 50 and 250 µg/ml might be induced by at least one or more of those components. The reason for this is not known, but it is not an uncommon phenomenon especially if the tested plant was previously situated in great concern. It was reported that an aqueous extract of *Teucrium polium* has some hepatotoxic effects and it is not suitable for use in humans as an antidiabetic agent [69]. Moreover, a significant increase was seen in both alanine aminotransferase and aspartate aminotransferase enzyme activities in female rats, also a significant increase in liver weight of male rats after

administration of *Teucrium polium* extract [70]. There are few reports about the possible toxicological actions of *T. polium*. Acute and chronic toxicity of *Teucrium stocksianum* in rats has been considered [71]. There is also a report about the hepatotoxic effects of *Teucrium polium* in rats [72]. Surprisingly, the essential oil of *T. Polium* has been effectively used as a bio-insecticide demonstrating its toxic content [73]. Much progress has been made in our understanding of the medicinal plant mechanisms that underlie cytotoxic, genotoxic, mutagenic, and carcinogenic actions. However, difficult challenges remain to be addressed to improve our understanding of their molecular mechanism.In summary, this research paper suggest mutagenic impacts of *Teucrium apollinis*.

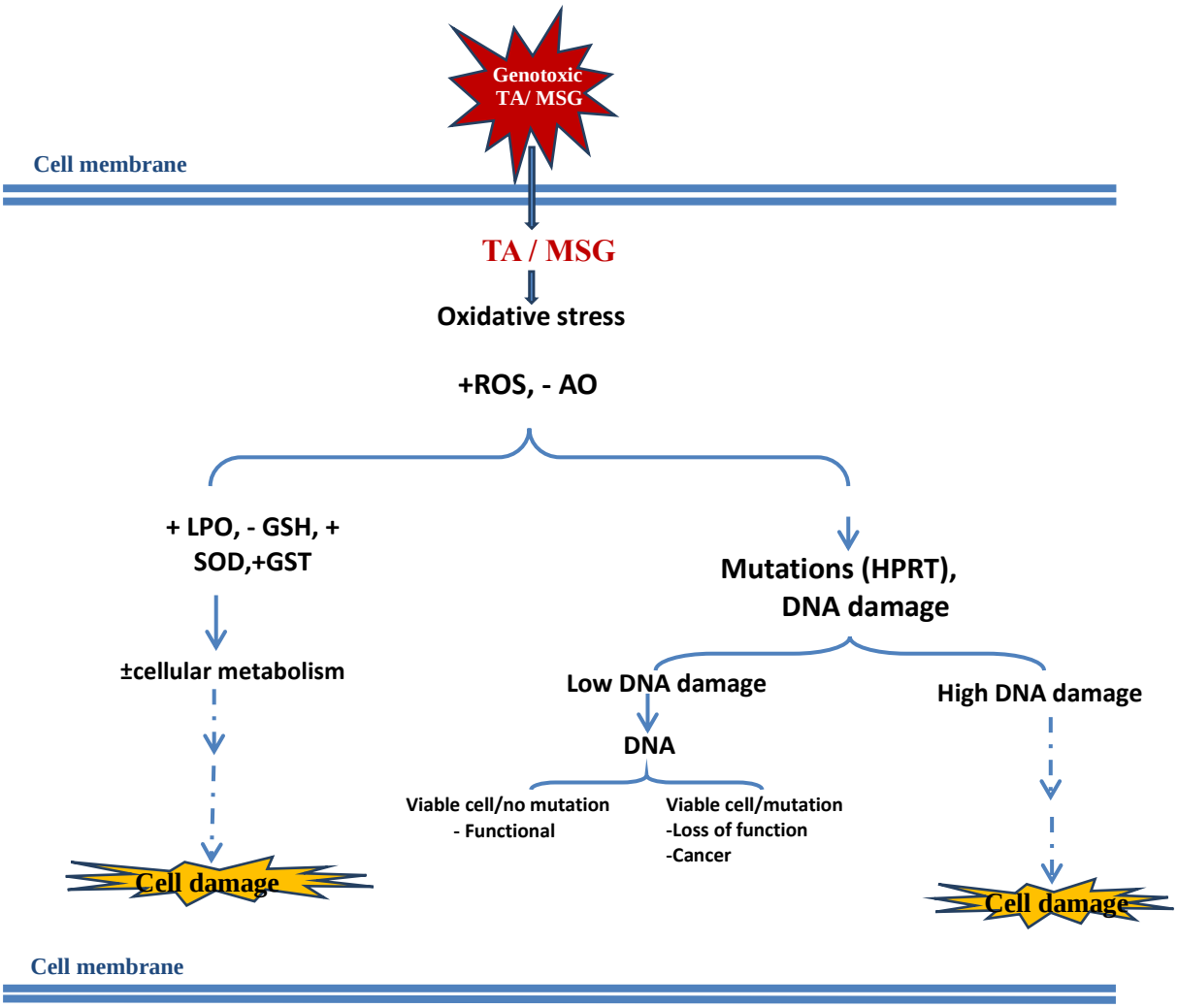

Figure 6. Proposed diagram of cytogenic/mutagenic of *Teucrium apollinis* (TA) and monosodium glutamate (MSG). LPO: lipid peroxidation, GSH: Glutathione, SOD: superoxide dismutase, GST: Glutathione-s-transferase, +: induction, -: reduction, ±: disruption, dotted arrows: unknown steps.

Figure 6. reflects possible genotoxic mechanisms of *Teucrium apollinis*. Current results revealed that TA has cytotoxic and mutagenic impacts on wild type in a similar way to MSG which is used as a positive control, mutant cell, and also on the genomic DNA in the tested model fungi. By understanding previous research and comparing their explanation with present data, proposed diagram 6 was generated assuming that TA and MSG have similar mutagenic impacts. At the molecular level, TA and MSG increase oxidative stress and reduce antioxidants leading to cell damage after multiple molecular processes involving mutation in the HGPRT gene and DNA damage.

5. Conclusion

Taken together, after assessing the genotoxicity of *Teucrium apollinis* extract on the selected experimental model, we conclude that the HGPRT gene mutation test is an operative method in the detection of the genotoxicity of chemicals. Also, the efficiency of studying the degradation of genomic DNA by using a spectrophotometer to investigate the induced damage caused by different applications. Finally, the findings of the present work support a genotoxic effect of TA in both of HGPRT mutation assay and DNA damage analysis. It is recommended to increase health education programs about medicinal plant usage.

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Conflict of interest

The trial is completely devoid of conflict of interest.

References

[1] S. Jafri, A. El-Gadi, "Flora of Libya", vol. 117. Al Faatheh University, Faculty of Science Publication, Tripoli, 30, (1985).

[2] J. Mejri, M. Abderrabba, M. Mejri, "Chemical composition of the essential oil of Ruta chalepensis L: Influence of drying, hydro-distillation duration and plant parts." Industrial Crops and Products, 32(3), (2010): 671-673.

[3] C. Fennell, K. Lindsey, L. McGaw, S. Sparg, G. Stafford, E. Elgorashi, O. Grace, J. Van Staden, "Assessing African medicinal plants for efficacy and safety: pharmacological screening and toxicology." Journal of Ethnopharmacology, 94(2-3), (2004): 205-217.

[4] J.P. Dzoyem, V. Kuete, J.N. Eloff, "Biochemical parameters in toxicological studies in Africa: significance, principle of methods, data interpretation, and use in plant screenings." Toxicological Survey of African Medicinal Plants, Elsevier, (2014):659-715.

[5] L. Verschaeve, J. Van Staden, "Mutagenic and antimutagenic properties of extracts from South African traditional medicinal plants." Journal of Ethnopharmacology, 119(3), (2008): 575-587.

[6] M. Déciga-Campos, I. Rivero-Cruz, M. Arriaga-Alba, G. Castañeda-Corral, G.E. Angeles-López, A. Navarrete, R. Mata, "Acute toxicity and mutagenic activity of Mexican plants used in traditional medicine." Journal of Ethnopharmacology, 110(2), (2007): 334-342.

[7] I.C. Fernandes de Sá Ferreira, V.M. Ferrão Vargas, "Mutagenicity of medicinal plant extracts in Salmonella/microsome assay." Phytotherapy Research, 13(5), (1999): 397-400.

[8] H. Rodrigues, C. Meireles, J. Lima, G. Toledo, J. Cardoso, S. Gomes, "Efeito embriotóxico, teratogênico e abortivo de plantas medicinais." Revista brasileira de plantas medicinais, 13(3), (2011): 359-366.

[9] R. Sehgal, S. Roy, V. Kumar, "Evaluation of the cytotoxic potential of latex of Calotropis procera and Podophyllotoxin in Allum cepa root model." Biocell, 30(1), (2006): 9.

[10] G. Dalla Nora, T. Pastori, H.D.L. Iv, T.S. "Do Canto-Dorow, S.B. Tedesco, Antiproliferative and genotoxic effects of Mikania glomerata (Asteraceae)." Biocell, 34(3), (2010): 95.

[11] A. Luz, I. Pretti, J. Dutra, M. Batitucci, "Avaliação do potencial citotóxico e genotóxico de Plantago major L. em sistemas teste in vivo." Revista Brasileira de Plantas Medicinais, 14(4), (2012): 635-642.

[12] Yuet Ping, I. Darah, U.K. Yusuf, C. Yeng, S. Sasidharan, "Genotoxicity of Euphorbia hirta: an Allium cepa assay." Molecules, 17(7), (2012): 7782-7791.

[13] A. Baradaran, Y. Madihi, A. Merrikhi, M. Rafieian-Kopaei, M. Nematbakhsh, A. Asgari, Z. Khosravi, F. Haghighian, H. Nasri, "Nephrotoxicity of hydroalcoholic extract of Teucrium polium in Wistar rats." Pakistan Journal of Medical Sciences, 29(S), (2013): 329-333.

[14] M. Rafieian-Kopaei, H. Nasri, A. Baradaran, "Teucrium polium: Liver and kidney effects." Journal of Research in Medical Sciences, 19(5), (2014): 478-479.

[15] A.R. Vahidi, R.M. DASHTI, S.M. BAGHERI, "The Effect of Teucrium polium boiled extract in diabetic rats." (2010).

[16] H.R. Rasekh, H. Yazdanpanah, L. Hosseinzadeh, N. Bazmohammadi, M. Kamalnezhad, "Acute and subchronic toxicity of Teucrium polium total extract in rats." (2005).

[17] W.T. Poon, T.L. Chau, C.K. Lai, K.Y. Tse, Y.C. Chan, K.S. Leung, Y.W. Chan, "Hepatitis induced by Teucrium viscidum." Clinical Toxicology, 46(9), (2008): 819-822.

[18] T.A. Çelik, Ö.S. Aslantürk, "Cytotoxic and genotoxic effects of *Lavandula stoechas* aqueous extracts." Biologia, 62(3), (2007): 292-296.

[19] L. Verschaeve, V. Kestens, J. Taylor, E. Elgorashi, A. Maes, L. Van Puyvelde, N. De Kimpe, J. Van Staden, "Investigation of the antimutagenic effects of selected South African medicinal plant extracts." Toxicology in vitro, 18(1), (2004): 29-35.

[20] P. Srivilai, P. Loutchanwoot, "Coprinopsis cinerea as a model fungus to evaluate genes underlying sexual development in basidiomycetes." Pakistan journal of biological sciences: PJBS, 12(11), (2009): 821-835.

[21] S. Swamy, I. Uno, T. Ishikawa, "Morphogenetic effects of mutations at the A and B incompatibility factors in Coprinus cinereus." Microbiology, 130(12), (1984): 3219-3224.

[22] R.P. Boulianne, Y. Liu, M. Aebi, B.C. Lu, U. Kües, "Fruiting body development in Coprinus cinereus: regulated expression of two galectins secreted by a non-classical pathwayThe GenBank accession number for the sequence reported in this paper is AF130360." Microbiology, 146(8), (2000): 1841-1853.

[23] Y. Liu, P. Srivilai, S. Loos, M. Aebi, U. Kües, "An essential gene for fruiting body initiation in the basidiomycete Coprinopsis cinerea is homologous to bacterial cyclopropane fatty acid synthase genes." Genetics, 172(2), (2006): 873-884.

[24] A. Li, J. Carver, W. Choy, A. Hsie, R. Gupta, K. Loveday, J. O'neill, J. Riddle, L. Stankowski Jr, L. Yang, "A guide for the performance of the Chinese hamster ovary cell/hypoxanthine-guanine phosphoribosyl transferase gene mutation assay." Mutation Research/Genetic Toxicology, 189(2), (1987): 135-141.

[25] D. Nikodijević, M. Milutinović, D. Cvetković, M. Stanković, M.N. Živanović, S. Marković, "Effects of *Teucrium polium* L. and *Teucrium montanum* L.: Extracts on mechanisms of apoptosis in breast and colon cancer cells." Kragujevac Journal of Science, (38), (2016): 147-159.

[26] M.L. Price, S. Van Scoyoc, L.G. Butler, "A critical evaluation of the vanillin reaction as an assay for tannin in sorghum grain." Journal of Agricultural and Food Chemistry, 26(5), (1978): 1214-1218.

[27] V.L. Singleton, J.A. Rossi, "Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents." American Journal of Enology and Viticulture, 16(3), (1965): 144-158.

[28] M.-H. Chi, S.-Y. Park, Y.-H. Lee, "A quick and safe method for fungal DNA extraction." The Plant Pathology Journal, 25(1), (2009): 108-111.

[29] D. Moore, "Developmental genetics of Coprinus cinereus: genetic evidence that carpophores and sclerotia share a common pathway of initiation." Current Genetics, 3(2), (1981): 145-150.

[30] D. Moore, M.M. Elhiti, R. Butler, "Morphogenensis of the carpophore of *Coprinus cinereus*." New Phytologist, 83(3), (1979): 695-722.

[31] C. Aaron, G. Bolcsfoldi, H.-R. Glatt, M. Moore, Y. Nishi, L. Stankowski, J. Theiss, E. Thompson, "Mammalian cell gene mutation assays working group report." Mutation Research/Environmental Mutagenesis and Related Subjects, 312(3), (1994): 235-239.

[32] M.M. Moore, K. Harrington-Brock, C.L. Doerr, K.L. Dearfield, "Differential mutant quantitation at the mouse lymphoma tk and CHO Hgprt loci." Mutagenesis, 4(5), (1989): 394-403.

[33] C. Aaron, L. Stankowski Jr, "Comparison of the AS52/XPRT and the CHO/HPRT assays: evaluation of 6 drug candidates." Mutation Research/Genetic Toxicology, 223(2), (1989): 121-128.

[34] S. OECD, OECD Structural Analysis Statistics (http://www. oecd-library. org/industry-and-services/data/stan-input–output_stan-in-out-data-en) in, Organisation for Economic Co-operation and Development, 29, (2012).

[35] M.F. Bellini, L.N. Cabrioti, A.P. Terezan, B.Q. Jordão, L.R. Ribeiro, M.S. Mantovani, "Cytotoxicity and genotoxicity of Agaricus blazei methanolic extract fractions assessed using gene and chromosomal mutation assays." Genetics and Molecular Biology, 31(1), (2008): 122-127.

[36] M.P. Machado, E. Rodrigues Filho, A.P. Terezan, L.R. Ribeiro, M.S. Mantovani, "Cytotoxicity, genotoxicity and antimutagenicity of hexane extracts of Agaricus blazei determined in vitro by the comet assay and CHO/HGPRT gene mutation assay." Toxicology in vitro, 19(4), (2005): 533-539.

[37] C.L. Crespi, F.J. Gonzalez, D.T. Steimel, T.R. Turner, H.V. Gelboin, B.W. Penman, R. Langenbach, "A metabolically competent human cell line expressing five cDNAs encoding procarcinogen-activating enzymes: application to mutagenicity testing." Chemical research in toxicology, 4(5), (1991): 566-572.

[38] C.L. Crespi, W.G. Thilly, "Assay for gene mutation in a human lymphoblast line, AHH-1, competent for xenobiotic metabolism." Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis, 128(2), (1984): 221-230.

[39] E.E. Furth, W.G. Thilly, B.W. Penman, H.L. Liber, W.M. Rand, "Quantitative assay for mutation in diploid human lymphoblasts using microtiter plates." Analytical biochemistry, 110(1), (1981): 1-8.

[40] E. Ernst, A. White, "The BBC survey of complementary medicine use in the UK." Complementary therapies in medicine, 8(1), (2000): 32-36.

[41] J.-P. Cosyns, M. Jadoul, J.-P. Squifflet, F.-X. Wese, C.v.Y. de Strihou, "Urothelial lesions in Chinese-herb nephropathy." American journal of kidney diseases, 33(6), (1999): 1011-1017.

[42] J.-L. Vanherweghem, J. Degaute, "The policy of admission to the education in medicine and dentistry in the French-speaking community of Belgium." Acta Clinica Belgica, 53(1), (1998): 2-3.

[43] I.M. Rietjens, M.J. Martena, M.G. Boersma, W. Spiegelenberg, G.M. Alink, "Molecular mechanisms of toxicity of important food-borne phytotoxins." Molecular nutrition & food research, 49(2), (2005): 131-158.

[44] M.S. Stanković, T.L. Mitrović, I.Z. Matić, M.D. Topuzović, S.M. Stamenković, "New values of Teucrium species: in vitro study of cytotoxic activities of secondary metabolites." Notulae Botanicae Horti Agrobotanici Cluj-Napoca, 43(1), (2015): 41-46.

[45] M.S. Stankovic, N. Niciforovic, V. Mihailovic, M. Topuzovic, S. Solujic, "Antioxidant activity, total phenolic content and flavonoid concentrations of different plant parts of Teucrium polium L. subsp. Polium." Acta Societatis Botanicorum Poloniae, 81(2), (2012).

[46] S. Merghache, M. Hamza, M. Bendahou, B. Tabti, "Chemical composition and antimicrobial activity of Ruta chalepensis L. essential oil from Algeria." Asian Journal of Chemistry, 20(4), (2008): 2989-2996.

[47] S. Kim, A. Liem, B. Stewart, J. Miller, "New studies on trans-anethole oxide and trans-asarone oxide." Carcinogenesis, 20(7), (1999): 1303-1307.

[48] G. Franzios, M. Mirotsou, E. Hatziapostolou, J. Kral, Z.G. Scouras, P. Mavragani-Tsipidou, "Insecticidal and genotoxic activities of mint essential oils." Journal of Agricultural and Food Chemistry, 45(7), (1997): 2690-2694.

[49] . Hasheminejad, J. Caldwell, "Genotoxicity of the alkenylbenzenes α− and β-asarone, myristicin and elemicin as determined by the UDS assay in cultured rat hepatocytes." Food and chemical toxicology, 32(3), (1994): 223-231.

[50] E.R. Nestmann, E.G.-H. Lee, "Mutagenicity of constituents of pulp and paper mill effluent in growing cells of Saccharomyces cerevisiae." Mutation Research Letters, 119(3-4), (1983): 273-280.

[51] P. Zuccarini, G. Soldani, "Camphor: benefits and risks of a widely used natural product." Acta Biologica Szegediensis, 53(2), (2009): 77-82.

[52] J. Leuschner, "Reproductive toxicity studies of D-camphor in rats and rabbits." Arzneimittel-forschung, 47(2), (1997): 124-128.

[53] E. Middleton, C. Kandaswami, T.C. Theoharides, "The effects of plant flavonoids on mammalian cells: implications for inflammation, heart disease, and cancer." Pharmacological reviews, 52(4), (2000): 673-751.

[54] T.H. Kang, Y. Park, J.S. Bader, T. Friedmann, "The housekeeping gene hypoxanthine-guanine phosphoribosyltransferase (HPRT) regulates multiple developmental and metabolic pathways of murine embryonic stem cell neuronal differentiation." PloS one, 8(10), (2013): e74967.

[55] C. Trapnell, L. Pachter, S.L. Salzberg, "TopHat: discovering splice junctions with RNA-Seq." Bioinformatics, 25(9), (2009): 1105-1111.

[56] C. Trapnell, B.A. Williams, G. Pertea, A. Mortazavi, G. Kwan, M.J. Van Baren, S.L. Salzberg, B.J. Wold, L. Pachter, "Transcript assembly and quantification by RNA-Seq reveals unannotated transcripts and isoform switching during cell differentiation." Nature Biotechnology, 28(5), (2010): 511-515.

[57] S. Bahramikia, R. Yazdanparast, "Phytochemistry and medicinal properties of *Teucrium polium* L.(Lamiaceae)." Phytotherapy Research, 26(11), (2012): 1581-1593.

[58] O.M. DJORDJEVIC, M.R. JAKOVLJEVIC, A. Markovic, M. Stankovic, A. Ciric, D. Marinkovic, D. Grujicic, "Polyphenolic contents of Teucrium polium L. and Teucrium scordium L. associated with their protective effects against MMC-induced chromosomal damage in cultured human peripheral blood lymphocytes." Turkish Journal of Biology, 42(2), (2018): 152-162.

[59] Z. Özer, T. Kılıç, S. Çarıkçı, H. Yılmaz, "Investigation of phenolic compounds and antioxidant activity of *Teucrium polium* L. decoction and infusion." Balıkesir Üniversitesi Fen Bilimleri Enstitüsü Dergisi, 20(1), (2018): 212-218.

[60] S.M. Galloway, J.E. Miller, M.J. Armstrong, C.L. Bean, T.R. Skopek, W.W. Nichols, "DNA synthesis inhibition as an indirect mechanism of chromosome aberrations: comparison of DNA-reactive and non-DNA-reactive clastogens." Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis, 400(1-2), (1998): 169-186.

[61] S.M. Galloway, "Cytotoxicity and chromosome aberrations in vitro: experience in industry and the case for an upper limit on toxicity in the aberration assay." Environmental and molecular mutagenesis, 35(3), (2000): 191-201.

[62] M. Stanković, M. Topuzović, A. Marković, D. Pavlović, S. Solujić, N. Nićiforović, V. Mihailović, "Antioxidant activity, phenol and flavonoid contents of different *Teucrium chamaedrys* L. Extracts." Biotechnology & Biotechnological Equipment, 24(sup1), (2010): 82-86.

[63] K. England, T. Cotter, "Direct oxidative modifications of signaling proteins in mammalian cells and their effects on apoptosis." Redox Report, 10(5), (2005): 237-245.

[64] B. Tepe, S. Degerli, S. Arslan, E. Malatyali, C. Sarikurkcu, "Determination of chemical profile, antioxidant, DNA damage protection and antiamoebic activities of *Teucrium polium* and Stachys iberica." Fitoterapia, 82(2), (2011): 237-246.

[65] X. Li, K. Hiramoto, M. Yoshida, T. Kato, K. Kikugawa, "Identification of 2, 5-dimethyl-4-hydroxy-3 (2H)-furanone (DMHF) and 4-hydroxy-2 (or 5)-ethyl-5 (or 2)-methyl-3 (2H)-furanone (HEMF) with DNA breaking activity in soy sauce." Food and chemical toxicology, 36(4), (1998): 305-314.

[66] K. Murakami, M. Haneda, T. Makino, M. Yoshino, "Prooxidant action of furanone compounds: Implication of reactive oxygen species in the metal-dependent strand breaks and the formation of 8-hydroxy-2′-deoxyguanosine in DNA." Food and chemical toxicology, 45(7), (2007): 1258-1262.

[67] T.T. Liu, T.S. Yang, "Effects of water-soluble natural antioxidants on photosensitized oxidation of conjugated linoleic acid in an oil-in-water emulsion system." Journal of food science, 73(4), (2008): C256-C261.

[68] N. Yamashita, M. Murata, S. Inoue, Y. Hiraku, T. Yoshinaga, S. Kawanishi, "Superoxide formation and DNA damage induced by a fragrant furanone in the presence of copper (II)." Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis, 397(2), (1998): 191-201.

[69] M.R. Shahraki, M.R. Arab, E. MIRIMOKADAM, M.J. Palan, "The effect of Teucrium polium (Calpoureh) on liver function, serum lipids and glucose in diabetic male rats." Iranian Biomedical Journal, 11 (1), (2007): 65-68.

[70] H.R. Rasekh, H. Yazdanpanah, L. Hosseinzadeh, N. Bazmohammadi, M. Kamalnezhad, "Acute and subchronic toxicity of *Teucrium polium* total extract in rats." Iranian Journal of Pharmaceutical Research, 4(4), (2005): 245-249

[71] M. Tanira, I. Wasfi, M.A. Homsi, A. Bashir, "Toxicological effects of Teucrium stocksianum after acute and chronic administration in rats." Journal of pharmacy and pharmacology, 48(10), (1996): 1098-1102.

[72] F. Zal, M. Rasti, M. Vesal, M. Vaseei, "Hepatotoxicity associated with hypoglycemic effects of *Teucrium polium* in diabetic rats." Archives of Iranian Medicine, 4(4), (2001):188-192

[73] A. Heydarzade, G. Moravvej, "Contact toxicity and persistence of essential oils from *Foeniculum vulgare*, *Teucrium polium*, and *Satureja hortensis* against Callosobruchus maculatus (Fabricius)(Coleoptera: Bruchidae) adults." Turkish Journal of Entomology, 36(4), (2012): 507-518.

الملخص العربى

التأثير السمي الوراثي لنبات الجعدة باستخدام جين HGPRT وتلف الـ DNA

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قسم النبات – كلية العلوم – جامعة عمر المختار – البيضاء - ليبيا

الملخص

في هذه الدراسة، نوثق نجاح تطبيق جين - Hypoxanthine Guanine Phosphoribosyl Transferase (HGPRT) لاختبار القدرة التطفيرية نبات الجعدة *Teucrium apollinis* . تم فحص التأثير السمي الوراثي باستخدام تركيزين هما التركيز المنخفض و المرتفع ٥٠ و٢٥٠ ميكروغرام / مل من الزيت الثابت المستخلص من *Teucrium apollinis* (TA).تهدف الدراسة الحالية للكشف عن الضرر الوراثي الذي قد تسببه الجرعات التي تم اختيارها على الخلية اللاجنسية (oidia) من فطر *Coprinopsis cinerea*.

أثبتت النتائج، طفرة الجين HGPRT، أن كلا التركيزين لهما تأثيرات سامة للخلايا/مطفرة من خلال تقليل الحيوية وزيادة معدل طفرة جين HGPRT في الفطر المدروس. والمثير للدهشة أن نسبة الحيوية انخفض بشكل ملحوظ إلى ٤٥,٨ و٢٩,٣٪ عندما حضنت الأويديا بجرعة منخفضة 50ميكرولتر وجرعة عالية 250ميكرولتر من مستخلص النبات على التوالي مما يوضح تأثيرها السام للخلايا. أيضا وصل معدل الطفرات إلى ٠,٣٣ و٠,٤ من كلتا الجرعتين المختارتين من الزيت الثابت مما يعني أن كلاهما كان مطفراً. تم الكشف عن تلف الحمض النووي الجيني لـفطر *Coprinopsis cinerea* عن طريق القياس الكمي باستخدام التحليل الطيفي. كانت هناك فروق ذات دلالة إحصائية (LSD = 0.05) بين المعالجات الضابطة وجميع المعاملات المذكورة أعلاه، حيث كانت أعلى قيمة ٢,٤١ ± ٩٤,٣٣ميكروغرام / مل لمجموعة السيطرة، وأدنى كمية من الحمض النووي (٣,٤٠ ± ٣٢,٥٣) تم الحصول عليها عند معالجة الاويديا بـ ٧ جم / لتر من جلوتامات احاديه الصوديوم الذي استخدم كسيطرة موجبة. بالتالي فان المستخلص النباتي بالجرعتين المنخفضة والعالية أدى الى خفض تركيز الحمض النووي إلى ٣,٢٦ ± ٣٣,٨٤ و ٢,٧٠ ± ٣١,٥٦على التوالي، مما يشير إلى السمية الوراثية لنبات الجعدة.

تم التعرف على عشرة مركبات كيميائية لزيت نبات الجعدة باستخدام مطياف الكتلة المرتبط بالكروماتوجرافي الغازي -GC MS كان من بينها كلا من حمض البروبانويك والكافور.