



Protective effects of fennel essential oil against oxidative stress and genotoxicity induced by the insecticide triflumuron in human colon carcinoma cells

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Abstract

The increased use of pesticides is the origin of multiple damages to the environment and to humans; thus, the search for new strategies to reduce or even protect the toxic effects caused by these synthetic products became a necessity. In this context, our study attempted to evaluate the protective effects of fennel essential oil (FEO), the main essential oil extracted from *Faeniculum vulgare* Mill., a plant with aromatic, flavorful, and medicinal uses, against toxicity induced by an insecticide—triflumuron (TFM)—in human carcinoma cells (HCT116). Our methodological approach consists of the cytotoxicity assay starting with the cell viability test, the ROS generation, the malondialdehyde (MDA) production, the DNA fragmentation, and the measurement of some antioxidant enzymes activities such as catalase (CAT) and superoxide dismutase (SOD). Also, we measured the mitochondrial transmembrane potential. The outcome of the current study showed clearly that after 2 h of HCT 116 cell pretreatment with FEO, there were increase in cell viability, reduction in ROS generation, and modulation in CAT and SOD activities induced by TFM. In the same manner, significant decreases in MDA levels were found. Mainly, the results indicated a perceptible decrease in DNA damages and a significant reduction in the mitochondrial membrane potential loss. Our work demonstrates that FEO can be an important protector against toxic effects induced by TFM in HCT 116 cells.

Keywords Triflumuron · Fennel essential oil · Oxidative stress · Cytotoxicity · DNA damages

Introduction

Excessive use of pesticides in the agricultural sector can cause chemical contamination leading to the loss of plant and animal lives (Ralph et al. 1996). Indeed, pesticides can pose problems for human health since pesticide residues are frequently detected in different compartments of the environment (surface water, groundwater, soil, etc.) and in products intended for human and animal food (Cerejeira et al. 2003; Yuan et al. 2014).

Numerous epidemiological studies suggest that pesticides may be involved in the induction of cancer, neurodegenerative diseases, fertility, and reproductive disorders (Akoto et al. 2015; Yoshida et al. 2015; Zhang et al. 2016). That is why it is necessary to look for new products with higher selectivity for the targets organisms and lower toxicity for human and for the environment. Insect growth regulators (IGRs) are among these new compounds, as they act on the growth and the development of insects. In fact, IGRs were introduced following the appearance of insect resistance to pyrethroids and organophosphate insecticides (Belinato et al. 2013).

Among these IGRs, we find the class of benzoylureas (disrupting molt) which interferes with the insect's exoskeleton causing eventually its death (Tunaz and Uygun 2004).

Benzoylurea insecticides are widely used for crops protection, especially fruits and vegetables. Technically, they could be classified such as insecticide of fourth generation having high selectivity, low acute toxicity for mammals, and high biological activity (Tomsej and Hajglov 1995). In this context, our interest in the current study will be focused on triflumuron

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(TFM), a benzoylurea insecticide commonly used in the world.

Triflumuron ((2-chloro-[N-4-trifluoromethoxy)phenyl]amino]carbonyl]; TFM) equally known as Starycid SC 480 (Batra et al. 2005) is used in order to protect crops, human, and animal health against diseases caused by some insects vectors (Van der Oost et al. 2003; Konradsen 2007; Winkaler et al. 2007; Alavanja 2009; Lowden et al. 2007).

Regarding the molecular level, TFM acts by inhibiting the synthesis of chitin which is a linear polymer composed of N-acetyl-glucosamine (Merzendorfer and Zimoch 2003). Thus, by blocking the transport of N-acetylglucosamine through the epithelial membrane, TFM acts as a general stressor, making the insect more susceptible to malformations and disease, for example, facilitating the entry of pathogenic fungi into insects by weakening the insect's cuticle (Irigaray et al. 2003).

Almost the majority of TFM toxic effects have been realized on insects. Indeed, TFM induced high mortality levels, delayed the development and inhibited molting in *Rhodnius prolixus* (Mello et al. 2008). In addition, TFM reduced larval density and caused inhibition of the emergence of adult mosquito larvae (Batra et al. 2005).

In addition to this, the treatment of *Aedes albopictus* with TFM inhibits the hatching of eggs. Also, abnormal morphology of the eggshell has developed (Suman et al. 2013). Furthermore, TFM acts on the offspring of insects, especially at the time of pupal formation. Like most IGRs, TFM causes malformations of pupae of treated females which die at the hatching moment or a few days later (Ouedraogo 1998) and causes embryo death (Itard 1986). It causes as well high rates of abortion in treated insects (Langley 1995).

However, TFM can cause toxic effects on non-target organisms, especially on aquatic organisms and invertebrates. For example, in rats, dogs, and rabbits, the administration of this compound causes spasms and skin and respiratory irritation resulting in sneezing and in several eye damages (Waller and Lacey 1986; EFSA 2011). Repeated administration of TFM is also known to cause hemolytic anemia (Tasheva and Hristeva 1993) and reproductive toxicity (Suman et al. 2013).

Because of TFM toxic effects on humans, animals, and environment, we focused our interest on antioxidant sources of molecules, as the fennel essential oil (FEO). Fennel is the commercial name of *Foeniculum vulgare*. It is an Apiaceae plant that is characterized by its medicinal properties. Indeed, this plant can be used to treat gastrointestinal and respiratory disorders (Agarwal et al. 2008). The essential oil extracted from this plant is known as FEO which is used in many sectors such as cosmetic, pharmaceutical, and perfume industries. Besides that, it is used as an additive in the preparation of food (Tinoco et al. 2007). Many studies have shown the important effects of FEO in the treatment of some human diseases, due to its medicinal properties such as a diuretic, anti-

inflammatory, analgesic, antioxidant (Gross et al. 2002) antiseptic, sedative, and stimulant activities (Tinoco et al. 2007; He and Huang 2011).

The antioxidant properties of this substance prompt us to study its protective effects against TFM-induced oxidative damages in human cell Carcinoma (HCT 116 cells).

Materials and methods

Chemicals

Triflumuron, fennel essential oil, and pyrogallol were purchased from Sigma–Aldrich (St. Louis, MO, USA). 3-[4,5-Dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT), cell culture medium (RPMI 1640), fetal calf serum (FCS), phosphate-buffered saline (PBS), trypsin-EDTA, penicillin and streptomycin mixture, and L-glutamine (200 mM) were from GIBCO-BCL (UK). 2,7-Dichlorofluorescein diacetate (DCFH-DA) was supplied by Molecular Probes (CergyPontoise, France). Low melting point agarose (LMA) and normal melting point agarose (NMA) were purchased from Sigma (St. Louis, MO). All other chemicals used were of analytical grade.

Cell culture and treatment

RPMI 1640 was used for the culture of human carcinoma cells (HCT 116). This medium is supplemented with 10% FBS, 1% L-glutamine (200 mM), 1% of mixture penicillin (100 IU/mL), and streptomycin (100 g/mL), at 37 °C with 5% CO₂. TFM was dissolved in DMSO and the FEO was dissolved in absolute ethanol (with a percentage that does not exceed 1% to avoid possible toxicity of absolute ethanol). The different concentrations of TFM (50 to 600 μM) in the presence or absence of the preventive substance (FEO) were added to the cell medium. Thus, the HCT 116 cells are pretreated for 2 h by FEO (1, 1.5, and 2% (v/v)) before their exposure to the TFM.

Cell toxicity assay (MTT assay)

The MTT assays (a tetrazolium salt reduction assay) measure if there are metabolic disorders in cell and more precisely in the mitochondria (Mosman 1983). For this, HCT 116 cells were seeded in 96-well plates at 2.5×10^4 cells/well and were treated with the FEO at 1, 1.5, and 2% (v/v) and different concentrations of TFM for 24 h at 37 °C. Wells containing untreated cells served as a negative control. After treatment, cells were incubated with the MTT solution for 3 h. At the end of the incubation, formed formazan crystals were dissolved in dimethyl sulfoxide and absorbance read at 570 nm using a microplate reader spectrophotometer (BioTek, Elx800). The results were expressed as the percentage of MTT reduction

relative to the absorbance's measured from negative control cells. All assays were performed in triplicate.

Reactive oxygen species determination and oxidative stress status

DCFH-DA is a fluorochrome that enters living cells and will be deacetylated by cell esterases into a non-fluorescent compound DCFH. The latter will be oxidized, in the presence of reactive oxygen species, into a fluorescent compound DCF (Cathcart et al. 1983; Le Bel et al. 1992; Chen and Wong 2009). The determination of free radicals is determined following the seeding of the cells in multi-well plates (96 wells) at a ratio of 2.10^4 cells/well. After 24 h, the cells were incubated for 30 min with 20 μ M DCFH-DA and were then treated with FEO (1, 1.5, and 2% (v/v)) combined or not with the TFM ($IC_{50} = 400 \mu$ M) and incubated for 24 h. Fluorescence was measured using a fluorometer (Biotek FLx800) with an excitation wavelength of 485 nm and an emission wavelength of 525 nm.

Protein extraction

HCT 116 cells (10^6 cells/well) were seeded in 6-well plates at 37 °C for 24 h. Then, we incubated cells for 24 h with 1, 1.5, and 2% (v/v) of FEO and with TFM at 400 μ M. A wash step with cold PBS was performed, and then cells were recuperated using a lysis buffer after a 30-min incubation period in ice. Centrifugation allowed obtaining cell extracts which were dosed using BioRad protein assay in order to determine protein concentrations in each sample (Bradford 1976).

Lipid peroxidation evaluation

To measure the level of MDA produced by the cells, 7.5×10^5 cells were plated on 6-well plates. The treatment of cells with both TFM (400 μ M) and FEO (1, 1.5, and 2% (v/v)) was carried out for 24 h at 37 °C. Thus, cells were recovered in PBS and incubated in the lysis buffer for 1 h. Centrifugation allowed the obtention of cell lysates, which were mixed with 200 μ L of KCl (1.15% w/v), 100 μ L of SDS (8.1% w/v), 750 μ L of acetic acid (20%; pH 3.5 v/v), and 750 μ L of thiobarbituric acid (0.8% w/v). The mixture is then incubated for 2 h at 90 °C. A cooling step for 10 min is necessary before adding a volume of 2.5 ml of n-butanol-pyridine (15:1/ v/v). The mixture obtained is then vortexed until the organic phase became up. Finally, a centrifugation allowed isolating the supernatant whose absorbance was read at 546 nm (Ohkawa et al. 1979).

Measurement of catalase (CAT) activity

To determine the activity of this enzyme, 780 μ L of phosphate buffer (100 mM; pH 7.0) is mixed with 20 μ L of proteins extracts and 200 μ L of H_2O_2 (20 mM). Then the absorbance was measured at 240 nm over a period of 3 min (Aebi 1984) at 37 °C. Using a molar extinction coefficient of 0.04/mM/cm, the calculus of this activity was effected and results were expressed as mmol/min/mg protein.

Measurement of superoxide dismutase (SOD) activity

SOD is enzyme that catalyzes the disproportionation of the superoxide anion into oxygen and dihydrogen peroxide. The assay of this activity is based on the ability of SOD to inhibit the autooxidation of pyrogallol by SOD. To do this, 970 μ L of Tris HCl (50 mM; pH 8.5), 10 μ L of pyrogallol (24 mM), and 20 μ L of protein extract are introduced into a curve, and the measurement of the optical density was effected at 440 nm for an interval of 3 min at 37 °C. In order to calculate the SOD activity, the amount of protein that inhibits the pyrogallol oxidation at 50% is determined. Results are expressed as U/mg protein (Marklund and Marklund 1974).

Mitochondrial membrane potential (MMP) assay

Rhodamine-123 is a fluorescent agent used to measure transmembrane mitochondrial potential (Debbasch et al. 2001). For this, cells, already seeded in a 96-well plate, were incubated with 1, 1.5, and 2% (v/v) of FEO in the presence or the absence of TFM for 24 h. A washing step with 100 μ L of PBS was primordial before putting 100 μ L of rhodamine 123 (1 μ M). Then, the plate was incubated for 15 min at 37 °C. Finally, the supernatant was discarded and was replaced by 150 μ L of PBS. Thus, a fluorimeter is used to determine the uptake of rhodamine 123. The results were expressed as the percentage of uptaken rhodamine fluorescence relative to the fluorescence measured from negative control cells.

DNA damage assessed by the comet assay

The DNA damage was quantified by using the comet test, which is an electrophoretic nuclei technique with a fluorescent agent, which allows the detection of DNA breaks on isolated cells after their inclusion in an agarose gel. To perform this test, cells were seeded in 6 well plates at 7.5×10^5 cells/well. After 24 h, cells were treated with 1, 1.5, and 2% (v/v) of FEO and TFM at 400 μ M. After recovering the cells in PBS, they were mixed with 60 μ L of low melting agarose (1.2%, w/v) and the solution was pretreated on coded slides and already covered with normal agarose (1%, w/v). Then slides were held in lysis

buffer for 1 h at +4 °C. Then, an electrophoresis step (30 min, 25 v, and 300 mA) (BioRad) made it possible to migrate DNA in an electric field. Finally, a neutralization step with Tris buffer was applied for all the slides for 15 min. At the time of DNA damage count, the slides were stained with ethidium bromide (20 mg/mL) and visualized by a fluorescence microscope. The DNA damage was classified into four classes according to the length of the DNA smear (Collins et al. 1996). Thus, a total score was calculated according to the following equation: (% of cells in class 0 × 0) + (% of cells in class 1 × 1) + (% of cells in class 2 × 2) + (% of cells in class 3 × 3) + (% of cells in class 4 × 4).

Statistical analysis

Data are expressed as the mean ± standard deviation (SD). The analysis parameters were tested for homogeneity of variance and normality, and they were found to be normally distributed. The data were therefore analyzed using a one-way analysis of variance (ANOVA) with a post hoc Tukey–Kramer test to identify significance between groups and their respective controls. In all cases, $p < 0.05$ was considered statistically significant.

Results

Cell viability determination

The exposure of HCT 116 cells to different concentration of TFM ranging from 100 µM to 1 mM for 24 h was established in order to determine the cell viability using MTT assay. Our results showed that TFM causes a decrease in the cell viability in a dose-dependent manner and with IC_{50} around 400 µM (Fig. 1a). For FEO, no cell toxicity was indicated. The pretreatment of HCT cells with 1, 1.5, and 2% (v/v) FEO, 2 h before the TFM treatment decrease significantly the cytotoxicity induced by TFM (Fig. 1b) ($*p < 0.05$ and $**p < 0.01$ vs. TFM alone).

Inhibition of ROS generation

Figure 2 shows the effect of FEO on ROS generation induced by TFM on human intestine cells. The level of the ROS produced by cells in the presence or the absence of TFM and FEO was measured according to the fluorescence of DCF. Indeed, in the presence of H_2O_2 in the intracellular medium, DCFH was oxidated to fluorescent DCF. Our results indicated that the level of ROS production increased from 5300 ± 500 in the control to $15,600 \pm 800$ in cells treated with TFM at 400 µM. Using different concentrations of 1, 1.5, and 2% (v/v) of FEO, the

intracellular ROS produced by TFM was completely abolished. Indeed, with the highest concentration of FEO, we showed that the level of ROS generation was around 4500 ± 200 (Fig. 2) ($##p < 0.01$ vs. control, $**p < 0.01$ and $***p < 0.001$ vs. TFM alone).

Effect of FEO on TFM-induced lipid peroxidation

To determine lipid peroxidation, we measured the rate of MDA which is an ultimate fragment of membrane lipid degradation. Our findings indicated that using TFM alone (400 µM), the level of MDA increased from 0.3 ± 0.03 (µmol/mg of proteins) in the control cells to 0.8 ± 0.03 (µmol/mg of proteins) in cells treated with TFM. The use of FEO at 1, 1.5, and 2% decreased respectively the level of MDA to 0.64 ± 0.03 , 0.51 ± 0.04 , and 0.38 ± 0.04 (µmol/mg of proteins) compared to the TFM-treated cells (Fig. 3). This decrease was of concentration-dependent manner ($###p < 0.001$ vs. control, $*p < 0.05$ and $**p < 0.01$ vs. TFM alone).

Effect of FEO on antioxidant enzymes activities

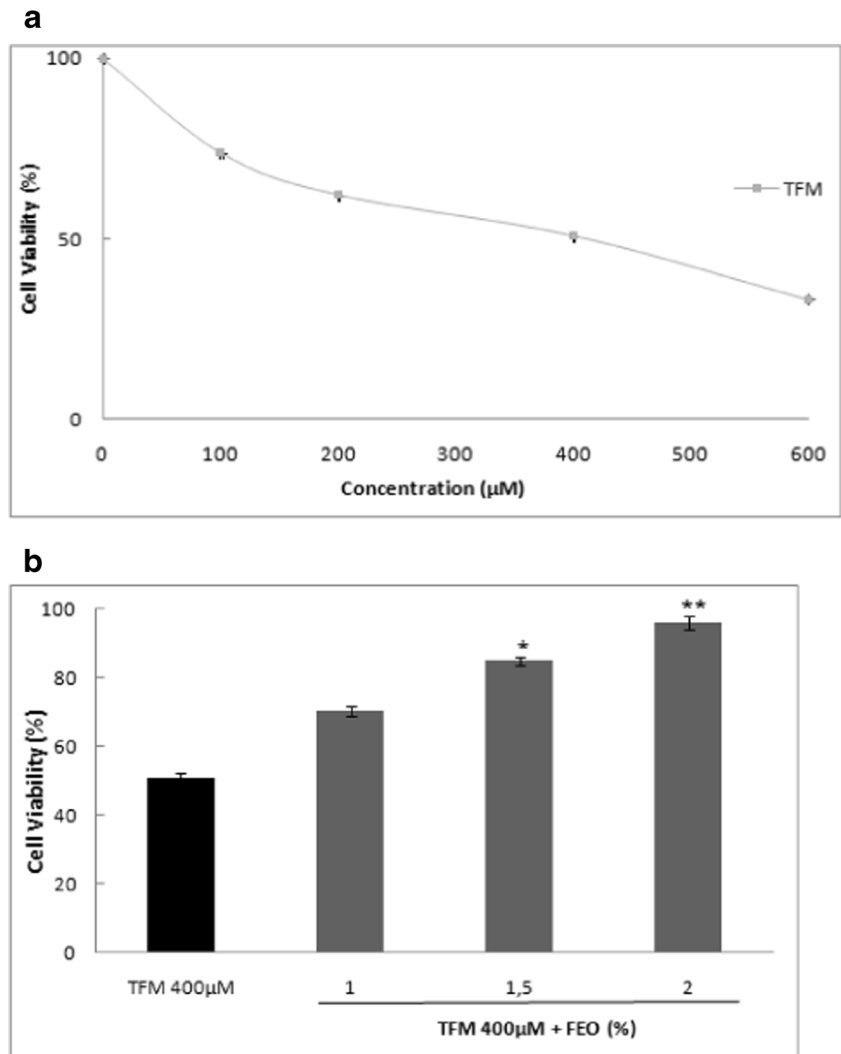
The change in the level of antioxidant enzymes can be considered as a marker of oxidative stress. Indeed, SOD catalyzes the dismutation of the highly reactive superoxide anion from oxidative stress to H_2O_2 , which can further be decomposed to water and oxygen by CAT or Gpx. Figure 4a, b shows the measurement of the activities of two antioxidant enzymes that are SOD and CAT.

Using TFM at 400 µM in HCT116 cells, we found a significant increase in the activities of these two enzymes. Indeed, in the untreated cells, CAT activity passed from 6 ± 0.08 to 47 ± 1 mmol/min/mg of proteins (Fig. 4a) in the TFM-treated cells group. Similarly, for the SOD activity, we noted an increase in this activity which passed from 48 ± 5 U SOD/min/mg of proteins in the control group to 127 ± 1 U SOD/min/mg of proteins (Fig. 4b). The pretreatment of cells with 1, 1.5, and 2% (v/v) of FEO reduced these increases in a dose-dependent manner ($###p < 0.001$ vs. control, $*p < 0.05$ and $**p < 0.01$ vs. TFM alone).

Effect of FEO on TFM mitochondrial alterations

To assess the effect of FEO on the mitochondrial alterations induced by TFM, we incubated cells with rhodamine 123. We noted a significant decrease in the Rh123 in cells treated with TFM (400 µM) alone with a percent of $53\% \pm 0.5$. These findings evidenced that in this case, cells lost their mitochondrial potential. Using FEO, a remarkable restoration in the percentage of Rh123 uptake was evidenced: $59\% \pm 1\%$, $78 \pm 4\%$, and $88\% \pm 0.4\%$

Fig. 1 a Cytotoxic effect of TFM and FEO on HCT116 cells. Cells were treated with TFM alone or combined with FEO for 24 h. Cell viability was determined using the MTT assay and expressed as percentages of viability. Values are significantly different ($p < 0.05$) from control. FEO reduces TFM-induced cytotoxicity in HCT116. Cells were pretreated for 2 h with FEO (1, 1.5, and 2%) before TFM treatment for 24 h (400 μM). **b** Cell viability was determined using MTT assay. Data are expressed as the mean \pm S.D. of three independent experiments. * $p < 0.05$ and ** $p < 0.01$ vs. TFM alone



using 1, 1.5, and 2% (v/v) of FEO respectively. Our results demonstrated the protective effect of FEO against mitochondrial alterations caused by TFM (Fig. 5) ($^{##}p < 0.01$ vs. control, $^{**}p < 0.01$ vs. TFM alone).

Effect of FEO on TFM-induced DNA fragmentation

The DNA fragmentation results are illustrated in Fig. 6. We showed that, compared to the control group, the total score

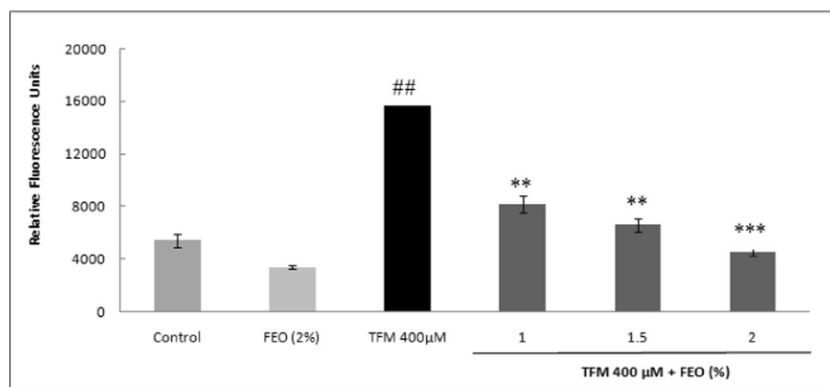
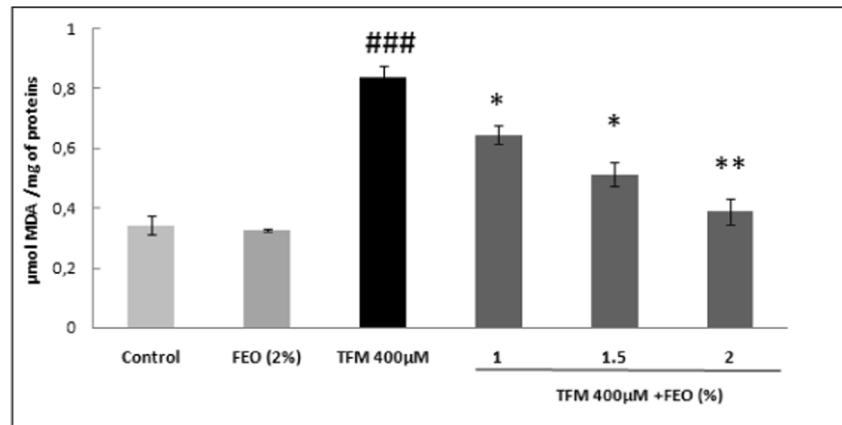


Fig. 2 Effects of FEO on TFM-induced ROS generation. HCT116 cells were pretreated with FEO (1, 1.5, and 2%) for 2 h before TFM treatment for 24 h (400 μM). The relative intracellular ROS production was evaluated by recording the fluorescence of DCF, the product of DCFH

oxidation mainly by H_2O_2 . Data are expressed as the mean \pm SD of three separate experiments. $^{##}p < 0.01$ vs. control, $^{**}p < 0.01$ and $^{***}p < 0.001$ vs. TFM alone

Fig. 3 Effects of FEO on TFM-induced lipid peroxidation. HCT116 cells were pretreated with FEO (1, 1.5, and 2%) for 2 h before TFM treatment for 24 h (400 μ M). The peroxidation of lipids was recorded by measuring the accumulation of MDA. Data are expressed as the mean \pm SD of three separate experiments. $###p < 0.001$ vs. control, $*p < 0.05$ and $**p < 0.01$ vs. TFM alone



increased from 44 ± 1 to 129 ± 6 in cells treated with TFM alone. Using different concentrations of FEO, a significant decrease in the DNA damages was noted: 117 ± 4 , 73 ± 2 , and 56 ± 4 respectively for 1, 1.5, and 2% (v/v) of FEO ($###p < 0.001$ vs. control, $**p < 0.01$ vs. TFM alone).

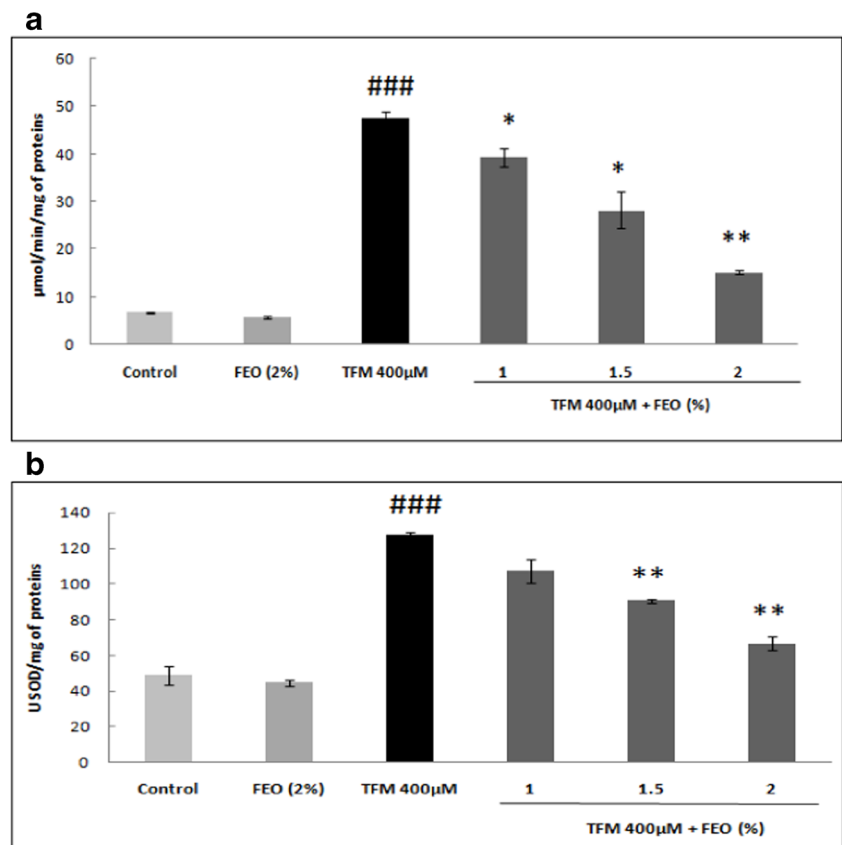
Discussion

Pesticides, also called plant protection products, are molecules used to protect humans and their environment against attacks caused by harmful vectors and predators (Marutescu and

Chifriuc 2007). Due to their increased use in many areas such as agriculture, industry, and traditional medicine, pesticides cause adverse effects on human health and threaten ecosystems (Farnesi et al. 2012). In this context, several studies have revealed the role of pesticides in the occurrence of numerous pathologies, namely, cancer and neurodegenerative and cardiac disorders (Kland 1988; Juricek and Coumoul 2014; Akoto et al. 2015).

Among those pesticides, we focused our interest on the Insect growth regulators (IGRs) which are a class of pesticide commonly used in agriculture and human and animal health in order to control damages caused by insects (Smith and Wall

Fig. 4 Effects of FEO on catalase (a) and superoxide dismutase activities (b). HCT116 cells were pretreated with FEO (1, 1.5, and 2%) for 2 h before TFM treatment for 24 h (400 μ M). Data are expressed as the mean \pm SD of three separate experiments. $###p < 0.001$ vs. control, $*p < 0.05$ and $**p < 0.01$ vs. TFM alone



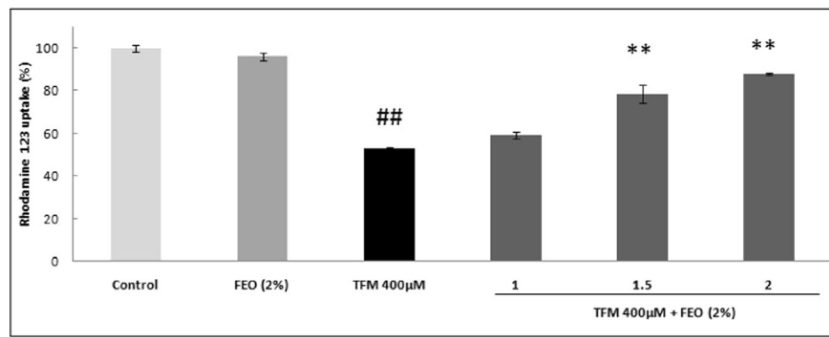


Fig. 5 Effects of FEO on TFM-induced loss of mitochondrial transmembrane potential. HCT116 cells were pretreated with FEO (1, 1.5, and 2%) for 2 h before TFM treatment for 24 h (400 µM). The mitochondrial

potential was assessed by measuring the uptake of rhodamine 123. Data are expressed as the mean ± SD of three separate experiments. ##*p* < 0.01 vs. control, ***p* < 0.01 vs. TFM alone

1998; and Parween et al. 2001; Zaim and Guillet 2002; WHO 2006).

TFM is one of the most known IGRs which is used to protect human, animal, and crops against insects (Kamsh et al. 1998; Parween et al. 2001). It acts by blocking the synthesis of chitin, the major constituent of the insect cuticle causing consequently its death (Vasuki and Rajavel 1992; Wilson and Cryan, 1997; Amir and Peveling 2004). It is characterized by its several toxic effects in most ecosystems. Also, it can affect the reproductive system of adult females of *Rhodnius prolixus* causing a high level of mortality in those insects (Henriques et al. 2016). Our previous study showed that TFM was an inducer of oxidative stress in male mice after short-term exposure (Timoumi et al. 2018).

Because of the toxic effects of TFM on the fauna and the flora, we tried to find in our work a natural substance that can reduce and prevent the toxicity induced by TFM. Thus, we worked with fennel essential oil (FEO) that is characterized by its antioxidant, antifungal, and antibacterial proprieties (Al-Amoudi 2017).

Our work aimed to study the protective effects of FEO against toxic effects induced by the insecticide, triflumuron (TFM), using human intestinal cell lines.

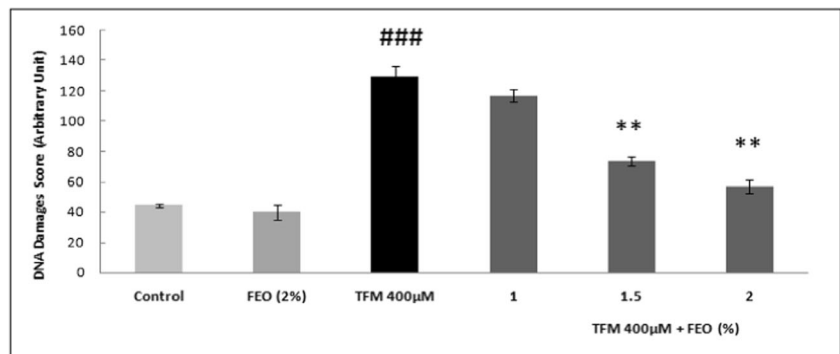
Treatment of HCT 116 cells with TFM (400 µM) decreased significantly cell viability by using the MTT assay. However, using FEO, cell death caused by TFM was significantly reduced.

In order to explain the decrease in cell viability induced by TFM, we performed some oxidative stress tests. Indeed, oxidative stress occurs when oxygen-reactive species (ROS) accumulate in cells, leading them to apoptosis (Battisti et al. 2008). ROS are essential intermediates in oxidative metabolism. Nevertheless, when oxidative stress occurs, ROS are generated in excess and consequently may damage cells by oxidizing lipids, disrupting DNA and proteins. Accordingly, we demonstrated that TFM-induced ROS generation and increased MDA levels and DNA damages. As a matter of fact, MDA is an ultimate fragment resulting from degradation of membrane lipids, which is frequently used as a marker of an oxidative state (Cini et al. 1994). The results of this state are the structural destruction in the cell membranes, DNA damages, and apoptotic death (Surapaneni and Venkataramana 2007).

Enzymatic and non-enzymatic antioxidants are cellular defenses having the role of protecting cells against environmental contaminants. These defense systems include catalase (CAT) and superoxide dismutase (SOD). Indeed, in case of stress, the SOD is the first enzyme that manifests. It catalyzes the dismutation of superoxide radicals into oxygen and hydrogen peroxide. The latter in the presence of CAT will be neutralized (Salvi et al. 2007). In our case, SOD, CAT activities, and mitochondrial potential were altered.

Any oxidative stress may be the cause of genotoxicity. Assuredly, in case of a reaction between pesticides and the

Fig. 6 Effects of FEO on TFM-induced DNA damage. HCT116 cells were pretreated with FEO (1, 1.5, and 2%) for 2 h before TFM treatment for 24 h (400 µM). Data are expressed as the mean ± SD of three separate experiments. ###*p* < 0.001 vs. control, ***p* < 0.01 vs. TFM alone



nuclear DNA, mutagenic and carcinogenic effects may arise. This is why we tried to quantify DNA damage caused by the TFM using the comet assay. Our results indicated the oxidative damages in the DNA level in the studied cells that were exposed to this compound.

These results confirm the share of oxidative stress in the toxicity induced by benzoylurea insecticides and in particular TFM. Moreover, our results showed that TFM is an oxidizing agent in HCT 116 cells, by its ability to increase the production of ROS and to alter the normal functioning of antioxidant enzymes such as CAT and SOD.

However, the use of FEO a natural antioxidant molecule protects against the oxidative stress and the genotoxicity induced by TFM. Indeed, we demonstrated that the use of FEO decreased the generation of ROS and reduced the level of MDA in comparison with the TFM-treated cells.

A modulation in CAT and SOD activities was observed when cells were exposed to FEO, 2 h before their intoxication with the TFM.

By combining the FEO with the TFM, we found an increase in the mitochondrial transmembrane potential accompanied by a decrease in DNA damage when comparing to cells treated with TFM only.

The toxicity study of TFM on cellular and animal models is very limited. However, recently, TFM has been shown to promote metastasis of liver cancer cells (Hep G2) by interfering with hypoxia-inducible factor 1 α (Ning et al. 2018).

Moreover, diflubenzuron (DFM), a benzoylurized insecticide as well as TFM, was found to be cytotoxic which decreases cell viability in CHOK1 cells (Bayoumi et al. 2003) and Hep G2 cells (Delescluse et al. 1998) using the MTT assay and the neutral red test. Similarly, the cytotoxicity of DFM has been demonstrated in BALBC/3T3 cells by inducing cellular transformations. Indeed, it increased the lipoperoxidation and activities of CAT, SOD, and Gpx enzymes (Ilboudo et al. 2014).

The investigation of the protective effects of the FEO in vivo has been over-clarified. Indeed, Tripathi et al. (2013) showed that FEO has anticytotoxic and anti-genotoxic effects in mice treated with cyclophosphamide. Thus, they founded that FEO reduced the cytotoxic and the genotoxic effects in mice bone marrow cells. Also, the activities of catalase, SOD, GSH, and the level of MDA measured at the livers of these mice were modulated.

In the same context, FEO was able to prevent hepatorenal damages caused by sodium valproate, a medicine used as an anticonvulsant and mood stabilizer, in albino rats (Al-Amoudi 2017).

It was previously known that fennel extracts are full of many types of polyphenolic compounds (Faudale et al. 2008; Chang et al. 2013) which are characterized by their antioxidant proprieties (Choi and Hwang 2004; Chatterjee et al. 2012). These antioxidant activities are involved in

absorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides (Sofowora 1993; Singh et al. 2006).

A study in human hepatoma cell line (HepG2) showed that estragole, a very common compound found in EFO, is neither cytotoxic nor genotoxic, nor even able to cause apoptosis (Villarini et al. 2014). Mizuno et al. (2015) showed that the FEO can protect neuronal cells (GT 1–7) against the oxidative stress caused by hydrogen peroxide.

In conclusion, our study clearly demonstrates the protective role of fennel essential oil, extracted from fennel, an aromatic plant frequently used in food. In no uncertain terms, FEO is able to protect cultured intestinal cells from toxicity and genotoxicity induced by TFM.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest.

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