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Preventive Activity of Ginger (*Zingiber officinale*) Against Myelotoxicity and Hepatotoxicity Induced by Cyclohexatriene and Identification of the Most Active Compounds by GC-MS

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Abstract

This study aimed to uncover the preventive capability of *Zingiber officinale* against myelotoxicity, leukaemia, and hepatotoxicity. For the most part, this work depended on the subcutaneous injection of cyclohexatriene in rabbits to cause the illness by a synthetic strategy. In parallel, another group of rabbits was exposed to the injection of cyclohexatriene under similar conditions with the feeding *Zingiber officinale*, where it was discovered that the cyclohexatriene-induced myelotoxicity was counteracted. The histological examination additionally uncovered the hepato-defensive intensity of *Zingiber officinale*. The most pharmacologically active molecules of *Zingiber officinale* were recognised by gas chromatography–mass spectrometry (GC-MS).

Keywords

Zingiber officinale, hepato-protective activity, cyclohexatriene, myelotoxicity, GC-MS

1 Introduction

Nowadays, the main treatment for leukaemia is chemotherapy. This treatment is dangerous as much as it is effective, because alkylating antineoplastic agents target cancer cells and healthy cells at the same time.[1] In addition, chemotherapy causes marrow depression, hence the risk of bacterial and viral infections.

Phytochemicals have been shown to possess anticarcinogenic and antimutagenic properties; thus, they can play an important role in lowering different types of neoplasia.[2] Natural and dietetic nutraceuticals may possess anticancer activities, such as carotenoids which promote gap-junctional communication, *in vitro*, through the amplification of "connexin 43", or flavonoids, which modulate xenobiotic detoxification in phase I and II , or vitamin E which inhibits protein kinase C, an essential enzyme in tumour progression of certain types of cancer.[3]

In another clinical study on Chinese herbs, ginger was significantly associated with longer survival time of patients suffering from metastatic breast cancer.[2] Numerous reports indicate that phytochemicals (*e.g.*, cumin, red pepper, and ginger) can potentially prevent cancer by suppressing the nuclear transcription factor-κB (NF-κB) pathway that correlates with cancer and many inflammatory diseases.[3]

This research represents continuation of the research from the work published in 2017. Our research aims to elucidate a natural treatment based on *Zingiber officinale* by maintaining the molecular compatibility present in the plant to eliminate or reduce the side effects. Therefore, the therapeutic potential *of Zingiber officinale* was tested to prevent or cure myelotoxicity or leukaemia, as well as leave the marrow intact. Dominant research is based on *in vitro* tests.[4,5,6] The purpose of all such research was to cause apoptosis in malignant cells by *Zingiber officinale*. This work attempted to accomplish an *in vivo* test in order to demonstrate that the pharmacological activity is quite different from that of an *in vitro* test.

2 Materials and methods

2.1 Animal materials

Rabbits were kindly supplied by the Laboratory of Pharmacotoxicology, Saidal Antibiotical of Médéa, Algeria. An albino race hybrid between New Zealanders and California – the mass of one rabbit was 1.5 kg. Three male rabbits in each group. Age of rabbits: 6 to 7 months.

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Lot (1): Negative control, without treatment.

Lot (2): *Zingiber officinale* group. These rabbits were used to study the influence of the chronic consumption of *Zingiber officinale* (Gavage).

Lot (3): Positive control. Rabbits, singled out to cause bone marrow toxicity and/or leukaemia, by subcutaneously injecting 0.2 ml kg^{-1} of cyclohexatriene, three times a week.

Lot (4): Cyclohexatriene + *Zingiber officinale*. The consumption of *Zingiber officinale* by mouth at the same time with subcutaneous injection of 0.2 ml kg^{-1} cyclohexatriene three times a week.

Blood cells interval of New Zealand rabbit were: red blood cells (RBC) $4.5–7.0 \cdot 10^6$/mm^3, white blood cells (WBC) $5–12 \cdot 10^3$/mm^3, and platelet (PLA) $250–750 \cdot 10^3$/mm^3.[7]

2.2 Plant materials

Used was the natural extract of *Zingiber officinale* (imported from China) taken from fresh rhizome by a mechanical method, and dried form of *Zingiber officinale*. The same doses were administered to Lot (2) *Zingiber officinale,* and Lot (4) cyclohexatriene + *Zingiber officinale,* during the experiment.

2.3 Gas chromatography–mass spectrometry (GC-MS)

This analysis is a quality control aimed to determine the most pharmacologically active compounds. In order to extract *Zingiber officinale* oil from the dried *Zingiber officinale* rhizome, acetone was used as the extraction solvent by maceration method. The analysis was carried out at the Laboratory of the Scientific Police of Algiers. Apparatus: GC-MS model CLARUS 500 brand Perkin-Elmer.

2.4 GC-MS method

The experimental conditions of the GC-MS system were as follows: standard non-polar column TR 5-MS capillary, dimension: 30 Mts, ID: 0.25 mm, film thickness: 0.25 μm. Flow of the mobile phase (carrier gas: He) was adjusted to 1 ml min^{-1}. Temperature program (oven temperature) was raised from 40 °C to 220 °C at 4 °C min^{-1}, and the injection volume was 1 μl. Samples were run fully at a range of 20 and 550 m/z, and the results were compared using the Wiley and NIST Spectral Library Search Programme.

2.5 Histology and histological stain (liver)

The tissue was fixed with 10 % formalin, dehydrated in successive alcohol baths at increasing concentrations 70 % – 95 % – 100 %, and making of paraffin blocks using inclusion cassettes, then stored at +4 °C for 24 h, according to two studies.[8,9] Cutting ribbons were produced by using the microtome (type 820 Rotary Microtome) set at 4 microns. Tissue was deparaffinised by hydration of the slides in a battery containing: 2 toluene baths, 3 alcohol baths at decreasing concentration: 100 % – 95 % – 70 %, 1 bath of distilled water. Finally, the tissues were stained with hematoxylin and eosin stain (H&E stain).[8,9]

2.6 Statistical analysis

Statistical analysis was performed using STATISTICA software, version 10.0.1 (STATISTICA Inc, LBMPT, Médéa, Algeria) for Windows. Data were analysed and presented as means ± SD. Differences between continuous data were analysed using one-way ANOVA. $P < 0.05$ was considered significant.

3 Results and discussion

3.1 Haematological behaviour during one year

The collection of blood was taken from the marginal ear vein of the rabbit. The blood was then put into EDTA tubes, and the *Automatic Hematology Analyser (ERMA PCE210)* at the Hematology Laboratory, Hospital of Médéa, was used for the analysis, which automatically gave CBC (complete blood count).

The analysis was carried out every 61 days.

3.1.1 Interpretation

The positive control Lot (3): made a leukocyte fall from the first three months. These values continued to fall significantly ($P < 0.005$) compared to the negative control Lot (1), until they had a severe leukopenia by the end of the experiment (Figs. 1(A) and 1(D)).The number of leukocytes fell below 2500/mm^3 for the positive control Lot (3). These values fell below the limit of healthy rabbits ($5–12 \cdot 10^3$/mm^3).[7] These values continued to fall until the end of the experiment, when a slight increase was noticed (Figs. 1(A) and 1(D)), indicating that the cyclohexatriene had affected the cell precursors.

The dominant side effect of the cyclohexatriene is myelotoxicity. This can harm the bone marrow, leading to anaemia and leucopoenia. Animals with aplastic anaemia often develop myelodysplasia, leading in general to acute myeloid leukaemia.[8]

It is essential to understand the metabolism of cyclohexatriene, in order to determine the mechanisms of toxicity. The metabolism of cyclohexatriene occurs mainly in the liver, although metabolism in the bone marrow is believed to play a crucial role in the myelotoxicity of cyclohexatriene, because bone marrow is rich in peroxidase activity. It is likely that oxidative stress contributes to the toxicity of cyclohexatriene in a way that the initial metabolic step is the oxidation of cyclohexatriene to an epoxide (*i.e.,* d. cyclohexatriene oxide), which is catalysed primarily by CYP2E1 liver.[8]

For Lot (4) cyclohexatriene + *Zingiber officinale*, it was observed that rabbits surmount the action of cyclohexatriene,

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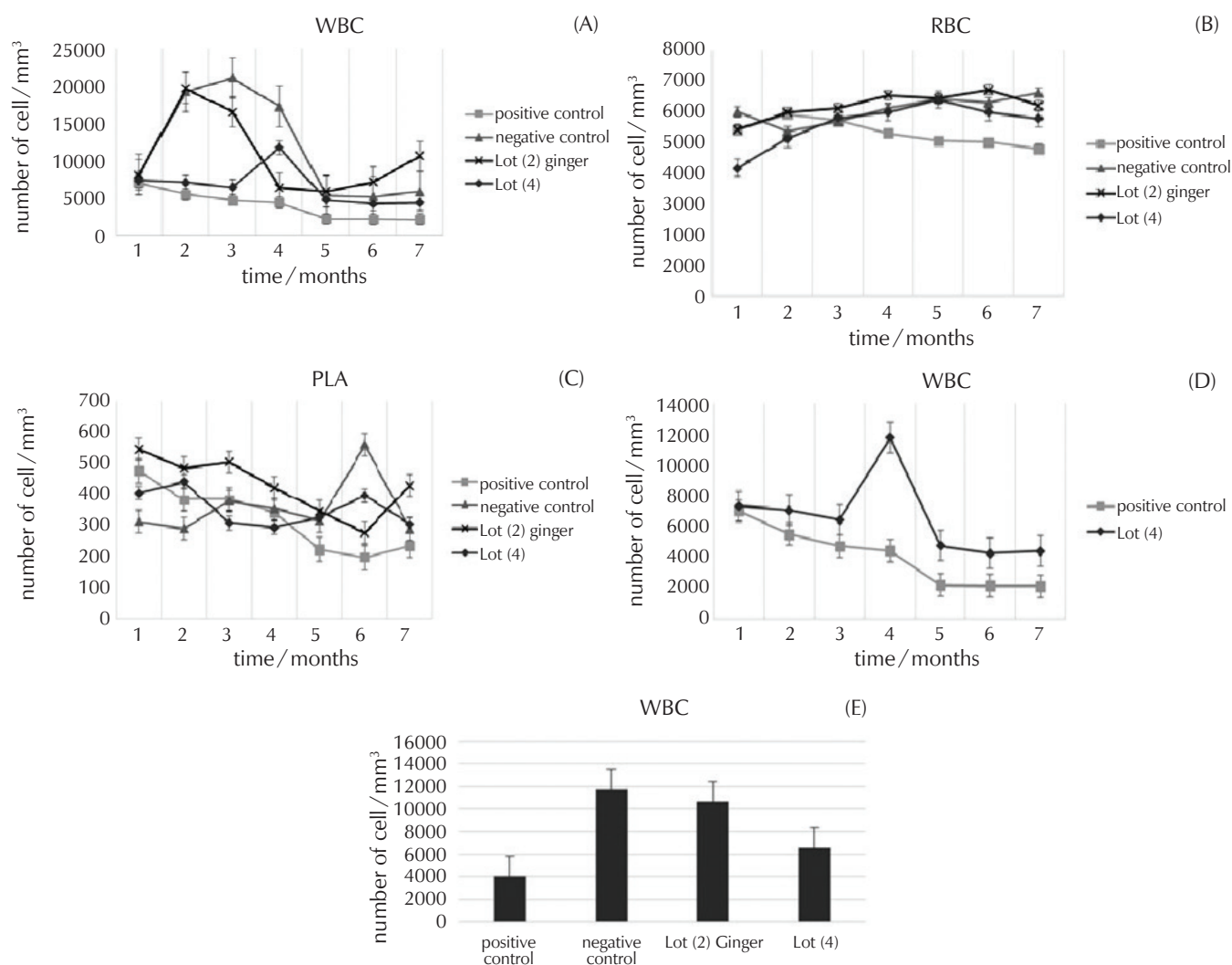
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Fig. 1 – (A) Curve representing the kinetics of white blood cells during the experiment. (B) Curve representing the kinetics of red blood cells during the experiment. (C) Curve representing the kinetics of platelet during the experiment. (D) Curve representing the kinetics of white blood cells during the experiment (positive control and Lot (4)). (E) Histogram represents the average level of white blood cells during the experiment.

as cellular parameters of blood were kept in the rabbits' hygienic interval, and we can save this resistance from the initial 3 months, so ginger thwarted myelotoxicity of cyclohexatriene in spite of the low portion administrated in this period hundredth LD_0 of the natural extract (1/100 LD_0: Adaptation of rabbits).[10]

N.B.: LD_0 (NOAEL) No Observed Adverse Effect Level is the most elevated portion that causes no lethality and no mortality.[7] It is 75 g kg^{-1} of fresh rhizome and 7.5 g kg^{-1} of powdered *Zingiber officinale*.[11] (1/x) LD_0: in order to find the lower dose that gives the best activity.[10]

In the next three months in which the rabbits received a high portion of ginger, 1/35 LD_0 of the natural extract (study of the preventive impact), it was observed that the amount of leukocytes (WBC) increased significantly ($P < 0.005$), indicating a safe upgrade prompted by ginger (Figs. 1(A) and 1(D)).

From that point forward, it was attempted to lower the portion of ginger to 1/70 LD_0 (natural extract) for three months, with the end goal to discover the connection between dose and activity accountable for the decline in leukocytes. However, the values failed to exceed the lower furthest reaches of the hygienic interval.[10]

From there on, the two forms of ginger (fresh natural extract + powder) were combined in a portion of 1/35 LD_0, allowing for the synergistic impact of the two plant forms, where the blood parameters were settled in the rabbits' hygienic interval.[10]

For negative control (Lot (1)) and *Zingiber officinale* group (Lot (2)) (Fig. 1(A)), a relatively indistinguishable leukocyte improvement was observed, indicating that ginger, in various dosages, had no haematological unsettling influence, on the first supposition that ginger action occurs particularly within the sight of myelotoxicity.[10]

3.2 Evaluation of the blood smear after bone marrow depression (positive control Lot (3)), and cyclohexatriene + *Zingiber officinale* group, Lot (4)

Morphological perception of polynuclear neutrophils (PNN) in all rabbits of this group, Lot (3), demonstrates a hyper poisonous granulation (Figs. 2(A) and 2(B)). This indication affirms that cyclohexatriene influenced the bone marrow by practicing its mutagenic impact and, along these lines, we recorded serious pancytopenia (3 lined falling WBC, RBC, PLA). This cell fall takes esteems underneath the lower interval value (Figs. 1(A), 1(B), and 1(C)). We noticed that the group injected with cyclohexatriene and subjected to a ginger gavage, Lot (4), reveals cell morphology flawless and non-pathogenic, in light of the fact that there is no indication of intoxication (Figs. 1(A), 1(D), and 1(E)) and (Figs. 2(C) and 2(D)). It should be pointed out that the 1/35 DL_0 from the ginger natural extract improved the best opposition to cyclohexatriene-instigated cell fall.[10]
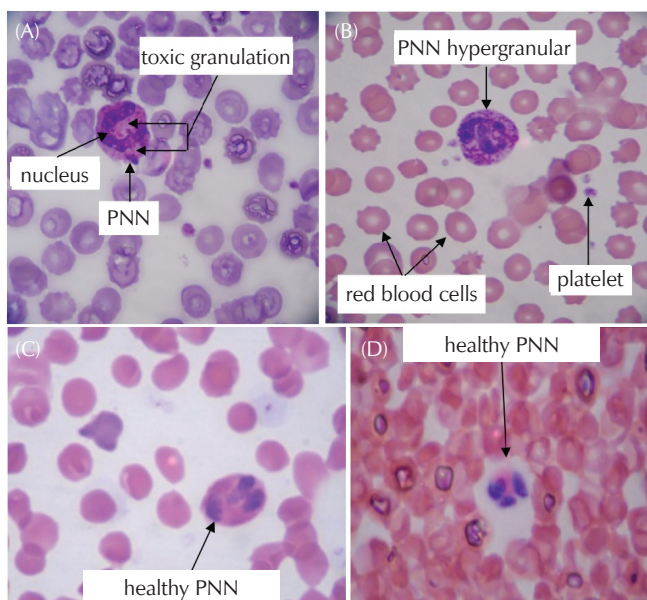

Fig. 2 – (A) and (B): Blood smear observed by optical microscopy, Lot (3) positive control (×100). (C) and (D): Blood smear shows healthy cells, Lot (2) ginger (×100).[10]

3.2.1 Perception of medullary gram after bone marrow injury

With the end goal to affirm the bone marrow injury, it is important to prepare the marrow smear. The specimen was taken using a sterile trocar by entering the iliac bone. At that point, the marrow was taken with a normal syringe (Fig. 3(E)). The smear demonstrates the morphology of the bone marrow antecedent cells.[10]

3.2.2 Interpretation

There was a hypertoxic granulation in the rabbits' marrow smears (positive control Lot (3)) (Figs. 3(A), 3(C), and 3(D)), and additionally, the observation of myelodysplasia phe-

nomena (Fig. 3(D)). Knowing these indications of myelodysplasia regularly precede development of leukaemia.[12,13,14] A few rabbits from the positive control Lot (3) had exceptionally poor marrow as a result of extreme bone marrow injury (Fig. 3(B)). Later, all animals from the positive control passed on following extreme pancytopenia, even before affirming the accomplishment of rabbits' leukaemia. However, the analysis revealed the dominance of acute leukaemia, which frequently showed as pancytopenia,[15] because all the rabbits of positive control Lot (3) had died.[10]
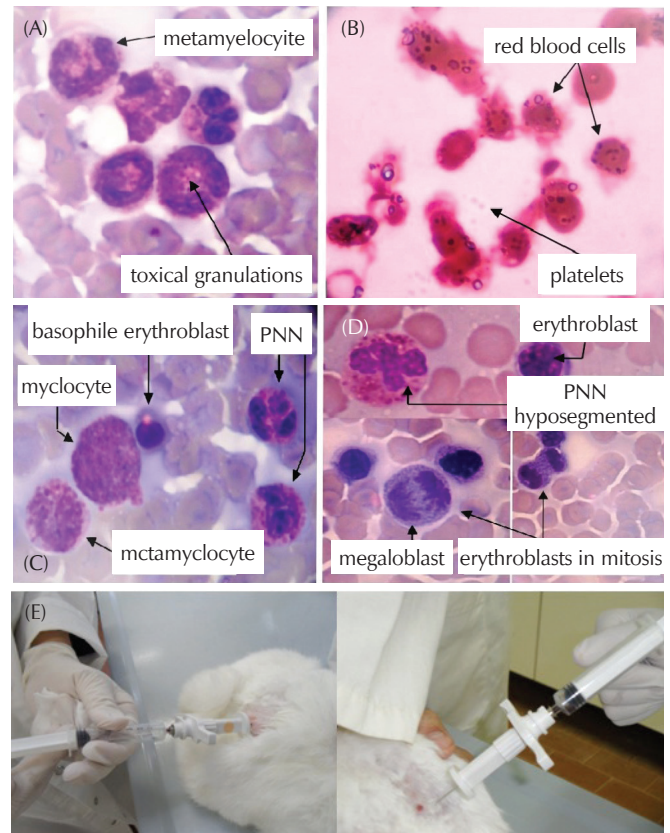

Fig. 3 – (A) Marrow smear: hyper toxic granulation Lot (3) Positive control. (B) Marrow smear: weak medullar smear in some rabbits Lot (3) positive control. (C) Marrow smear: hyper toxic granulation Lot (3) positive control. (D) Marrow smear: sign of myelodysplasia Lot (3) positive control. (E) Marrow sampling technique.[10]

3.3 Anatomy investigation of tissue (dissection)

3.3.1 Liver

The microscope revealed a normal liver parenchyma in negative control Lot (1) (Fig. 4(A)). In the group submitted to chronic gavage of ginger Lot (2): the hepatic parenchyma showed a normal appearance, without injury (Fig. 4(B)). In rabbits exposed to injection of cyclohexatriene (positive control Lot (3)), there was a cytoplasmic cell elucidation, with poisonous granulation in the whole specimen. These indications of inebriation affirm the hepatotoxic activity of cyclohexatriene (Figs. 4(C) and 4(D)). It might flag the non-appearance of tumour invasion.

Concerning the gavage of *Zingiber officinale* and injection of cyclohexatriene in parallel, a heterogeneous appearance can be seen because of ordinary liver cells, healthy in their greater part, and a few cells with cytoplasmic granulations and elucidation (Figs. 4(E)). In this way, the progressions and cell harm instigated by cyclohexatriene in positive control Lot (3), were fundamentally diminished in the Lot (4) by the accompanying activity of *Zingiber officinale*, subsequently the term hepato-defensive ginger.
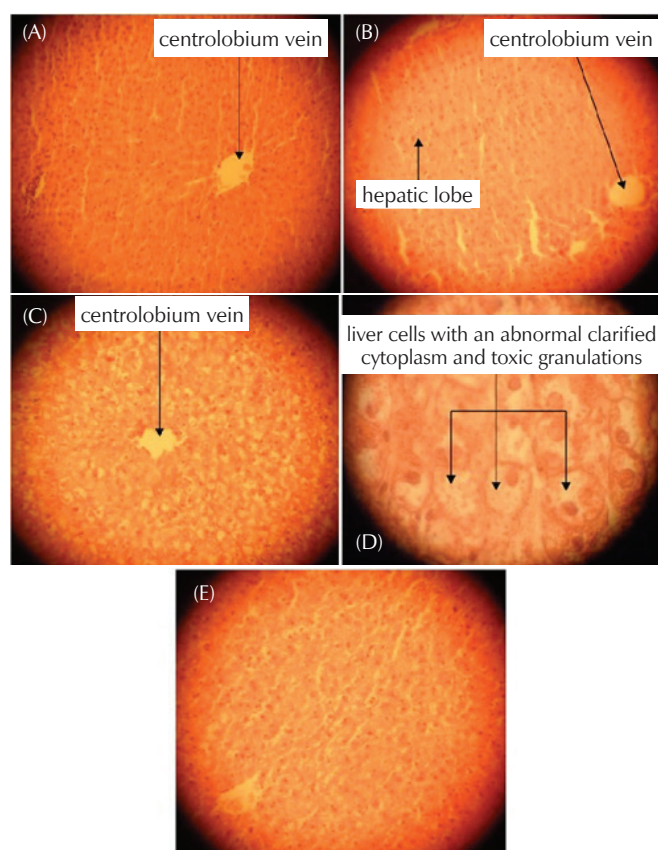

Fig. 5 – GC-MS spectrum of ginger acetone extract

Fig. 4 – (A) Hepatic parenchyma of a healthy histological aspect Lot (1) negative control (×10). (B) Hepatic parenchyma of a healthy histological aspect Lot (2) ginger (×10). (C) Hepatic parenchyma cell showing an interesting clarification in almost all tissues Lot (3) positive control (×10). (D) Hepatic parenchyma cell showing an interesting clarification in almost all tissues Lot (3) positive control (×40). (E) Hepatic parenchyma with few clarified cells, Lot (4) cyclohexatriene + ginger (fewer cells with clear cytoplasm) (×10).[10]

3.3.2 Analysis of Zingiber officinale extract with GC-MS

The gas chromatography mass spectrum GC-MS, revealed that acetone *Zingiber officinale* extract contained eight different molecules (Fig. 5), with a characteristic retention time and molecular weight of each molecule (Table 1).

Major constituents of *Zingiber officinale* extract identified by gas chromatography and mass spectrometry were β-sesquiphellandrene, curcumene, Zingiberene, and 6-shogaol.
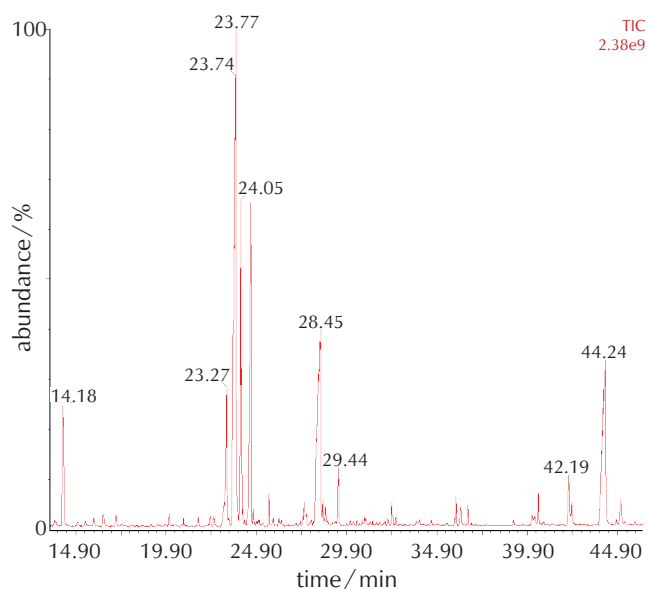
Table 1 – Molecules composing the ginger extract obtained by GC-MS analysis

Molecule name	Retention time / min	Molecular weight / g mol⁻¹
β-sesquiphellandrene	17.24	204
curcumene	23.24	202
zingiberene	23.76	204
α-farnesene	24.03	204
zingerone	28.36	195
6,10-Dodecadien-1-yn--3-ol,3,7,11-trimethyl	29.43	220
cis-6-Shogaol trans-6-Shogaol	42.15 44.19	276 276

4 Conclusion

This investigation reveals that ginger contains a compelling preventive potential against the blood-harmfulness and hepatotoxicity initiated by cyclohexatriene. In addition, as cyclohexatriene fundamentally targets the bone marrow by causing DNA mutations that harm hematopoietic cells, which end up being the wellspring of expansion of cancer-causing cells and the beginning of leukaemia, ginger can protect against leukaemia and against marrow harm instigated by xenobiotic factor. Thus, it is obvious that ginger is a compelling defender against natural factors. The remedial action of ginger against leukaemia should be further investigated.

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SAŽETAK

Preventivna aktivnost đumbira (*Zingiber officinale*) u suzbijanju mijelotoksičnosti i hepatotoksičnosti izazvane cikloheksatrienom i identifikacija najaktivnijih spojeva metodom GC-MS

Khaled Otmanine,[a,*] *Salah Hanini*,[b] *Othmane Benkortbi*,[b] *Razika Bekri*,[c] *Karim Amroun*,[d] *Merouane Djellal*,[e] *Sara Kohli* [a] i *Karine Oumouna-Benachour*[e]

Cilj ove studije bio je otkriti preventivnu sposobnost *Zingiber officinale* u suzbijanju mijelotoksičnosti, leukemije i hepatotoksičnosti. Ovaj je rad većim dijelom temeljen na potkožnom ubrizgavanju cikloheksatriena u kuniće da bi se sintetskom strategijom izazvala bolest. Usporedno s time, druga skupina kunića bila je izložena injekciji cikloheksatriena u sličnim uvjetima s hranjenjem *Zingiber officinale*, gdje je otkriveno da je suprotstavljena mijelotoksičnost izazvana cikloheksatrienom. Histološkim pregledom dodatno je otkriven hepatoprotektivni intenzitet *Zingiber officinale*. Najviše farmakološki aktivnih molekula *Zingiber officinale* prepoznato je metodom plinske kromatografije–masene spektrometrije (GC-MS).

Ključne riječi
Zingiber officinale, hepatoprotektivno djelovanje, cikloheksatrien, mijelotoksičnost, GC-MS

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