



## ***In Vivo* and *In Vitro* Evaluation of Genotoxicity of four Common Umbelliferous Plants**

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### **ABSTRACT**

This study aimed to evaluate the safety of some plants that belongs to the Family Apiaceae on human health which are used daily as food spices. Four plants had been chosen namely, *Foeniculum vulgare*, *Cuminum cyminum*, *Coriandrum sativum* and *Carum carvi*. An *in vivo* study had been conducted with modern molecular techniques to determine the genotoxicity and mutagenicity for the study plants on rat's genome through random amplification of polymorphic DNA analysis. The rats were orally administered the aqueous extracts of the plants for a daily single dose of 5ml for 28 days. Results have shown that all the four plant extracts do not cause any genotoxic or mutagenic effects on the rat's genome. Furthermore, an *in vitro* study was also performed on the human lymphocytes model by using Comet assay and evaluate the antigenotoxic or antimutagenic effects of the four plant extracts against a mutagen (*Rhazya stricta* leave extract). The safety and prophylactic effects of this mutagenic plants on human health and the antigenotoxic or antimutagenic effects of all the four plant extracts against *R. stricta* was evaluated by exposing the human blood lymphocytes to each plant extract alone and in combination with the extract of the mutagenic agent. The results showed that all the four plant extracts are genetically safe on the human lymphocytes when used alone, and showed preventive properties against mutagenic agents when mixing it with mutagenic materials. It was concluded from the study that the daily use of these plants as medicinal or culinary purposes has no harm to humans. Additionally, it can confer protective effects upon the genetic material against many harmful physical and chemical conditions. It has been recommended that all the four plant extracts are neither genotoxic nor a threat to people who use them for various medicinal or culinary purposes. Furthermore, it has also been recommended more expanded researches on these plants to take advantage of the positive aspects of the curative and preventive properties possess by these plants.

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## INTRODUCTION

Genotoxicity describes a lethal effect on the genetic material (DNA, RNA) of a cell that affects its integrity. Genotoxin is a substance which possess the ability of genotoxicity. Genotoxins are mutagens and can induce mutations. Genotoxins include both radiation and chemical genotoxins.<sup>1</sup> Genotoxins can be carcinogens, mutagens or teratogens. Mostly genotoxicity induces mutations in different cells and other systems of the body. Mutations can be the main cause of other problems, including cancer and a wide variety of diverse diseases.<sup>1</sup>

Nature has bestowed us with a vast variety of medicinal plants. Active components in medicinal plants could be bio-antimutagenic (protective against initiation, promotion or progression of carcinogenesis) and demutagenic (blocking or destroying the DNA-damaging mutagens outside the cells), hence averting cell mutations.<sup>2</sup> Medicinal plants being a source for a wide variety of natural antioxidants have also antigenotoxic, antimicrobial, anti-cancer, anti-diabetic, anti-atherosclerosis, immunomodulatory, and even reno-protection or hepatoprotective properties.<sup>3</sup>

Umbelliferous (Apiaceae family) medicinal plants- Fennel (*Foeniculum vulgare* Mill.), Cumin (*Cuminum cyminum* Linn.), Coriander (*Coriandrum sativum* Linn.) and Caraway (*Carum carvi* Linn.) possess antigenotoxic and antioxidant properties.<sup>4,5,6</sup> *C. carvi* and *C. cyminum* are among the ancient herbs cultivated in Africa, Asia and Europe. Cumin and caraway seeds have been considered as favorite culinary spices and are also robustly used in folklore therapy since ancient past in different geographical regions. Essential oils are produced from the cumin and caraway seeds. Therefore, these seeds have been keenly examined for their biological activities and for chemical composition. Cumin and Caraway have antioxidant, antimicrobial, anticarcinogenic/antimutagenic, antidiabetic, diuretic, immunomodulatory, estrogenic/anti-osteoporotic, gastrointestinal and other biodynamic properties. It has been researched that food rich in cumin and caraway was found to block the onset of colon cancer in rats, caused by a colon-specific carcinogen, 1, 2-dimethylhydrazine (DMH).<sup>6</sup> The debilitation of carcinogenicity by caraway and cumin has been ascribed to their potential antioxidative action in the target tissues.<sup>4,7</sup> Various studies have connected the anticarcinogenic actions of caraway and cumin to their potential antimutagenic, antiproliferative and apoptotic properties.<sup>4</sup>

*Coriandrum sativum*, a popular culinary spice is medicinal herb belonging to Apiaceae family. Every part of coriander is used as flavoring agent. It has been used as folklore medicine or traditional remedy for the treatment of different diseases in different civilizations.<sup>8</sup> *C. sativum* root also carries the anticancer and antioxidant properties. It has a potential to prevent

diseases related to oxidative stress and can be used as a beneficial supplement in combination with conventional drugs to improve the treatment of diseases like cancer.<sup>9</sup> Studies have also clearly demonstrated that *Corriandrum sativum* (coriander) oil, a member of the Apiaceae family, has an antibacterial and antifungal activities against all a host of tested microbes with the notable exception of *Pseudomonas aeruginosa*.<sup>10</sup> *Foeniculum vulgare* is a solid perennial herb with feathery leaves and yellow flowers. The foliage, bulb and seeds of this plant are used in different culinary traditions around the world. Aqueous extracts of fennel proved to have antioxidant activity higher than some well-known antioxidant such as ascorbic acid.<sup>11</sup> The fennel extract as an antioxidant shows effects in reducing malondialdehyde (MDA) level and increasing plasma superoxide dismutase (SOD) as well as catalase activities.<sup>12</sup> In addition, *F. vulgare* is claimed to possess analgesic, hepatoprotective, diuretic, immune booster and bronchodilatory properties. It is also reported that Fennel extract may have slight genotoxic effects on mice rather than *Drosophila*.<sup>13</sup>

Antioxidants play a major defensive role against oxidative stress caused by reactive oxygen species (ROS) generation during genotoxicity.<sup>14</sup> These antioxidants obstruct lipid peroxidation by supplying hydrogen atom to ROS in lieu of polyunsaturated fatty acids present in lipid membrane.<sup>15</sup> Since the extracts from all the four plants of Apiaceae family mentioned above have antioxidant properties. But the product from some medicinal plants are genotoxic as well.<sup>13</sup> Detecting genotoxic effects of various compounds and their mixtures in different components of environment has called for constant surveys in pharmacy and biology. A group of accepted *in vitro* and *in vivo* genotoxicity test systems has been set up on various organisms, such as *Drosophila*, mammals, bacteria and yeast.<sup>16,17</sup> A genotoxic effect can result from a large variety of possible injuries to genetic material in cells, from single-strand DNA breakages to chromosomal changes, and it is of major importance in genotoxicity testing to develop methods that can reliably, with sufficient sensitivity, detect either such a vast array of damage or a general cellular response. No single test can detect the effect of every substance, and the concept of a battery of tests has therefore been implemented in many regulatory guidelines.<sup>18</sup> The test protocols used for genotoxicity testing are used to test the antimutagenic and antigenotoxic effects of substances.<sup>19</sup> This can uncover the capacity of substances for preventing or repairing damage to the genetic material in a cell.

*Rhazya stricta*, is an important medicinal plant that are widely used in popular medicine to treat a wide range of diseases across many countries. Many studies have highlighted its biological effects on rats, rabbits and guinea pigs. Despite the importance of the *R. stricta* extract, a number

of reports indicated its toxic effects and mutagenic behavior.<sup>20</sup> hence; this plant was selected for this study as a mutagenic agent which was used with lymphocytes in humans in order to understand the mutagenic abilities of the aqueous extract and its impact on the human genetic system. The plant was used in its widely-administrated dose in a 48-hour period along with other phytogetic extracts from the Apiaceae family as means to study the Apiaceae plants' resistance to *R. stricta* since these plants contain antioxidants as well as antimutagenic, antifungal and antimicrobial agents. The aim of this study was to evaluate genotoxicity, antigenotoxic and mutagenic effects of *Rhazya stricta* and Fennel (*Foeniculum vulgare*), Cumin (*Cuminum cyminum*), Coriander (*Coriandrum sativum*), and Caraway (*Carum carvi*) of Apiaceae family by using *in vivo* and *in vitro* studies.

## MATERIALS AND METHOD

### Chemicals

All chemicals were purchased from BHD laboratory supplies (Analar<sup>®</sup>, England) and were of molecular biology grade.

### *In vivo* study

#### Plant seeds aqueous extraction

Seeds of the four Apiaceae plants (*Foeniculum vulgare*, *Cuminum cyminum*, *Coriandrum sativum*, and *Carum carvi*) were purchased from a local market in Jeddah, Kingdom of Saudi Arabia (KSA). The seeds were cleaned with tap water to remove dust and dried at 37°C for 36 hours. The cleaned seeds were then ground to soft powder and packed in nylon bags. Ten percent extract solution was prepared by dissolving 10 gm of powder in 100 ml of boiled distilled water for 10 minutes. The extracted solution was then filtered by using filter papers and stored in a refrigerator up to the time of use.

#### *Rhazya stricta* leave extraction (Mutagenic agent)

Samples of *Rhazya stricta* leaves were collected from Mecca-Jeddah highway. The leaves were immediately stored in an icebox and transferred to the laboratory. Leaves were dried in shades for a few days and 40 grams of the dried leaves was weighted, ground to fine powder by using an electric blender. The weight required was the common dose of 6 grams/liter. The solution was put on an automatic shaker for 24 hours then filtered by using filter paper. The extract was then stored in a refrigerator up to time of use.

### Test Animals

A total of 25, 90 days old female Wister Albino rats, weighing 150-250 gm were purchased from King Fahad Medical Research Center (KFMRC) in Jeddah, KSA. The rats were kept in groups of five animals per polycarbonate cage with wood shaving as bedding at a temperature of  $22 \pm 2^\circ\text{C}$  and relative humidity of 40-65% with 12hrs/12hrs light/dark cycles throughout the experimental period. The rats were fed laboratory chow, which was supplied by Grain Silos and Flour Mills Organization (Jeddah, KSA), Tap water in plastic bottles with steel sipper tubes were used for an ad-libitum supply of water. All the animals were allowed to acclimatize at the experimental environment for 3 days before the treatment initiation. The use of animals and experimental design were approved by unit of biomedical ethics, King Abdulaziz University Medical College (Jeddah, KSA), which are in compliance with the national and international laws and policies (NIH Guiding Principles in the care and Use of Animals).

### **Experimental design**

The rats were divided into five groups, each group contained five rats. Group 1 (control) was given 5ml of distilled water, group 2 was given *F. vulgare* seed extract, group 3 was given *C. cyminum* seed extract, group 4 was given *C. sativum* seed extract and group 5 was given *C. carvi* seed extract. Aqueous extracts of all the four plant seeds and distilled water (for the control) were orally gavaged to the respective groups at a daily single dose of 5ml for 28 days.

The administration of aqueous plant extracts was done by using a metallic needle curved-ball ended (Size PS-20).

### **Rat blood collection and DNA extraction for RAPD analysis**

After 28 days of exposure, 1ml of blood samples was collected from retro orbital sinuses of all the rats in EDTA tubes and stored immediately at  $80^\circ\text{C}$  until the time of analysis. DNA from the rat blood samples were extracted by using QIAamp DNA mini kit.

### **RAPD Analysis**

A total of 25 random primers (Operon Technologies, USA) of RG and Ra series were used as primers for the RAPD analysis (Table 1). The PCR amplification was carried out using PCR parameters as follows. PCR amplification reactions were contained 30 ng of genomic DNA, 10nM Tris HCl (pH9), 4.0mM  $\text{MgCl}_2$ , 50mMKVI and 0.01% gelatin, 2.0 of 1.25 mM each of dNTP's, 15 ng of the primer, 1.5 U *Taq* DNA polymerase in 30ul reaction volume. DNA amplification was performed in a Perkin Elmer Cetus 480 DNA Thermal Cycler programmed for 45 cycles as follows: 1st cycle of 2.0 min at  $94^\circ\text{C}$ , 1 min at  $45^\circ\text{C}$ , 2 min at  $72^\circ\text{C}$ ; followed by 44 cycles each of 1 min at  $92^\circ\text{C}$ , 1 min at  $35^\circ\text{C}$ , 2 min at  $72^\circ\text{C}$  followed by one final extension cycle of 8 min at  $72^\circ\text{C}$ . The amplification products were resolved by electrophoresis in 1.2% (w/v)

agarose gels with 0.5×TBE buffer gel at 60V for 4h. Controls lacking templet DNA were included in each primer reaction mix. Amplification products were stained with ethidium bromide, DNA ladder (3 kb) was used as molecular standards and visualized under UV light and screened with gel documentation system. Each band was considered as RAPD marker. All the reactions were repeated at least twice.

### *In vitro* study

#### Human blood collection and DNA extraction for COMET assay

To further evaluate the genotoxicity of the tested plants extracts on human blood lymphocytes, 1ml blood sample was collected from a volunteer, 26 years old healthy female. The blood sample was collected in EDTA tube and stored immediately at 80°C until the time of assay. The human DNA was extracted from the blood sample by using QIAamp DNA mini kit.

**Table 1: Sequences of the primers used in this experiment**

| Primers No. Code | Nucleotide sequences | G+C % | Primers No. Code | Nucleotide sequences          | G+C % |
|------------------|----------------------|-------|------------------|-------------------------------|-------|
| 1 RG1            | 5`AGT CAG CCA C`3    | 60%   | 14 RG14          | 5`GTG AGG CGT C`3             | 70%   |
| 2 RG2            | 5`AAT CGG GCT G`3    | 60%   | 15 RG15          | 5`CCG CAT CTA C`3             | 60%   |
| 3 RG3*           | 5`AGG GGT CTT G`3    | 60%   | 16 RG16          | 5`GTG GAT GCG A`3             | 60%   |
| 4 RG4            | 5`GAA ACG GGT G`3    | 60%   | 17 RG17          | 5`AGG ACC AGG`3               | 66.7% |
| 5 RG5            | 5`GTG ACG TAG G`3    | 60%   | 18 RG18          | 5`AGG GCC CGG G`3             | 90%   |
| 6 RG6            | 5`TCT GTG CTG G`3    | 60%   | 19 RG19          | 5`ATC GAG GAG`3               | 55%   |
| 7 RG7            | 5`TTC CGA ACC C`3    | 60%   | 20 RG20          | 5`GGC AAG CTG GTG GGA GGT AC3 | 65%   |
| 8 RG8            | 5`AGC CAG CGA A`3    | 60%   | 21 Ra1           | 5`CGG CCACTGC`3               | 80%   |
| 9 RG9            | 5`GAC CGC TTG T`3    | 60%   | 22 Ra2           | 5`CGG CCA CTG N-Wobbles`3     | 75%   |
| 10 RG10          | 5`AGG TGA CCG T`3    | 60%   | 23 Ra3           | 5`CGG CCA CTN C`3             | 75%   |
| 11 RG11*         | 5`GTT GGT GGC T`3    | 60%   | 24 Ra4*          | 5`CGG CCA CNG C`3             | 85%   |
| 12 RG12          | 5`GTC CAC TGT G`3    | 60%   | 25 Ra5           | 5`CGG CCA NTG C`3             | 75%   |
| 13 RG13          | 5`TCG GCA CGC A`3    | 70%   |                  |                               |       |

\*Primer containing result

#### Alkaline COMET Assay

Alkaline comet assay was performed according to the protocol of Baeshin, et al.<sup>21</sup> and was analyzed using Loat's Comet Assay Software with extended dynamic range imaging (EDRI). A total of 2 µL of whole blood taken from the rats, initially collected in EDTA tubes, was mixed with 100 µL of 1% low melting point agarose, after it was cooled to 37°C. Plating of 75µL of agarose mixture was performed in a dimmed light using Trevigen comet slides, which are specially treated to promote adherence of low melting point agarose, then slides were placed on ice for 8 minutes to allow agarose to solidify. After that, a gentle cell lysis was performed by immersing the slides in cold lysis buffer (2.5M NaCl, 100 mM EDTA, 10 mM Tris base, 1%

sodium lauryl sarcosinate, pH 10 and 1% Triton X-100 and 10% DMSO added just before use) for 30-60 minutes at 4°C. The excess buffer was tapped off the slides and they were immersed in freshly prepared alkaline solution, (300 mM NaOH pellets and 1mM EDTA, pH 14 and allowed to cool before use) for 20-60 minutes at room temperature in the dark, to unwind the DNA and hydrolyze sites of damage, and then slides were removed from the alkaline solution with gentle removal of excess buffer as before. Slides were then transferred to a horizontal electrophoresis apparatus (CH420, UK) and alkaline solution added. Electrophoresis was conducted for 20 minutes at 1 Volt/cm, 300 mA, in an ice bath and under dim light to minimize light induced DNA breaks. Finally, after gentle removal of excess electrophoresis solution, the slides were placed in Tris buffer (pH7) for 10 minutes to neutralize alkali, and then immersed in absolute ethanol for 5 minutes to complete precipitation of DNA and accelerate dehydration. Slides were then drained, air dried, and then stained with 1µg/ml of ethidium bromide for 5 minutes, followed by washing for 5 minutes with distilled water. Analysis of comet tail was done by Loat's single gel comet assay software with EDRI and observed with fluorescent microscope, Olympus, BX-51(Japan). Two parameters were used to determine genotoxicity: (a) comet frequency (nuclei with DNA damage) in 50 randomly selected nuclei on each slide (two slides per treatment); and (b) comet tail length (DNA fragmentation), evaluated by measuring the distance (in µm) from the nuclear region to the end of the tail in 100 consecutive nuclei.

### Statistical analysis

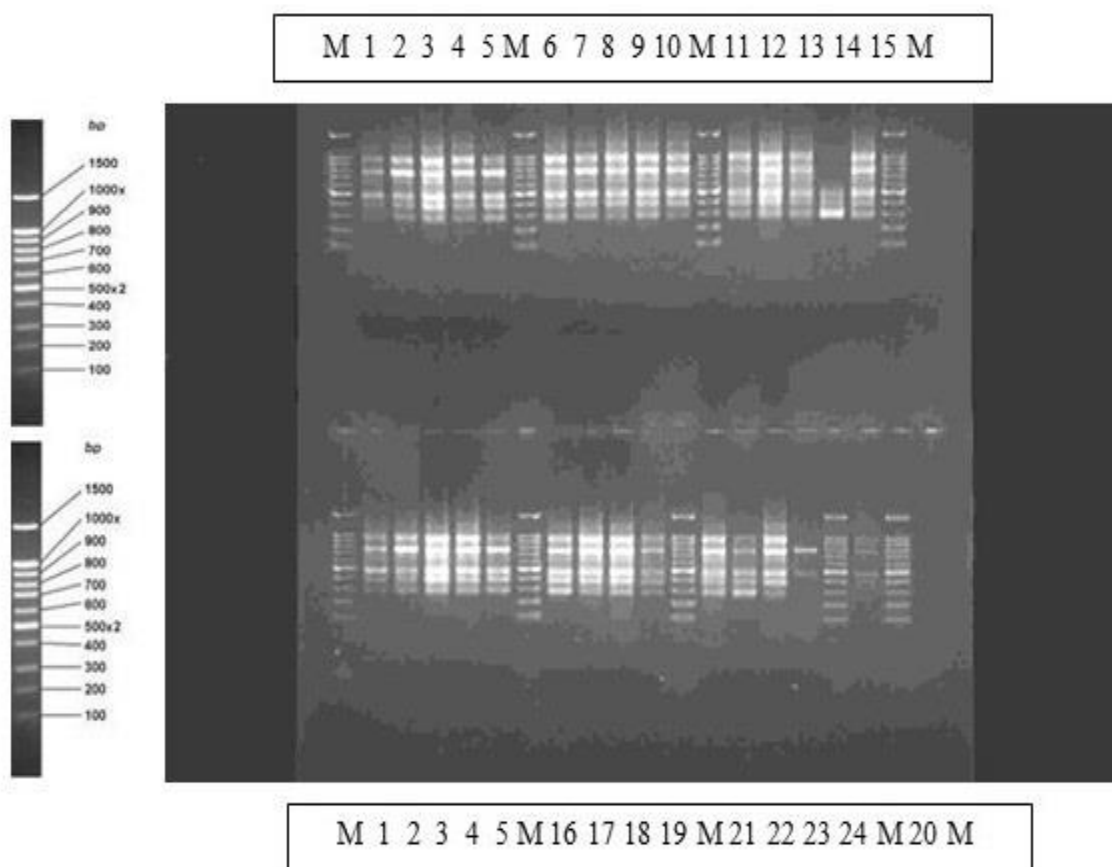
Tail length, and cell viability are reported as the mean and an analysis of variance (t-test). Differences between obtained values (mean ± SD) were compared. A P-value less than 0.05 were used as the criterion for a statistically significant difference.

## RESULTS AND DISCUSSION

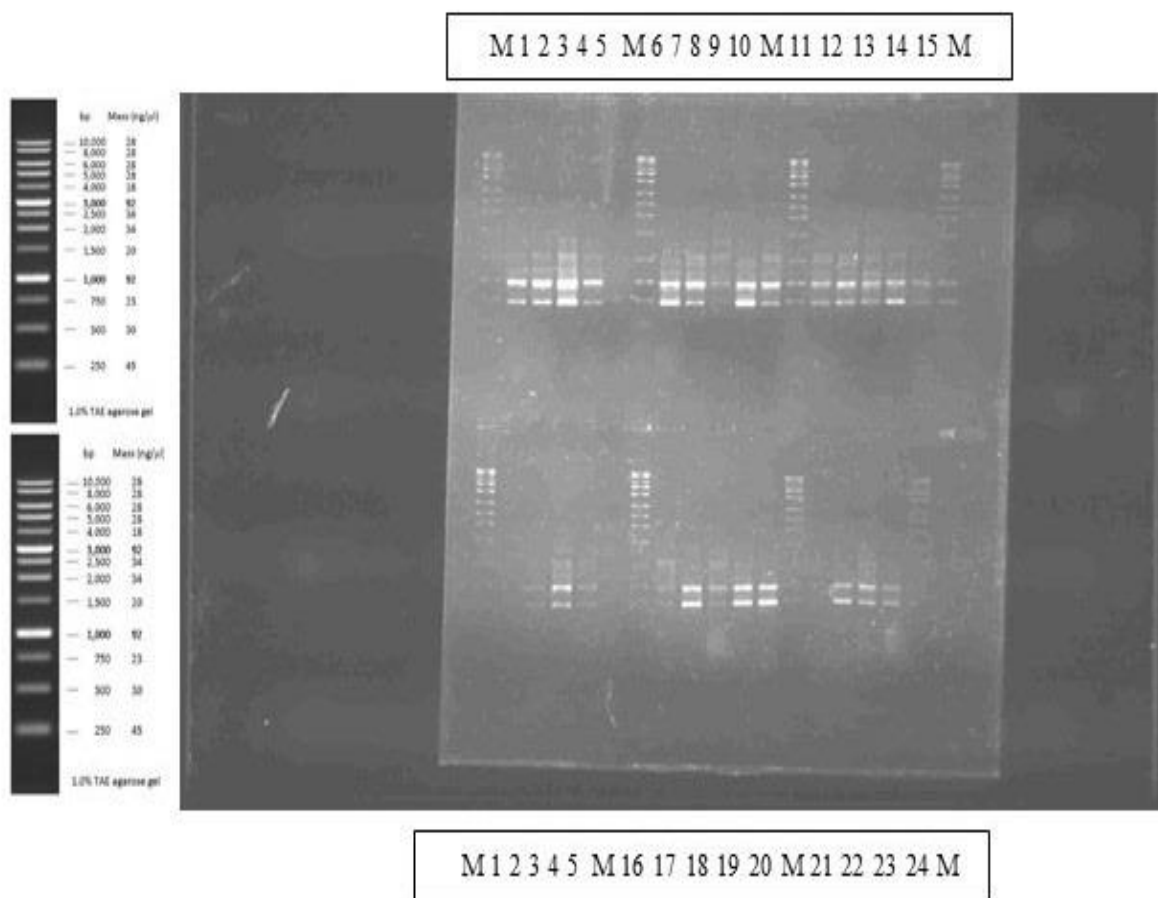
### Assessment of Genotoxicity of the plant extracts on Wistar rat

The outcomes of polymerase chain reaction using RG11 primer showed appearance of 5 groups in different volumes ranging between 1500 and 800 nucleotide pairs resulting from aqueous extracts interactions with the rat genome. It was possible to differentiate between bundles resulting from the reaction, while genome cells treated with *F. vulgare* (21, 22, 23, 24), *C. cyminum* (11, 12, 13, 14, 15), *C. sativum* (16, 17, 18, 19, 20) *C. carvi* (6, 7, 8, 9, 10) and controlled-experiment genome cells (1, 2, 3, 4, 5) showed 6 bundles. This is significant of safety when using aqueous extracts on *F. vulgare*, *C. cyminum*, *C. sativum* and *C. carvi* compared to the controlled experiment which provided the same volumes. (Figure 1.)

Twenty bundles appeared for the 5 sets at the level of amino acid. Outcomes of polymerase chain reaction using RG3 bundles in different volumes ranging between 1000 and 250 nucleotide pairs resulting from aqueous extracts interactions with the rat genome. It was possible to differentiate between bundles resulting from the reaction, while genome cells treated with *F. vulgare* (21, 22, 23, 24), *C. cyminum* (11, 12, 13, 14, 15), *C. sativum* (16, 17, 18, 19, 20) *C. carvi* (6, 7, 8, 9, 10) and controlled-experiment genome cells (1, 2, 3, 4, 5) showed 4 bundles. This is significant of safety when using aqueous extracts on *F. vulgare*, *C. cyminum*, *C. sativum* and *C. carvi* compared to the controlled experiment which provided the same volumes. (Figure 2.)



**Figure 1: Outcomes of RG11 in Rats Genome with extracts of fruits of *F. vulgare*, *C. cyminum*, *C. sativum* and *C. carvi*. M = DNA marker (1 kb)**



**Figure 2: Outcomes of RG3 in Rats Genome with seed extracts of *F. vulgare*, *C. cyminum*, *C. sativum* and *C. carvi*. M = DNA marker (10.000 pb)**

### COMET Assay

The period of the human body's lymphatic cells' exposure to *R. stricta* concentration has a significant effect on the formulation of comets. Table 2 shows the arithmetic means and standard deviations of the species of all the groups before and after adding *R. stricta*. Data shows that the highest arithmetic mean was of *F. vulgare* which was very closely followed by the control and the lowest was of *C. carvi* before adding *R. stricta*. After adding *R. stricta*, it is evident that the highest of these arithmetic means is of *R. stricta*, followed by that of the *R. stricta* with *C. sativum* and *R. stricta* with *F. vulgare*, while the lowest mean was that of the *R. stricta* with *C. cyminum*. It was noticed that the arithmetic means increase after adding *R. stricta*, 48 hours after the exposure compared to the control experiment with significant difference of  $p > 0.05$ .

**Table 2: Arithmetic means and standard deviation of all the groups**

| Species  | Standard Deviation ± Arithmetic means |
|--|---------------------------------------|
| <b>Before adding <i>Rhazya stricta</i></b>           |                                       |
| Control  | 1.22±7.99                             |
| <i>Foeniculum vulgare</i>                            | 1.2±8                                 |
| <i>Cuminum cyminum</i>                               | 1.3±5.94                              |
| <i>Coriandrum sativum</i>                            | 3.2±6.35                              |
| <i>Carum carvi</i>                                   | 0.80±5.24                             |
| <b>After adding <i>Rhazya stricta</i></b>            |                                       |
| <i>Rhazya stricta</i>                                | 13.5±121.63                           |
| <i>Rhazya stricta</i> with <i>Foeniculum vulgare</i> | 12.0±48.98                            |
| <i>Rhazya stricta</i> with <i>Cuminum cyminum</i>    | 0.94±5.75                             |
| <i>Rhazya stricta</i> with <i>Coriandrum sativum</i> | 27.5±60.78                            |
| <i>Rhazya stricta</i> with <i>Carum carvi</i>        | 6.13±6.13                             |

**Table 3. Differences between plant extracts using T test**

| Group                     | Before adding <i>Rhazya stricta</i> . |                    | After adding <i>Rhazya stricta</i> . |                    | Test Value | Statistical Significance Level |
|---------------------------|---------------------------------------|--------------------|--------------------------------------|--------------------|------------|--------------------------------|
|                           | Arithmetic Means                      | Standard Deviation | Arithmetic Means                     | Standard Deviation |            |                                |
| <i>Foeniculum vulgare</i> | 8                                     | 1.2                | 49                                   | 12                 | -12.65     | 0.000                          |
| <i>Cuminum cyminum</i>    | 5.8                                   | 1.3                | 6                                    | 0.94               | -0.22      | 0.838                          |
| <i>Coriandrum sativum</i> | 6.3                                   | 3.2                | 114.9                                | 8.5                | -30.30     | 0.000                          |
| <i>Carum carvi</i>        | 5.2                                   | 0.8                | 6.1                                  | 1.1                | -1.39      | 0.237                          |

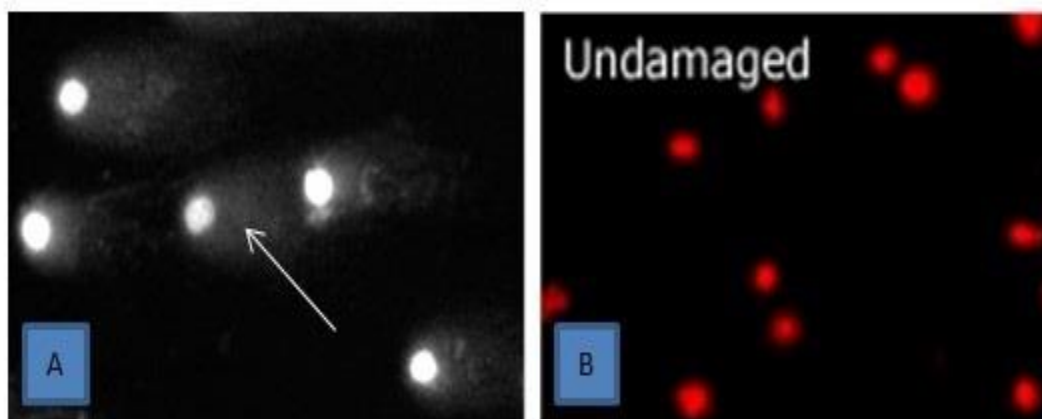
The table 3 shows the differences between the two groups before and after adding *R. stricta*. A T test was used and the differences between the two groups of *F. vulgare* was statistically significant. It was observant that the test value was  $t=-12.65$  with statistical significance of 0.000 below 0.05, which means the two groups are statistically significant. Which suggest that adding *R. stricta* to *F. vulgare* leads to significant differences between the two groups of *F. vulgare*. Whereas the test value T for the two groups of *C. cyminum* was  $t=-0.22$  with statistical significance of 0.838 above 0.05 which means that there are no statistically significant differences between the two groups. Demonstrating that adding *R. stricta* to *C. cyminum* does not lead to statistically significant change. The test value T for the *C. sativum* group was -30.30 with statistical significance of 0.000 below 0.05 which means that adding *R. stricta* to *C. sativum* leads to statistically significant difference of 0.05 between the two groups. Finally, the test value T for the two groups of *C. carvi* is -1.39 with statistical significance of 0.237 above 0.05 which suggest that there are no significant differences between the two groups of *C. carvi* after adding *R. stricta*.

## DISCUSSION

Four Umbelliferous plants namely *F. vulgare*, *C. cyminum*, *C. sativum*, and *C. carvi* were evaluated for any possible genotoxicity to rats by *in vivo* study. Studies have demonstrated the antibacterial, insecticidal, anti-inflammatory, antioxidant, anti-carcinogenic, analgesic and antihypertensive activities of *C. cyminum*.<sup>22</sup> This result bears resemblance with an earlier study in which a group of laboratory rats were exposed to *Azadirachta excels* leaves' extract yielding positive results.<sup>23</sup> The present study has also relied on this rat model as rats share a similar life cycle to humans, which is convenient for research. According to another study, extracts of *C. carvi* and *C. cyminum* are resistant to genetic toxicity while extract of *Psacalium palladium* is anticytotoxic as evident in the spleen cells of rats.<sup>24</sup> Extracts of *F. vulgare*, *C. cyminum*, *C. sativum* and *C. carvi* pose no genotoxic threats in similarity with *C. senna* extract which has been proven not genotoxic in a study relying on micronucleus assay. *F. vulgare* has been traditionally used as medicine to inflammatory diseases and recently the anti-inflammatory and antioxidant activities of the plant have been proven with the extraction of methanol from the fruits of.<sup>25</sup> It has already been established the nutritional value of *Ganoderma* plant while emphasizing the non-genotoxicity of its aqueous extract and its failure in preventing the migration of cancerous cells such as Ethyl Methanesulfonate and hence the improbability of using it as an anti-mutagenic agent.<sup>26</sup> Our present study has also demonstrated the similar failure of *F. vulgare* and *C. sativum* to act as protective agent against the mutagenic effects of *R. stricta*. The study has eventually recommended that all the four plant extracts is neither genotoxic nor a threat to people who use them for various medicinal or culinary purposes.

Aqueous extracts of *F. vulgare*, *C. cyminum*, *C. sativum*, and *C. carvi* plants were evaluated for their antigenotoxic and antimutagenic effects on human blood lymphocytes against mutagen (*R. stricta* leave extract) by *in vitro* study. Results of different reactions of the *R. stricta* leaves aqueous extract on human lymphocytes have indicated that the phyto-genic extract's concentrations have a symbolic impact on the formation of the comet cells. This has been noted through tracking the comet cells during their formation and comparing them with the control sample. Symbolic differences of  $p > 0.05$  have been observed in the average total of comet cells per each concentration upon comparison with the control experiment. These results support prior research in observing the unpacking of the comet cells and the formation of strings in the sequenced polymerization tests to Apiaceae plants' extracts. *R. stricta* is evidently capable of breaking the DNA nuclear acid strands in a peculiar way. This break is thought to take place in

one DNA interchangeably while generating chromatid gaps as a result of the deletion of one nucleotide or more, accompanied with a failure of the repair mechanisms to correct such an aberration. Moreover, it is believed that one component (or more) of the *R. stricta* extract can infiltrate the DNA or activate another component to infiltrate the DNA. Where such a compound occupies a space among the nucleotides in one of the DNA strands with the comet appearance of the active 24gm/l *R. stricta* extract. This extract is diluted to the usable popular common concentration of 6 gm/l, along with the *F. vulgare* and *R. stricta* extract as well as the *C. sativum* and *R. stricta* extract, in huge quantities. Results indicate that *C. carvi* and *R. stricta* extract as well as *C. cyminum* extract have a preventive effect against the activity of one component or more of the *R. stricta* extract. This finding is at odds with what has been reported in an earlier study of the nutritious value of the *Ganoderma* plant and its genetic toxicity using comet assays on Albino Swiss rats. The study suggested that there has been no sign of any genetic toxicity of the extract at the chromosomes level and the cell level on applying the aqueous extract to the rats. The extract has not shown any protective activity to the cells against carcinogenic substances such as Ethyl Methanesulfonate. Therefore, there could not be any protective edge to this extract against mutagenic substances.<sup>26</sup> On applying the comet cells' destruction test (comet assay), the matrix of the DNA strands is unpacked, and then breaks in areas infiltrated by *R. stricta* extract.



**Figure 3: Photographic Images of human leukocytes.**

(A) after 48hr incubation with *R. stricta*; shows DNA damage with comet tail (arrow). (B) after 48hr incubation with tested plants extracts solution, shows normal cell without DNA damage. Subjected to Single Cell Gel Electrophoresis, subsequently analyzed by Image Analysis System.

## CONCLUSION

In the light of this research, we recommend paying due attention to the importance of

constructing a gene bank for medicinal plants that are rife in our country through employing molecular techniques in documenting and preserving plants and preparing highly qualified cadres to utilize the latest technologies in this field. We further recommend conducting clinical trials in the future along with expanded research in the field to make use of the curative and preventive aspects of medicines given that preliminary studies indicate that these trials are safe as long as it adheres to aqueous extracts of medicinal plants.

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