Genotoxicity and Antigenotoxicity Activities of *Rhazya stricta* and *Zingiber officinale* Single and in Combination

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Abstract: *Rhazya stricta* and *Zingiber officinale* rhizomes Ginger are commonly used in folk medicine in the Arabian Peninsula for the treatment of many diseases. In many studies, relatively large doses of the plant extract were used to determine the pharmacological and toxicological actions. Therefore, it was necessary to study the biochemical effects of these plants using low doses, almost near the dose that is used by humans in the folk medicine. In the present study we investigated the genotoxicity and antigenotoxicity of *Rhazya stricta* leaves and *Zingiber officinale* rhizomes aqueous extracts as single and in combinations using *Salmonella typhimurium* mutagenicity (Ames) test and single cell gel electrophoresis (comet) assay at the maximum tolerant dose for each assay. Although these plants are heavily investigated but their mixtures have has not. So we intended to investigate the possible occurrence of antagonistic, additive or synergistic interactions implications for risk assessment. Result showed that none of *R. stricta* and *Z. officinale* extractions in single and in combinations showed any mutagenicity effect on the battery of *Salmonella typhimurium* mutagenicity (Ames) test and single cell gel electrophoresis (comet) assay at the maximum tolerant dose for each assay. Although these plants are heavily investigated but their mixtures have has not.

INTRODUCTION

The use of plants and plant products as medicines could be traced as far back as the beginning of human civilization [1]. Now, in the last century, the practice of traditional medicine became main stream throughout the world. In spite of great advances observed in modern medicine, plants still make an important contribution to health care. This is due to the recognition of the value of traditional medical systems. Medicinal plants are distributed worldwide, but they are most abundant in tropical countries [2, 3].
Today, herbs and medicinal plants are most important treatment for human diseases such as gastrointestinal disorders, cardiovascular disease, pain, diarrhea, fungal and bacterial infections and cancer. The needs for reliable information on herbal medicinal products are considerable. In the United States, the popularity of complementary and alternative medicine is growing at a remarkable speed and herbal medicine has grown faster than any other “alternative” treatment method in the United States [4].

*Rhazya stricta* Decne is a medicinal plant commonly used in the Arabian Gulf region and in the Sub-Indian and Saudi Arabia to alleviate a number of unrelated conditions—that include diabetes mellitus, inflammatory conditions and helminthiasis the phytochemistry, pharmacology and toxicity of the plant have recently been reviewed contains mainly alkaloids, glycoside, flavonoids [5].

Human lymphocyte exposure to *Rhazya stricta* extraction showed necrosis of most of the treated cells at all used concentrations, an indicative possibility of anticancer activity, by using single cell gel electrophoresis (Comet) test it caused DNA breaks that were proportionally to the concentration and exposure time to the aqueous extract of the *R. stricta* leaves [6]. Also, it has been shown that the indole alkaloids of *Rhazya stricta* has an anticancer effects [7]. Moreover the genotoxicity of *Rhazya stricta* leaves aqueous extract was demonstrated for the first time by Baeshen in *Saccharomyces cerevisiae* auxotrophic mutant test [8]. *Zingiber officinale* is another medicinal plant that has, been widely used in Chinese, Ayurvedic and TibbUnani herbal medicines all over the world. It has antibacterial, antiviral, antioxidant, antiemetic, antitumorigenic, antiinflammatory, anticancer and antigenotoxic effect [9].

The antioxidant activity and antimutagenic activity of water extract of *Zingiber officinale* was studied using Ames test [10]. Comet tests were developed in recent years in addition to Ames test. It is widely used and successfully assay for the scoring of DNA damage and repair. This test is now considered a very important alternative for the cytogenetic tests and is much less labor intensive, more rapid and less expensive [11]. In the comet assay individual cell are lysed and their DNA unwounded. The DNA is then subjected to a gel electrophoresis procedure at high pH results in structures resembling comets, DNA “comets” can be visualized with a fluorescent microscope after staining with a fluorochrome (e.g. ethidium bromide). The tail length, tail moment of DNA and other parameters are used to measure the extent of DNA damage. We shall measure the DNA damage and DNA moment using a specialized image analysis system [12].

In this research we evaluated the genotoxicity and antigenotoxicity of *R. stricta* and *Z. officinale* single and in a combination by the ames and comet tests. Also, we explored the possible involvement of the cytochrome p450 1A1 in the action of the plant extracts.

**MATERIALS AND METHODS**

**Animals:** Eighty locally bred adult male and female Swiss mice weighing 25-35 g were obtained from King Fahd Medical Research Center (KFMRC), King Abdulaziz University, Jeddah, KSA. They were housed in groups of five animals at a temperature of 22°C under a 12 h dark-light cycle and given a standard pelleted diet (Grain Soils and Flourmills Organization Jeddah, KSA) and water add libitum.

**Experimental Design:** The animals were divided into two major groups A for comet assay and B for cyp 1A1 and were housed for 2 days prior to start of experimentation to adapt to the conditions of the experiment. Group A were divided into four sub groups, groups A 1 (n= 10) was the control and was dosed orally by gavage with distilled water (1 ml). Group A2 (n= 10) was treated orally by gavage with 1 ml *Rhazya stricta* extract at single doses of 1.5 g/kg, group A3 (n= 10) was gavage with 1ml *Zingiber officinale* extract at single doses of 10 g/kg and group A4(n= 10) the combination group was gavage with Rhazya stricta 1.5 g/kg and 0.5ml of *Zingiber officinale* extract 10 g/kg respectively. After 24 hours blood samples were obtained from mouse by penetrating the retro-orbital plexus with a glass capillary tube, it was collected in EDTA tub.

Group B were divided into four subgroups , groups B1 (n= 10) was the control and was dosed orally by gavage with distilled water 1 ml. GroupB2 (n= 10)was treated orally by gavage with 1 ml *Rhazya stricta* extract at single doses of 1.5 g/kg, group B3 (n= 10) was gavage with 1ml *Zingiber officinale* extract 10g/kg and group B4(n= 10) the combination group was gavage with *Rhazya stricta* (1.5 g/kg) and *Zingiber officinale* (10 g/kg). After 48 hour blood samples were collected in EDTA tubs and livers were collected for cytochrome assays.

**Rhazya stricta** Plant Extract Preparation: The plant was collected in plastic bags and transferred to the lab in the fridge from the nearby areas of Jeddah-Makkah Highway, KSA.
The leaves were washed, freeze dried (lyophilized) for 48 hours at -30°C and grounded to a fine powder with a blender and the resulting powder was stored at 4°C. Aqueous solutions were prepared weekly from this powder and used freshly in all tests. One gram powdered leaves was incubated with 10 ml of distilled water for 12 hours at room temperature, with occasional shaking. The extract was filtered stored at -20°C. The aqueous extract was always administrated orally in a volume of 1 ml of the prepared dose and 10 mg/plate in the Ames test.

**Extraction of Zingiber officinale:** Fresh Zingiber officinale rhizomes was collected in plastic bags and transferred to the lab in the fridge from the local market of Jeddah, KSA. Zingiber officinale was washed, cut into small slices and freeze dried (lyophilized) for 48 hours at -30°C and grounded them to a fine powder with a blender. The resulting powder was stored at 4°C. Aqueous solutions were prepared weekly from this powder and used freshly in all tests. One gram powdered leaves was incubated with 30 ml of distilled water for 2 hours at 90°C, with occasional shaking. Later the extract was filtered and stored at -20°C. The aqueous extract was always administrated orally in a volume of 1 ml of the prepared dose and 10 mg/plate in the Ames test.

**Determination of LDm of R. stricta Leaves and Z. officinale Water Extracts:** Stock solution of R. stricta (1 g/10 ml) and Z. officinale (1 g/30 ml) was prepared. Dilutions of herbal extracts were made to permit the administration of increasing doses in suitable volume, up to a maximum of 0.5 ml orally.

**Genotoxicity Tests**

**Single Cell Gel Electrophoresis (Comet) Assay:** Comet assay was conducted as described by Ellassouli et al., [13]. Briefly, 1 ml of peripheral blood was exposed to different amount of the extracts for different periods of time. After treatment, 1x10^6 cells combined with 100 µl of molten low melting agarose (LMA) and immediately pipetted 75 µl onto comet slide (Trevigen, USA), then the slides were immersed in freshly prepared cold lysing solution (2.5 M NaCl, 100 mM Na2 EDTA, 10 mM Tris, pH 10, 1% sodium saccharinate), 1% Triton X-100 and 10% DMSO was added just before use for a minimum of 1 h at 4°C. Slides then were laid in horizontal electrophoresis apparatus filled with freshly prepared alkaline solution, pH=13, the slides were immersed in the lysing solution for 20 minutes, then electrophoreses was performed at 1 Volt/cm for 20 minutes. Slides were rinsed for 5 minutes three times and then immersed in 70% ethanol for 5 minutes. After air drying slides were stained with 50 µl ethidium bromide (20 µg/ml), covered and placed in a humidified air-tight container to prevent drying of the gel and were analyzed within 3-4 hours.

**Comet Scoring:** A total of 50 cells from each of the duplicated slides were examined randomly under fluorescence microscope (Olympus BX-51, Japan). The extent of DNA damage was measured quantitatively using comet analysis system (Loats, USA) based on extended dynamic range imaging (EDRI) technology, which expressed the comet tail moment automatically. Parallel to each experiment a series of negative controls were done in order to determine any non-specific formation or reduction in the comet.

**Antigenotoxicity Rhazya Stricta and Zingiber Officinale Single and in Combination as Determination by Comet Assay:** The ability of Rhazya stricta and Zingiber officinale single and in combination to inhibit the MNNG induced genotoxicity were evaluated by comet test. Blood cells from healthy, nonsmoker donor was first treated with the plant extracts at concentration (10 mg/ml of peripheral blood) for 2 hours, MNNG was added to the blood cells, the mixer was incubated at 37°C. After that peripheral blood was tested in duplicate in Single cell gel electrophoresis (SCGE) assay the percentages of cells with comet and comet tail moment in 50 cells were scored.

**Genotoxicity of Rhazya Stricta and Zingiber Officinale Determined by as Salmonella Mutagenicity Test (Ames Test):** We used a variety of different mutant strains that have important distinct mutations that make them suitable for detecting different types of mutations. Mutagenicity test was performed by the plate incorporation technique using the Salmonella typhimurium strains TA97a, TA98, TA100, TA102 and TA1535 as described by Maron and Ames [14] with and without metabolic activation S9 fraction. The liver S9 fraction was prepared as described by Adam [15]. Revertants colonies were scored after 48 h incubation at 37°C using image pro-plus software. Standard mutagens were used as positive controls including 4-nitroquinoline-1-oxide, 0.5 pg/plate; sodium azide, 5 pg/plate (without S9); 2-aminoanthracene, 2.5 pg/plate (with S9). Positive response was defined by at least a two fold increase in revertants over the negative control.
Estimating of Cytochrome P450 CYP 1A1 Concentration in Mice Liver: The water extracts of *Rhazya stricta* and *Zingiber officinale* was given orally to mice for two day as described. Liver homogenates from all animals were used to assess the microsomal activity of cytochrome P450 by Enzyme-linked immunosorbent assay (ELISA).

Statistical Analysis: Statistical analysis of the data was performed by the Statistical Package for Social Science (SPSS 10 for Windows, version 10, 1999, SPSS, Inc, Chicago, IL) program. The data were expressed as means ± standard deviation. Comparison of variables between groups was performed using one way analysis of variance (ANOVA). The least significance difference test (LSD) was employed to compare means for pairs of groups. Differences were considered to be statistically significant when $p$-value ≤ 0.05 and highly significant when $p$ ≤ 0.01.

RESULTS

Genotoxicity of *Rhazya stricta* and *Zingiber officinale* Extracts as Determined by Comet Assay: Statistical analysis showed that there were significant differences between the control mice percentage of cells with comet (2±1.1) and their tail moment was (0.8-1.6) and all the treated mice after 24hours of treatment with *Rhazya stricta* and *Zingiber officinale* and combination group (Table 1 Fig, 1, 2, 3, 4). After 24hours of treatment with *Rhazya stricta* and *Zingiber officinale* and combination, there were significant differences between the control mice and group 2 treated orally with *Rhazya stricta* extract (1.5 gm/kg) percentage of the cells with comet was 12±5.9 and their tail moment was 18-23. Statistical analysis showed significant ($p$<0.05) increase in comet *Zingiber officinale* extract (10 gm/kg) and percentage of the cells with comet was (48±7.1) and their tail moment was (48-77), in group 4 treated orally with *Rhazya stricta* and *Zingiber officinale* extract showed significant ($p$<0.05) increase in the percentage of the cells with comet (21±5.6) and caused severe DNA damage as indicated by the increase in the comet tail moment and their tail moment was 44-67.

Antigenotoxicity of Plants Extracts as Determined by Comet Assay: Statistical analysis showed that there were no significant differences in comet between the control group and all the treated groups after 2hours. The pre incubation with extract did not inhibit or decrease the genotoxic potency of the mutagen and cells showed a huge DNA damage as compared to the positive control (cells treated with 30$\mu$mol MNNG). There were no significant differences between the control and group 2 treated (10mg/ml of peripheral blood) with *Rhazya stricta* extract. Statistical analysis showed non-significant ($p$>0.01) and *Zingiber officinale* extract (10mg/ml of peripheral blood) and combination (Table 2, Fig. 5, 6, 7).

Genotoxicity of *Rhazya stricta* and *Zingiber officinal* Extracts as Determined by the Salmonella Mutagenisity Test (Ames Test): None of extracts showed any cytotoxicity and mutagenicity effects on the battery of *Salmonella typhimurium* mutant strains TA97a, TA98, TA100, TA102 and TA1535 at the concentrations used in presence of the metabolic activation system S9 (Fig. 8).

A possible mutagenic potential was assumed if the quotient ranges of revertants is between 1.7 to 1.9 that of the control.

Effect of the *Rhazya stricta* and *Zingiber officinal* Extracts on Cytochrome P450 CYP 1A1 Level in Mice Liver Measured by ELISA: The water extracts of the *Rhazya stricta* and *Zingiber officinale* and their combination were given orally to mice, in a single dose of *Rhazya stricta* and *Zingiber officinale* and in combination. Liver homogenates from treated animals were used to assess the microsomal activity of cytochrome P450 CYP 1A1by ELISA.

Statistical analysis showed significant ($p$<0.01) increase in CYP 1A1 concentrations in mice liver, in animals treated orally with *R. stricta* extract (1.5 gm/ml) and significance ($p$<0.01) in *Z. officinale* treated animals and in animals treated with combination (Table 4).

**LD**$_{50}$ of *R. stricta* Leaves and *Z. officinale* Water Extract: The LD50 of *R. stricta* in mice was found to be 2 g/kg, after (2-10min) of oral gavage the animal had neurological symptoms convulsion, vomiting, palpitation and syncope and death. The LD50 of *Z. officinale* in mice was found to be 20 g/kg after (120 min) of oral gavages, animal had the abdominal muscle contractions and itching and diarrhea and death. The main goal of LD50 is to find the maximum tolerable dose (MTD) which does not cause death or and toxic symptoms for the animal and later will be used. In our studies we found that the maximum tolerable dose for the first time for the water extract of *R. stricta* was 1.5g/kg and in of *Z. officinale*10g/kg in mic.
Table 1: Genotoxicity of *Rhazya stricta* and *Zingiber officinale* and combination groups as determined by comet assay.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Percentage of the cells with comet</th>
<th>Comet tail moment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1 Control</td>
<td>2.0±1.10</td>
<td>0.8-1.6</td>
</tr>
<tr>
<td>Group 2 Rhazyastricta</td>
<td>12±5.9</td>
<td>18-23</td>
</tr>
<tr>
<td>Group 3 Zingiberofficinale</td>
<td>48±7.1</td>
<td>48-77</td>
</tr>
<tr>
<td>Group 4 Combination</td>
<td>21±5.6</td>
<td>44-67</td>
</tr>
<tr>
<td>LSD 5%</td>
<td>4.35</td>
<td>----</td>
</tr>
</tbody>
</table>

Values are presented as means with their standard deviations

Number of mice for each group is 10

Table 2: Antigenotoxicity of plants extracts as determined by comet assay. Percentage of cells with comet and comet tail moment of positive control cells (treated with 30µmol MNNG) and cells preincubated with plant extracts.

<table>
<thead>
<tr>
<th>Groups</th>
<th>% of cells with comet</th>
<th>Comet tail moment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (treated with 30µmol MNNG)</td>
<td>79±2</td>
<td>12-33</td>
</tr>
<tr>
<td>Rhazyastricta extract</td>
<td>85±7</td>
<td>20-40</td>
</tr>
<tr>
<td>Zingiberofficinale extract</td>
<td>98±5</td>
<td>33-45</td>
</tr>
<tr>
<td>Extracts combination</td>
<td>92±3</td>
<td>35-41</td>
</tr>
<tr>
<td>LSD 5%</td>
<td>8.78</td>
<td>----</td>
</tr>
</tbody>
</table>

Values are presented as means with their standard deviations.

Table 3: Genotoxicity of *RhazyaStricta* and *Zingiberofficinale* and combination groups as determined by Ames test.

| Test strain  | Revertants (Negative control) Mean revertants at 10mg *R. stricta* extract/plate Mean revertants at 10mg *Z. officinale* extract/plate Mean revertants at 10mg combination extract/plate |
|--------------|---------------------------------------------------------------|---------------------------------------------------------------|-----------------------------------------------------------------|
| TA97a        | 185±7                                                         | 168±8                                                         | 175±6                                                           | 170±8                                                           |
| TA98         | 55±5                                                          | 50±5                                                          | 55±4                                                           | 50±7                                                           |
| TA100        | 240±10                                                        | 206±8                                                         | 250±6                                                          | 235±6                                                          |
| TA102        | 550±20                                                        | 545±18                                                        | 530±20                                                         | 535±20                                                         |
| TA1535       | 43±5                                                          | 35±5                                                          | 44±4                                                           | 40±5                                                           |
| LSD 5%       | 19.91                                                         | 18.09                                                         | 18.26                                                          | 19.40                                                          |

Values are presented as means with their standard deviations.

Table 4: Effect of the plant extracts on the CYP 1A1 concentration level in mice liver.

<table>
<thead>
<tr>
<th>Animal treatment</th>
<th>CYP1A1 concentration ng/mg of protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated (Control)</td>
<td>0.36±0.16</td>
</tr>
<tr>
<td><em>Rhazya stricta</em> (1.5 g/kg)</td>
<td>0.68±0.04</td>
</tr>
<tr>
<td><em>Zingiber officinale</em> (10g/kg)</td>
<td>0.88±0.03</td>
</tr>
<tr>
<td>Combination (1.5 g/kg <em>R. stricta</em> &amp; Z. officinale10g/kg)</td>
<td>0.73±0.04</td>
</tr>
<tr>
<td>LSD 5%</td>
<td>0-16</td>
</tr>
</tbody>
</table>

Values are presented as means with their standard deviations.

Fig. 1: Photographic image of the control group, whole blood cells subjected to single cell gel electrophoresis subsequently analyzed by image analysis system.
Fig. 2: Photographic image of the *Rhazya stricta* group, whole blood cells subjected to single cell gel electrophoresis subsequently analyzed by image analysis system.

Fig. 3: Photographic image of the *Zingiber officinale* group, whole blood cells subjected to single cell gel electrophoresis, subsequently analyzed by image analysis system.

Fig. 4: Photographic image of the combination group, whole blood cells subjected to single cell gel electrophoresis subsequently analyzed by image analysis system.
Fig. 5: EDRI fluorescent images of leukocyte pre incubated with 10 mg/ml of *R. stricta* extract, than treated with 30µmol MNNG, no protection in the DNA damage occurred

Fig. 6: EDRI fluorescent images of leukocyte pre incubated with 10 mg/ml of *Z. officinale* extract, than treated with 30µmol MNNG, no protection in the DNA damage occurred

Fig. 7: EDRI fluorescent images of leukocyte pre incubated with 10 mg/ml of *R. stricta* and *Z. officinale* extract, than treated with 30µmol MNNG, no protection in the DNA damage occurred
Fig. 8: Mutagenicity of the plants extracts Ames test in the presence of activation liver fraction S9 and plant extracts using TA100 strain, plate without mutagen (control), plate with 10mg/plate of *Rhazya stricta*, plate with 10mg/plate of *Zingiber officinale*, plate with combination 5mg of *Rhazya stricta* and 10mg/plate of *Zingiber officinale*. Number of bacteria revertants was counted automatically by image pro-plus software. Spontaneous revertants colons was 65-200

**DISCUSSION**

*Rhazya stricta* is commonly used in folk medicine of the Arabian Peninsula for the treatment of many diseases. In many studies, relatively large doses of the plant extract were used to determine the pharmacological and toxicological actions [16, 17]. Therefore, it was necessary to study the genotoxicity effects of this plant using maximum tolerable doses.

A number of strategies have been considered for the evaluation of mutagenicity. These include testing whole mixtures (integrative), fractionation of mixtures to determine mutagenic components (dissective, top-down approach) and investigations of interactions by testing simple combinations, recombined fractions and spiking of mixtures/fractions (synthetic, bottom up approach). Mutagenic potentiation of *R. stricta* has been demonstrated in vitro by [8] for the first time, they evaluated the genotoxicity of *R. stricta* leaves water extract by the *Saccharomyces cerevisiae* auxotrophic mutagenicity test, using different concentrations of the leaves extract (13.4, 15 and 20.1g/10ml). They were found that the extract has potent lethal and mutagenic activities. Survival percentage decreased as concentration or time of exposure increased on yeast cells.

In this study mutagenicity and antimagnetic of crude extract from *R. stricta* and *Z. officinale* and combination was investigated in *Salmonella typhimurium* TA100, TA 79a, TA 98, TA 102, TA1535 in the presence of exogenous metabolic activation system S9. This study showed that none of these extracts showed any cytotoxicity and mutagenicity effect on the battery of *Salmonella typhimurium* mutant strains TA97a, TA98, TA100, TA102 and TA1535 in presence of the metabolic activation system at concentration of 10µg/plate. The different result between our study and other is according to different assay system *Salmonella typhimurium* mutagenicity test is assay using bacteria which is prokaryotic cells, *Saccharomyces cerevisiae* is eukaryotic cells. They have different metabolism system. Other study was carried out to evaluate the mutagenic activity of *Z. officinal* ethanol extract in *Salmonella typhimurium* strains TA 98, TA 100 and TA 1535 and TA 1838 at concentrations of 25-50 mg/plate and 5-10 mg/plate, ethanolic extract at concentrations between 10 and 200 µg/plate and they found that gingerol and shogaol were mutagenic in strains TA 100 and TA 1838 with metabolic activation by rat liver S9 fraction, while zingerone did not have mutagenic effects [18].

The result of our study showed that the water extract of *Z. officinal* did not have any mutagenic potential. Possible reasons for this range of variation results by different research groups may be due to the herbal doses, experimental period, sample size, herb extraction method that could explain the disparity of the findings. The assessment of genotoxicity and antigenotoxicity can be performed at different steps of the interaction of the effects of the mutagen on DNA. The direct damage to DNA can be assessed by comet assay, but depending on the stage of cell cycle, repair capacity, genetic background of cells and type of mutagen, only a fraction of induced DNA damage will lead to fixed mutations. However, we designed this study to determine if oral exposure of water extract from *R. stricta* and *Z. officinal* and combination taken at high levels had any genotoxicity and antigenotoxicity effect by comet assay.

In our study Statistical analysis showed that, there were significant differences in comet between the control mice and all the treated mice after 24hours of treatment with *Rhazya stricta* and *Zingiber officinale* and
combination group. There were significant differences between the control group and group 2 treated orally with Rhazya stricta extract (1.5 gm/kg). Statistical analysis for group 3 showed significant (p<0.05) increase in comet Zingiber officinale extract (10 gm/kg), group 4 treated orally with Rhazya stricta and Zingiber officinale extract showed significant (p<0.05) increase in comet caused severe DNA damage as indicated by the increase in the comet tail moment.

The antigenotoxicity statistical analysis showed that there were no significant differences in comet between the control and all the treated groups after 2hours, extracts did not inhibit or decrease the genotoxic potency of the mutagen and cells showed a huge DNA damage as compared to the positive control (cells treated with 30µmol MNNG). There were no significant differences between the control and group 2 treated (10mg/ml of human peripheral blood) with Rhazya stricta extract. Statistical analysis showed non-significant (p < 0.01) and Zingiber officinale extract (10mg/ml of human peripheral blood) and combination.

Our results, agreed with the study conducted by Baeshen, [6] who reported that the aqueous extract of the R. stricta leaves has mutagenic, clastogenic and possibly anticancerous activities on human lymphocytes in vitro by comet assay at three different concentrations (6, 12 and 24 g/liter) of R. stricta aqueous leaf extract. On the other hand, other studies investigating the effects of Z. officinale on DNA damage and development of urothelial tumors in a mouse bladder carcinogenesis model, they found that Z. officinale did not alter the DNA damage levels induced by the BBN/MNU treatment during the course of the exposure by comet assay [19]. Another, study investigated the genotoxic effect of 6-gingerol on human hepatoma G2 cells by comet assay and they found that exposure of the cells to 6-gingerol caused significant increase of DNA migration [20]. Study of the effect of aqueous extract of Zingiber officinale to prevent genotoxicity in human peripheral blood lymphocytes was investigated. The aqueous Zingiber officinale extract was used at three concentrations namely 2.5, 5 and 10 µg were tested for protective effect against B(a)P (300 µM) induced DNA damage by comet assay and When Zingiber officinale extracts were present in incubation mixtures the comet ratios have increased depending on the dose of Zingiber officinale extract and they found at the highest concentration of Zingiber officinale extract namely 10 µg, the comet ratios were similar to their respective controls demonstrating the protective effect of Zingiber officinale on carcinogen induced DNA damage [20].

We investigated the cytochrome P450 1A1 enzymes which is the most prominent enzymes involved in such activation reactions in human cytochrome P450 enzymes can be an activation of procarcinogens material (Güengerich et al., 1998). Results of the present study found that significant (p<0.01) increase in CYP 1A1 concentration in mice liver and in group 2 treated orally with Rhazya stricta extract (1.5 gm/ml) and significant (p<0.01) increase in group3 Zingiber officinale and group 4 treated orally combination. In agreement with our results [21]. studied the effect of alkaloid fraction of Rhazya stricta on cytochrome P450-mediated metabolism of theophylline in mice, they found that alkaloidal fraction had no significant effect on the total amount of microsomal cytochrome P450, but it caused a significant increase in the cytochrome P450 isoforms CYPs 1A1 and 1A2. It also significantly increased the concentrations of some metabolites of theophylline.

CONCLUSIONS

Herbals become more popular, there will be increased concern for their safety. Therefore, this study was designed to examine the genotoxicity and antigenotoxicity effects of Rhazya stricta and Zingiber officinale single and in combination at high levels doses given orally to mice. The investigation through studying of the interaction activates of the combination was performed by different genotoxicity assay viz., Ames test, comet assay and cytochom p450 cyp 1A1. This results, suggest that R. stricta water extract did not has the potential to interact with Z. officinale. However, more work is needed, with that huge growing interest of the world in the alternative medicines. Further study is needed to investigate the possible mechanisms of action of Rhazya stricta leaves, by the same method of extraction, that have been used by humans in the folk medicine to find an explanation heeling potential Rhazya stricta.

Competing Interest: All the authors declare that there is no any conflict of interests regarding the publication of this paper and we do not gain financially from the publication of this article.

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