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Assessment of Mutagenicity of Herbal Preparations from Al-Baha Region, Saudi Arabia

Al-Zubairi, Adel Sharaf*
Department of Laboratory Medicine, Faculty of Applied Medical Sciences, Al Baha University, Al-Baha, KSA
E.Mail : adelalzubairi@hotmail.com

INTRODUCTION
Cancer-related and cancer deaths are increasing worldwide as reported by the World Health Organization (WHO) in 2012, in which 14 million new cancer cases and 8.2 million cancer-related death has been recorded (Ferlay et al. 2013). Lifestyles and environmental factors represent the causes of 30% of cancer deaths and herbal preparations are among the possible causes of these death cases. Using of medicinal herbs has been continuously increasing for the recent decades throughout the world as complementary and alternative medicines particularly in Western countries (Cheng and Leung, 2012). According to the WHO, reports, approximately 80% of the population still relies on the traditional use of herbal medicine for primary healthcare (Mukherjee 2002; Bodeker ET AL. 2005). The use of traditional medicinal herbs is due to the disappointment of the patients with
standard treatment and the beliefs that herbal medicines, being natural and the use of herbs is associated with a healthier lifestyle and are therefore harmless (Ekor 2014). Toxicological studies on traditional medicines are still scarce, as it is estimated that toxicological data are missing for up to 90% of traditional Chinese herbal medicines (Cheng and Leung 2012), and the situation appears even worse for herbs used in developing countries.

Acalypha species belongs to the Euphorbiaceae family are utilized in folk medicine as a diuretic, anthelmintic and for treatment of respiratory problems such as bronchitis, asthma and pneumonia (Emeka et al. 2012) and are used in food for consumption. It has been reported that Acalypha fruticosa used as antidiarrheal, antioxidant, anti-inflammatory, anticancer, antiplasmodial, wound healing and cytotoxic (Madlener et al. 2009; Gopalakrishnan et al. 2010), as well as possessing antibacterial activity (Mothana et al. 2008). Aloe vera (L) family Aloaceae is another plant, traditionally used for the treatment of hyperglycemia in diabetic patients as well as hyperlipidemia in hyperlipidemic patients (Vogler and Ernst 1999). It has been found to ease the healing of wounds and helpful in relieving many gastrointestinal disorders as well as a laxative and in the treatment of hemorrhoids (Foster 1999). Aloe extract has been shown to possess anti-inflammatory properties, cellular protection, restoration, and immune and mucus stimulating activities (Davis et al. 1994; Davis 1997; Hamman 2008.

Jatropha species is a medicinal plant that is among the traditional medicinal plants used in Saudi Arabia due to its anti-inflammatory, antiulcer, antioxidant, antiseptic, analgesic and wound healing properties (Yusuf and Maxwell 2010; Oskouiean et al. 2011). Antiprotozoal activities against malaria, leishmanial and trypanosoma have been attributed to Jatropha species (Sabandar et al. 2013). Ricinus communis Linn. plant (family Euphorbiaceae) found growing wild in Saudi Arabia, commonly known as castor plant. In traditional medicine, Ricinus communis has been reported to have antibacterial, anti-inflammatory, antidiabetic, hepatoprotective, anticancer, purgative and lubricant activities as well as a remedy for various ailments (Ilaivarasan et al. 2006).

Genotoxicity refers to the deleterious effects of a chemical substance or a physical action on the genetic material of the cell and such genotoxic effects are considered a characteristic feature of cancer risk. Genotoxicity assessment of herbal preparations becomes of great importance due to the wide consumption of the herbal remedies, modern medicinal products and household as well as environmental chemicals. Several plant metabolites and environmental pollutants and toxicants are known to cause various diseases or even death in animals and humans (Rates 2001; Ames and Gold 1997). Many natural and synthetic compounds have been reported to cause mutagenesis and/or carcinogenesis (Ames 1983; Vargas et al. 1990). Several in vitro genotoxicity assessment tests have been developed including the cultured mammalian cells such as human peripheral blood lymphocytes (PBL) or Chinese hamster ovary (CHO) cells and bacterial mutation assay as the Ames test, for the screening of potentially mutagenic, carcinogenic and/or teratogenic agents. Mutagenicity is a DNA alteration leading to the genotoxicity, which results in events that cause changes to the DNA and/or chromosomal structure, that are
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subsequently passed to the following generations. The bacterial reverse mutagenicity assay uses Salmonella typhi (S. typhi) and Escherichia coli (E. coli) strains which contain specific gene mutation in the pathway of amino acid biosynthesis preventing the bacterial growth. Substances capable of inducing mutation (mutagenic substance) may induce a second mutation (a reversion) leading to restoration of the functional capability of the bacteria to resynthesize the non-essential amino acid.

To date, there is no mutagenicity/genotoxicity studies have been carried out on the most herbal plants used in Al-Baha region as traditional medicines and even no reports have been found about the genotoxicity of Saudi medicinal plants. The flora of Saudi Arabia and in particular of Al-Baha region is extraordinarily rich and diverse. There are many plants that the Saudi people use either in rural or urban areas for the treatment of different ailments (El-Shanawani 1996; Gushash 2006). This study reports for the first time, the genotoxicity of herbal plants methanolic extracts growing in Al-Baha region and used in traditional medicine. We used Ames bacterial mutation assay to assess the genotoxicity of four herbal methanolic extracts from Acalypha fruticosa, Aloe vera, Jatropha and Ricinus communis.

MATERIALS AND METHODS

Plant Materials:
The plant material was collected between February – April 2018 from different locations in Al-Baha region and outskirts of Albaha (Table 1). The plants were taxonomically identified by Dr. Abdulwali Ahmed Al-Khulaidi, the botanists at the Department of Biology, Faculty of Sciences and Arts, Baljrushi, Albaha University.

Processing of the Plant Material:
Plant leaves were washed thoroughly, finely ground using an electrical grinder and stored for further use. The powdered plant material was extracted with MeOH (3x100 ml) under shaking at room temperature. The separated extracts were then filtered through Whatman's No. 1 filter paper and evaporated to dryness in vacuo at 40°C. Dried extracts were collected in airtight containers and stored at 4 °C till further analysis. Stock solutions have been prepared in DMSO at a concentration 20 mg/mL (Al-Musayeib et al. 2012).

Ames Bacterial Mutation Assay:
The Ames test was conducted according to the Organization for Economic Co-operation and Development (OECD) guidelines no. 471 (OECD 1997). The bacterial mutagenicity test has been carried out using the Muta-chromoplate™ Kit using the bacterial strain TA100 was purchased from Environmental Biodetection Product Inc. (EBPI), Canada. One day prior to the day of assay, one vial of growth media was thoroughly mixed with one vial of bacterial strain followed by incubation in 37°C incubator for 16 to 18 h as described by the manufacturer. The mutagenicity test is based on the well-known ‘Ames test’ (Ames et al. 1975) that utilises a mutant strain of Salmonella typhimurium (TA100) which is defective in the operon gene coding for histidine. However, the mutant bacterial strain that will be exposed to the mutagenic agents can undergo reverse mutation from being auxotrophic (histidine-dependent or -his) to prototrophic (wild-type that can synthesize histidine on their own). The mutagenicity test was
carried out without any metabolic activation system. All procedures were performed using aseptic technique in a safety cabinet. The growing Salmonella typhimurium TA100 strain culture was added to 96-well flat-bottomed tissue culture plates each containing a fixed concentration (0.1, 1.0 and 5.0 mg/ml) of the plant extracts dissolved in DMSO. The plates were incubated at 37°C for 6 days. A plate containing sterile water (without Salmonella typhimurium TA100 strain culture) was included as the blank and for sterility checking of the assay setup. A background plate containing sterile water and Salmonella typhimurium TA100 strain was also included to show the level of spontaneous or background mutations. The plate containing standard, the positive mutagen Sodium azide, was included as the positive controls in the assay.

The number of wells showing bacterial growth (the positive wells) in each plate was scored. Wells that turn yellow, partially yellow or turbid indicate that the viability of the bacteria due to the reverse mutation (positive results) activity of the material under examination. Purple wells indicate that the bacteria did not survive (negative results). The statistical significance of the differences in the number of positive wells in the plant extract-treated plates and the positive control plate against the corresponding background plate was determined using a table provided by the manufacturer. The scores for the blank plate, the background control plates and the positive control plates obtained were within the manufacturer’s recommendation, indicating the validity of the results obtained for the aqueous extract.

RESULTS

The assay was carried out in vitro using one histidine-requiring strain of Salmonella typhimurium, strain TA100 without a metabolic activating enzyme (S9). The bacterial strain was treated with the plant extracts at 0.1, 1.0 and 5.0 mg/ml, respectively. Plants extracts mutagenicity at various concentrations tested was determined from the statistical table provided by the manufacturer. According to the manufacturer table, the level of mutagenicity was classified as strongly mutagenic if p < 0.001, moderately mutagenic if p < 0.01 and mildly mutagenic if p < 0.05. The results obtained in the absence of S-9 metabolic activation are presented in Table 1. The lowest concentration 0.1 mg/ml of the four plants extracts (Acalypha fruticosa, Aloe vera, Jatropha and Ricinus communis) were found to be non-mutagenic in the absence of S-9 metabolic activation as the numbers of positive wells in the treated plates were not statistically different from the results obtained in the background plate, in contrast at 1.0 mg/ml, the methanolic extracts of Aloe vera and Jatropha were shown to be significantly different (p < 0.05) compared to the background non-treated plat. Meanwhile, 1.0 mg/ml of methanolic extract of Ricinus communis was found to be cytotoxic at this concentration since there was no bacterial growth as well as at the greater (5.0 mg/ml) concentration. However, the plants methanolic extracts of the three plants, Acalypha fruticosa, Aloe vera and Jatropha at 5.0 mg/ml the number of positive wells were significantly higher (p < 0.001) compared to the background. The positive controls, sodium azide was strongly mutagenic as the number of positive wells in the positive control plates were significantly higher than that in the background plate (p < 0.001).
Table 1; Number of positive wells in the background control plates, the positive control plates (Sodium azide) and the plates treated with various concentrations of the plants methanolic extracts in the absence of metabolic activation

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Positive scores</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.1 mg/ml</td>
</tr>
<tr>
<td>Positive control Sodium azide</td>
<td>94</td>
</tr>
<tr>
<td>No treatment (Background)</td>
<td>15</td>
</tr>
<tr>
<td>A. fruticosa</td>
<td>-</td>
</tr>
<tr>
<td>Aloe vera</td>
<td>-</td>
</tr>
<tr>
<td>Jatropha</td>
<td>-</td>
</tr>
<tr>
<td>Ricinus communis</td>
<td>-</td>
</tr>
</tbody>
</table>

* p < 0.05, **p < 0.01, ***p < 0.001

**DISCUSSION**

Traditional medicine is commonly used by Saudi people either in rural or urban areas for the treatment of different ailments (El-Shanawani 1996; Gushash 2006), however toxicological studies and in particular mutagenicity/genotoxicity studies, still scarce for many herbal preparations from Saudi Arabia. This study reports for the first time, the mutagenicity/genotoxicity of herbal plants methanolic extracts growing in Al-Baha region and used in the traditional medicine. We used Ames bacterial mutation assay to assess the mutagenicity/genotoxicity of the methanolic extracts from Acalypha fruticosa, Aloe vera, Jatropha integerrima and Ricinus communis.

Genotoxic substances have the potential to interact with the DNA and may cause DNA and chromosomal damage which is referred to as genotoxicity or mutagenicity. The Muta-Chromo Plate™ kit is a mutagenicity test based on the bacterial reverse mutation, the Ames mutagenicity test, which is commonly used as a screening test for determining the mutagenic potential of the new chemicals and drugs (Chang et al. 2012). It detects point mutations, which involve substitution, addition or deletion of one or a few DNA base pairs.

Acalypha fruticosa has various traditional uses, a cure for stomachache, dyspepsia, venom antidote, rheumatism and dermatitis (Senthilkumar et al. 2006; Sripathi and Uma 2010), antiepileptic (Govindu and Adikay 2014), cytotoxic, antioxidant (Rajkumar et al. 2010; Thambiraj et al. 2012; Mothana et al. 2010), anti-bacterial (Vinoth 2013), anti-inflammatory (Gupta et al. 2003), antitumor (Sivakumar et al. 2010) and wound healing properties (Gopalakrishnan et al. 2010). There is no toxicological study has been found in the literature on Acalypha fruticosa plant extracts. However, the bacterial reverse mutation Ames test showed methanolic extract of Acalypha fruticosa at low concentrations used in this study (0.1 and 1.0 mg/ml) to possess no mutagenic potential. In contrast, higher concentration (5.0 mg/ml) was found to be significantly different (p <0.05) compared to the background simultaneous mutation indicating it ability to cause genetic alterations to the tested bacterial (the
Salmonella typhimurium strain TA100) at least under the experimental conditions used in this study.

Aloe vera is another herbal plant widely distributed in Saudi Arabia and especially in Al-Baha region. Aloe vera has been therapeutically used for the treatment of several health problems such as wound healing (Visuthikosol et al. 1995; Vogler 1999; WHO 1999), constipation and widely used in cosmetic and folk medicine (WHO 1999; Capasso et al. 1998; Boudreau and Beland 2006). A number of studies have reported that Aloe vera plant extracts constituents shown to be non-mutagenic and non-genotoxic in different tests systems (Heidemann et al. 1993,1996; NTP 2001; Mengs et al. 2001). Aloe vera methanolic extract has been found to be non-mutagenic at low concentration (0.1 mg/ml) in the absence of metabolic activator, while at a higher concentration significantly increased the number of revertant bacteria. However in a study by Kayraldiz and coworkers (Kayraldiz et al. 2010) significant increase in the revertant bacteria observed after treatment of TA98 strain of Salmonella typhimurium in the absence of S9 mix, while treatment of TA198 with metabolic activator S9and TA100 strain with and without metabolic activation didn’t induce mutation.

Jatropha species are traditionally used in the treatment of many diseases, such as stomachache, skin inflammation, eye infection, chest pain and itching, as well as antiprotozoal activities for treatment of malaria, leishmanicidal and trypanocidal or as ornamental plants and energy crops in Latin America, Africa, and Asia (Sabandar et al. 2013). Jatropha integerrima traditionally used for the treatment of tumours, herps warts, rheumatism, pruritis, scabies, eczema, ringworm, as purgative, styptic and emetic (Burkhill 1994; Heller 1996). Jatropha has been shown to contain compounds with cytotoxic, anticancer, antimicrobial, antioxidant, antiprotozoal and anti-inflammatory activities (Sabandar et al. 2013; Hartwell 1996; Chatterjee and Das 1980; Singh and Singh 2002; Panda et al. 2009). The genotoxic effects of Jatropha have been reported by (Singh et al. 2014) who reported plant extract to induce micronucleus formation in fish. The results of our study showed that high concentrations of Jatropha methanolic extracts were mutagenic in Ame’s bacterial reverse mutagenicity test in the absence of metabolic activator. The lowest concentration (0.1 mg/ml) was found to be non-mutagenic while the higher (1.0 and 5.0 mg/ml) concentrations were strongly mutagenic.

Ricinus communis Linn. was reported to have antibacterial, anti-inflammatory, antidiabetic, hepatoprotective, anticancer, purgative and lubricant activities as well as a remedy for various ailments (Ilavarasan et al. 2006). All parts of the plant have been used in traditional medicine for treatment of several ailments (Scarpa and Guerci 1982 ). Leaves juice has been reported for its use as an emetic in the poisoning of narcotics like opium, useful against jaundice too and with antifungal activity (Rana and Dhamija 2012; Vandita et al. 2013). Roots also found to have purgative activity and for a toothache (Rana and Dhamija 2012 ). The methanolic extract of Ricinus communis roots has been found to exhibit anti-inflammatory, free radical scavenging activity, and anti-
fertility properties (Ilavarasan et al. 2006; Sandhyakumary et al. 2003). The ethanolic extracts of *Ricinus communis* has been reported to have an antimicrobial property (Alamri and Mustafa 2012). *Ricinus communis* methanolic extract was found to be non-mutagenic in this study at low concentration, 0.1 mg/ml in the bacterial reverse mutagenicity test in the absence of metabolic activator. While higher concentrations (1.0 and 5.0 mg/ml) were found to have cytotoxic activity as there was no bacterial growth observed when incubated with these concentrations suggesting that the methanolic extract was cytotoxic to the bacteria.

**Conclusion**  
Among the medicinal plants used in Saudi Arabia are *Acalypha fruticosa*, *Aloe vera*, *Jatropha integerrima* and *Ricinus communis*. The methanolic extracts of these plants have been studied for their mutagenicity/genotoxicity against the bacterial reverse mutagenicity assay (Ames’ test). The results were found vary from plant to another, the lowest concentrations of the four plant extracts were found to be non-mutagenic, while only the highest concentration (5.0 mg/ml) of *Acalypha fruticosa* was found to have the ability to induce mutagenicity in Salmonella typhimurium (TA100). Meanwhile, both high concentrations of *Aloe vera* and *Jatropha* methanolic extracts were shown to have the ability to induce mutagenicity in Salmonella typhimurium (TA100) in the absence of metabolic activation. On the other hand, the extract of *Ricinus communis* at low concentrations was non-mutagenic, while the higher concentrations were cytotoxic to the bacteria.

**Acknowledgment**  
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**Conflicts of Interest:** The authors declare no conflict of interest.

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تقييم طفرات مستحضرات عشبية من منطقة الباحة بالمملكة العربية السعودية

عادل شرف الزبيري
قسم طب المختبرات، كلية العلوم الطبية التطبيقية، جامعة الباحة، المملكة العربية السعودية

الخلفية والأهداف: الاستخدام التقليدي للنباتات الطبية العشبية في المملكة العربية السعودية شائع وخاصة في منطقة الباحة إما في المناطق الريفية أو الحضرية لعلاج العديد من الأمراض وليس هناك تقارير حول سمية هذه النباتات على المورثات الجينية. في هذا البحث فحصنا بعض التأثيرات السمية على الموراثات أربعة مستخلصات عشبية بالميثانول وهي: نبات الصبار Aloe vera، نبات الزحر Acalypha fruticosa، نبات العباب Jatropha ونبات الخروع Ricinus communis. باستخدام اختبار Ames لقياس سمية مستخلصات النباتات الأربعة على الموراثات الجينية استخدمنا اختبار الطرقات العكسية البكتيرية (اختبار Ames) في غياب أي نظام تنشيط أيضي. كانت الترتكز المستخدمة من مستخلصات النباتات المختلفة هي 0.1 و 1.0 و 5.0 ملغ/مل.

المنهجية: لتقييم سمية مستخلصات النباتات الأربعة على الموراثات الجينية استخدمنا اختبار الطرقات العكسية البكتيرية (اختبار Ames) في غياب أي نظام تنشيط أيضي. كانت الترتكز المستخدمة من مستخلصات النباتات هي 0.1 و 1.0 و 5.0 ملغ/مل. كانت النتائج التي شملت الترتكزات المنخفضة من المستخلصات (0.1 ملغ/مل) من النباتات الأربعة ليست ذو تأثير على جينات بكتريا Aloe vera والصابرا Jatropha. وجد أن تركيز 1.0 ملغ/مل غير سام للخلايا عند هذا التركيز. عند تركيز 1.0 ملغ/مل كانت النباتات Acalypha fruticosa وJatropha وAloe vera مطفرة. عند تركيز 5.0 ملغ/مل كانت النباتات Acalypha fruticosa وJatropha وAloe vera مطفرة. وجد أن تركيزات عالية فقط من المستخلصات 1.0 و 5.0 ملغ/مل من النباتات العشبية الزحر، الصبار Aloe vera، العباب Jatropha، الزحر Acalypha fruticosa للطرقات البكتيرية العكسية.

النتائج: كانت نتائج التركيزات منخفضة من مستخلصات النباتات أربعة أثارت سمية على جينات بكتريا TA100 أب. وجد أن تركيزات 0.1 ملغ/مل كانت غير مطفرة في نباتات Acalypha fruticosa وJatropha وAloe vera. وجد أن تركيزات 1.0 ملغ/مل كانت مطفرة في نباتات Acalypha fruticosa وJatropha وAloe vera. وجد أن تركيزات 5.0 ملغ/مل كانت مطفرة في نباتات Acalypha fruticosa وJatropha وAloe vera.