Cytotoxic and Genotoxic and Oxidative Effects of Aqueous Extracts of Some Frequently Used Medicinal Plants in Pakistan

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Abstract: The unlimited use of Medicinal plants due to lack of information about their possible toxicities is increasing day by day in Pakistan. Therefor aqueous extracts of Thyme seed, Neem leaf, Neem seed and Eucalyptus leaf were evaluated for cytotoxicity, genotoxicity (DNA damage) and oxidative stress using chromosomal aberration assay in Chickpea (Cicer arietinum L.) root tip cells. Chickpea seeds were incubated in 10% solutions of aqueous extracts for 0.5 hour and 1 hour. All the extracts inhibit germination of chickpea seed. In 0.5 hour treatment maximum percent decrease was observed in eucalyptus (11%) and in 1 hour treatment in thyme seed (37%). In 0.5 hour treatment minimum M.I. was observed in Thyme seed (93%) and in 1 hour treatment Neem seed (32%) exhibited minimum M.I. In 0.5 hour treatment maximum abnormality index (A.I) was observed in Neem seed (26%) and in 1 hour treatment Thyme seed (97%) showed maximum abnormality index. Thyme seed and Neem seed extracts were more genotoxic than Neem leaf and Eucalyptus leaf extracts. Among all the applied extracts most frequent chromosomal aberration was fragmentation. Oxidative damage was also induced by all the extracts. It is concluded that unlimited use of these medicinal plants should be avoided. Their safe dose must be administered.

Key words: Medicinal plants • Cytotoxicity • Genotoxicity • Oxidative damage

INTRODUCTION

Though medicinal plants have been used since antiquity for the prevention and treatment of various ailments. At the turn of the century, approximately 170 herbal drugs were officially recognized in the U.S.A. and the director of W.H.O. Traditional medicine reported in 1993 that 80% of the world population relies chiefly on traditional medicine, mainly plant based, especially for their primary health care needs. In some Asian and African countries, up to 80% of the population relies on traditional medicine for their primary health care needs. When adopted outside of its traditional culture [1].

Pakistan scenario is that there are around 46,000 registered "hakims". Around 200 medicinal plants are found to be extensively used by the traditional healers evidenced by their annual consumption by ten leading herbal drug manufacturers in Pakistan. The worldwide alternative herbal medicine use has grown tremendously in the last three decades [2]. Amongst several factors contributing towards the increasing use of herbal drugs is the safety due to lack of information about adverse reactions and side effects. Whereas their accessibility, affordability, historical and cultural background besides safety promotes their use in the developing countries. A noteworthy population of Pakistan is using complementary and alternative medicine (CAM) therapies from different practitioners, irrespective of any education and awareness about their disease and long-term drawbacks that ends up in serious consequences and even loss of lives [3]. In Pakistan the use of CAM therapies are more common in patients with knee Osteoarthritis [4]. Out of many Medicinal plant families, 15 are associated with cytotoxic and genotoxic effects [5-11]. These are some examples of problems apparently associated with the uncontrolled use of "natural" products and traditional herbal medicines [2].

Few of most commonly used medicinal plants in Pakistan are Thyme seed (seed) Neem (leaf and seed) and Eucalyptus (leaves). Neems (Azadirachta indica) belong to family Meliaceae. Although aqueous extracts of neem are anti-fungal, anti-bacteria and anti-viral [12, 13, 14] and...
widely used for skin diseases but 150 mL of neem oil can cause bilateral vision loss after 5 days of application on scalp [15]. Neem leaf and seed extracts are reported to cause micronuclei, dinuclei, multinuclei, stickiness, laggards, bridges and tripolar anaphase in Allium cepa root tip cells [16, 17]. Eucalyptus (Eucalyptus globulus) another frequently used medicinal plant belongs to family Myrtaceae. It is widely used for treatment of fever, upset stomach, coughs, respiratory tract infections, asthma, pulmonary tuberculosis, osteoarthritis, joint pain (rheumatism), acne, wounds, burns, bacterial dysentery, ringworms, liver, gallbladder problems and loss of appetite. The major active ingredient of eucalyptus is Eucalyptol (Monoterpenoids) also known by a variety of synonyms: 1,8-cineole, limonene oxide, cajeputol, 1,8-epoxy-p-menthane, 1,8-oxido-p-menthane, 1,3,3-trimethyl-2-oxabicyclo (2,2,2) octane, cineole [18]. Eucalyptol are reported to cause transient coma, epigastric burning with nausea and usually vomiting, vertigo, ataxia, muscle weakness, stupor, pallor and sometimes cyanosis, respiratory stridor (edema) and myosis. Exposure to Eucalyptol vapor causes eye irritation in humans and corneal swelling. It can cause skin and mucous membranes irritation in human. If consumed internally at low dosage as a flavoring component or in pharmaceutical products at the recommended rate, cineole-based 'oil of eucalyptus' is safe for adults. However, systemic toxicity can result from ingestion or topical application at higher than recommended doses. Probable oral dose is lethal for humans is 50-500 mg/Kg or between 1 teaspoon and an ounce for a 70 kg person [19]. Its vapor causes transient injury of the corneal epithelium [20]. Thyme (Thymus vulgaris) belongs to family Lamiaceae. Thyme seed usage is almost confined to central Asia and Northern India. In Pakistan Thyme seeds are first aid medicine, present in every home. It is mostly used for the treatment of digestive tract, fever, cough, throat irritations, releasing stomach acids and Asthma. It has also anti-bacterial and anti-fungal properties [9, 21]. Chemical composition of Thyme seed includes Thymol (35 to 60%); furthermore, α-pinene, γ-terpinene, β-pinene, p-cymene and limonene have been found. Thymol, P-cymene and β-pinene are reported to cause human diseases [22, 23]. Thymol can cause pulmonary edema, respiratory failure, myocardial damage, circulatory failure, further complication include cardiac arrhythmias, depression, acidosis, cyanosis. Over dosage of Thymol include coma, dizziness and headache.

Mostly of people in Pakistan and specially Sindh are poor there for relay solely on herbs for common disorders for being cheap. According to them there are no side effects of herbs as compared to allopathic medicines. There for unlimited use of medicinal herbs has been a common practice in Pakistan. On the other hand rate of cancer patients with no previous drug addiction history is increasing all over the Pakistan. Uncontrolled use of herbal medicine may be one of the cancer risk factor. There for four herbal extracts will be tested for cytotoxicity, genotoxicity and oxidative damages (membrane damage) in chickpea (Cicer arietinum L.).

**MATERIALS AND METHODS**

**Plant Extract Preparation:** Neem fruits were soaked in hot water for 30 minutes to soften pulp. After removing pulp manually seeds were washed thoroughly. Then seed were dried overnight in Laboratory. The weighed quantities of medicinal plants were ground in metallic pestle and mortar until become pest for preparation of percentage solutions. Plant parts were simply soaked overnight and sieved to get extracts.

**Treatment:** Seeds were soaked in 10% solutions of four aqueous extracts of three medicinal plants (Thyme seeds, Neem seeds, Neem leaves, Eucalyptus leaves) for 0.5 and 1 hours, untreated seeds were used as positive control were soaked in dH₂O for 0.5 and 1h respectively and 0.5 hour soaked in 0.6% EMS treated seeds were used as negative control. After incubation seeds were wash from dH₂O (water) for 2 to 3 times than seeds sow in the pots for roots germination.

2cm root sample were collected andined in (3:1 glaciered acetic acid and alcohol) for 24 hour. Roots were transferred to 70% alcohol until used. Root tips were spread by using the squash technique [24, 25] slides were stained with 2% acetocarmine (2% in 45% glacial acetic acid).

6 slides per treatment were used to score chromosomal aberrations with the help of microscope (Olympus 51x) at 100x magnification. The photographs were taken with the digital camera (USB-2.0) Dino eye.

**Oxidative Damages:** Oxidative damages were observed under microscope in the form of Nuclear membrane damages, Cell membrane damages and Ghost cells.

**Data Analysis:** Initially germination percentage was obtained and then percent increase and decrease was calculated by the following formula:
% Increase/ decrease = \( \frac{\text{Treatment} - \text{Positive control}}{\text{Positive control}} \times 100 \)

**Mitotic Index (M.I.):** Mitotic index is ratio between the number of total dividing cells and the total cells analyzed. It was calculated by following formula:

\[ M.I. = \frac{\text{Total dividing cells}}{\text{Total cells analyzed}} \times 100 \]

**Abnormal Index (A.I.):** It is ratio of abnormal cells per was calculated by the method of Râcuciu [26] according to following formula:

\[ A.I. = \frac{\text{No. of abnormal dividing cells}}{\text{Total dividing cells}} \times 100 \]

**RESULTS AND DISCUSSION**

The results of germination percentage are compiled in Fig. 1. All the extracts inhibit germination of chickpea seed. In 0.5 hour treatment maximum percent decrease was observed in eucalyptus (11%) where as remaining three extracts caused 6% decline in germination percentage. In 1 hour treatment maximum percent decrease was recorded in thyme seed (37%) while minimum decline was found in neem leaf extract. The inhibitory effects of extracts were more pronounced as the incubation increases except neem leaf extract. Alteration in germination response is a clear indication of cytotoxicity of applied extracts. Present results are supported by number of workers who reported inhibitory effects of various medicinal plant aqueous extracts on seed germination [7, 27, 28, 29 ].

Fig. 2 shows the intact normal cells and Fig. 3 shows the negative control root tip cells with broken segments of chromosomes during mitosis. Figs. 4-11 shows the chromosomal aberrations induced by the aqueous extracts in the form of translocations, scatting bridges, micronuclei, dinuclei and fragmentation and high level of cell membrane damage and ghost cells.

The data of mitotic index and abnormality index is compiled in Table 1. In 0.5 hour treatment maximum mitotic index (M.I.) was observed in Eucalyptus (98%) and minimum in Thyme seed (93%). In 1 hour treatment maximum mitotic index was observed in Neem leaf (75%) and minimum in Neem seed (32%). Where as in 0.5 hour treatment maximum abnormality index (A.I.) was observed in Neem seed (26%) and minimum in Eucalyptus (7%).

In case 1 hour treatment mix abnormally index (A.I.) was observed in Thyme seed (97%) and minimum in eucalyptus (72%). On the basis of mitotic index and abnormality index Thyme seed and Neem seed extracts were the more genotoxic than Neem leaf and Eucalyptus leaf extracts. Soliman [16] also reported inhibitory effects of neem extracts on cell division.
Table 1: Effect of aqueous extracts of medicinal plants on mitotic index and abnormality index in chickpea root tip cells

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Name of treatment</th>
<th>Non dividing</th>
<th>Normal</th>
<th>Abnormal</th>
<th>M.I</th>
<th>A.I</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0.5 h 1 h</td>
<td>0.5 h 1 h</td>
<td>0.5 h 1 h</td>
<td>0.5 h 1 h</td>
<td>0.5 h 1 h</td>
</tr>
<tr>
<td>1</td>
<td>Neem Leaf</td>
<td>12 122</td>
<td>410 30</td>
<td>78 348</td>
<td>97 75</td>
<td>16 92</td>
</tr>
<tr>
<td>2</td>
<td>Neem Seed</td>
<td>12 338</td>
<td>361 28</td>
<td>127 134</td>
<td>97 32</td>
<td>26 82</td>
</tr>
<tr>
<td>3</td>
<td>Eucalyptus Leaf</td>
<td>10 282</td>
<td>456 60</td>
<td>34 158</td>
<td>98 43</td>
<td>7 72</td>
</tr>
<tr>
<td>4</td>
<td>Thyme Seed</td>
<td>33 208</td>
<td>411 7</td>
<td>56 285</td>
<td>93 58</td>
<td>12 97</td>
</tr>
<tr>
<td>5</td>
<td>EMS (.6%)</td>
<td>80</td>
<td>- 200</td>
<td>- 220</td>
<td>- 84</td>
<td>- 52</td>
</tr>
</tbody>
</table>

(EMS=Ethylemethan sulfonate).

Fig. 4: Neem Leaf (0.5 h) showing (A) Micronuclei; (B) Scattered nuclei; (C) Fragmentation; (D) Early anaphase; (E) Mid anaphase; (F) Anaphase bridge; (G, H & I) Ghost cell

Fig. 5: Neem seed (0.5 hour) showing (A) Ghost cells; (B) Proliferation

Fig. 6: Eucalyptus (0.5 hour) showing (A) Dinuclei; (B & C) Fragmentation

Fig. 7: Thyme seed (0.5 hour) showing (A) Dinuclei; (B, C & D) Ghost cells

Fig. 8: Neem leaf (1 hour) showing (A) Micronuclei; (B) Laggard; (C) Fragmentation (D & E) Ghost cells

Fig. 9: Neem seed (1 hour) showing (A) Cell membrane damage; (B) Micronuclei; (C & D) Fragmentation; (E) Dinuclei ghost; (F) Translocation ring
Table 2: Chromosomal aberration induced by aqueous extracts of medicinal plants in chickpea root tip cells.

<table>
<thead>
<tr>
<th>S.No</th>
<th>Abnormalities</th>
<th>Neem Leaf 0.5 h</th>
<th>Neem Seed 0.5 h</th>
<th>Eucalyptus Leaf 0.5 h</th>
<th>Thyme Seed 0.5 h</th>
<th>EMS (.6%) 0.5 h</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0.5 h</td>
<td>1 h</td>
<td>0.5 h</td>
<td>1 h</td>
<td>0.5 h</td>
</tr>
<tr>
<td>Metaphase</td>
<td>1 Sticky Metaphase</td>
<td>0</td>
<td>0</td>
<td>50</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>2 Translocation rings</td>
<td>0</td>
<td>6</td>
<td>0</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>3 Laggard</td>
<td>0</td>
<td>3</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Anaphase</td>
<td>4 Anaphase bridge</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>5 Scattered Nuclei</td>
<td>3</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>6 Fragmentation</td>
<td>67</td>
<td>200</td>
<td>71</td>
<td>74</td>
<td>28</td>
</tr>
<tr>
<td>Telophase</td>
<td>7 Micronuclei</td>
<td>4</td>
<td>98</td>
<td>4</td>
<td>18</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>8 Multinuclei</td>
<td>1</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>9 Di-nuclei</td>
<td>0</td>
<td>13</td>
<td>1</td>
<td>43</td>
<td>3</td>
</tr>
<tr>
<td>Total number of abnormal cells</td>
<td>78</td>
<td>348</td>
<td>127</td>
<td>134</td>
<td>34</td>
<td>158</td>
</tr>
</tbody>
</table>

(EMS=Ethylemethan sulfonate)

Table 3: Oxidative damages induced by aqueous extracts of medicinal plants in chickpea root tip cells.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Abnormalities</th>
<th>Neem Leaf 0.5 h</th>
<th>Neem Seed 0.5 h</th>
<th>Eucalyptus Leaf 0.5 h</th>
<th>Thyme Seed 0.5 h</th>
<th>EMS (.6%) 0.5 h</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0.5 h</td>
<td>1 h</td>
<td>0.5 h</td>
<td>1 h</td>
<td>0.5 h</td>
</tr>
<tr>
<td>1</td>
<td>Ghost cells</td>
<td>100</td>
<td>100</td>
<td>26</td>
<td>87</td>
<td>34</td>
</tr>
<tr>
<td>2</td>
<td>C.M damage</td>
<td>20</td>
<td>100</td>
<td>102</td>
<td>200</td>
<td>10</td>
</tr>
</tbody>
</table>

(C.M. = Cell membrane; EMS = Ethylemethan sulfonate)

Types of chromosomal aberrations induced by four aqueous extracts are complied in Table 2. In 0.5 hour treatment with aqueous extracts frequent abnormality found in Neem leaf was multinuclei (1 cell) while most frequent abnormality was fragmentation (67 cells). Least frequent abnormality found in Neem seed were laggard, scattered nuclei and dinuclei (1 cell) while most frequent abnormality was fragmentation (71 cells). Least frequent abnormality found in eucalyptus leaf was micronuclei (1 cell) while most frequent abnormality was fragmentation (46 cells). Least frequent abnormality found in Thyme seed were sticky metaphase and micronuclei (1 cell) while most frequent abnormality was fragmentation (200 cells). Least frequent abnormality found in EMS was multinuclei (20 cells) while most frequent abnormality was fragmentation (30 cells). In case of 1 hour treatment with aqueous extracts least frequent abnormality found in Neem leaf was laggard (3 cells) while most frequent was fragmentation (200 cells). Least frequent abnormality found in Neem seed was translocation ring (4 cells) while most frequent abnormality was fragmentation (74 cells). Least frequent abnormality found in eucalyptus were sticky metaphase and micronuclei (1 cell) while most frequent was fragmentation (156 cells). Least frequent abnormality found in Thyme seed were sticky metaphase and translocation rings (5 cells) while most frequent abnormality was again fragmentation (250 cells). The occurrence of high level of most deleterious chromosomal
abnormalities in cytology suggest the genotoxic effects of all the extracts applied and conforms the cytotoxicity observed in germination. Yumnamcha et al. [30] reported *Croton tiglium* aqueous extract induced plasmid DNA strand breakage. Soliman [17] also reported that 24 and 48 h treatment of Neem extracts can cause micronucleus and multinucleated cells, bridges, thickness, laggards and polyploidy.

Date of oxidative damages in presented in Table 3. In 0.5 hour treatment maximum ghost cell was found in Neem leaf (100 cells) and minimum in Neem seed (26 cells). Maximum cell membrane damage was found in Thyme seed (200 cells) and minimum were Eucalyptus and EMS (10 cells). In case of 1 hour treatment with aqueous extracts maximum ghost cells was found in Eucalyptus (126 cell) and minimum was Neem seed (87 cells). It can be inferred from results that besides being usefulness all the applied extracts have ability to destroy cell and can damage DNA when applied prolonged.

**CONCLUSIONS**

It is concluded that all the tested aqueous extracts of medicinal plants caused cytotoxic, genotoxic and oxidative damages. Due to DNA damaging capabilities of tested plant extracts their long term use might result in cancer. There for theses medicinal plants should not be used frequently for prolonged duration. In order to elucidate possible safe dose low concentration of these medicinal plants should be tested.

**REFERENCES**


