Research article

In vitro cytogenetic and cytotoxic activity of Xanthium strumarium plant extract on human breast cancer cell line

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ABSTRACT

Introduction and Aim: The enormous greater parts of people rely on conventional medicinal plants for their everyday health care desires. One of these medicinal plants is Xanthium strumarium. The purpose of this study was to assess the total phenolic content and antioxidant activity of X. strumarium extract as well as test its activity against micronucleus formation and cytotoxicity against breast cancer cell lines.

Materials and Methods: An estimation was made of the total flavonoid antioxidant content in the ethanolic extract of X. strumarium. The plant extract was assessed for its DPPH radical scavenging activity and compared to the conventional vitamin C. The plant extract's cytotoxicity was evaluated by conducting an MTT test on MCF-7 cancer cell lines.

Results: The total flavonoid content estimated in the plant extract was 215.63± 5.85 µg/ml. The DPPH results varied depending on concentration and found to be significantly reduced at 100 and 200 mg/ml of the extract in comparison to vitamin C. In contrast to the negative control, the micronucleus generation in the blood of breast cancer patients was reduced, notably at 200 mg/ml concentration of plant to (0.0065 ± 0.0006 mn/10⁶ cells), when it was higher in untreated culture (0.0230 ± 0.0013 mn/10⁶ cells). When plant concentration was increased (from 6.25 to 200 µg/ml), there was a drop in cell viability (86.27± 0.70% to 50.04± 3.32%).

Conclusion: This research indicates that the ethanolic extract of X. strumarium exhibits antioxidant capabilities and has the capability to cause cytotoxic effects on breast cancer cells. These findings were the result of an investigation that was conducted.

Keywords: Xanthium strumarium; DPPH; MCF-7 cancer cells; antioxidant; micronucleus; total flavonoid.

INTRODUCTION

The genus Xanthium (Family: Asteraeaceae) commonly known as ‘cockleburrs’ are flowering herbs that are widely distributed throughout America and Eastern Asia (1). Xanthium species have been used in traditional Chinese and Indian medicine since ancient times (2). Pharmacological and phytochemical studies have shown the plant X. strumarium to have anti-inflammatory, analgesic, antibacterial, anticancer, antifungal, antihyperglycemic, antiinflammatory, antitumoral, antimalarial, and diuretic properties (1-4). Even though X. strumarium is used medically, few research studies have shown X. strumarium ingestion to cause deleterious toxic side effects resulting in death in farm animals (5,6) and hepatotoxicity in humans (7). Recent studies have shown that plants with potential cytotoxic activity to be successfully used in treating human cancers (8, 9). Among 100s of cancer, breast cancer is the most prevalent cancer worldwide (10-13).

Several cytotoxic phytochemicals have been reported from Xanthium species (2). Xanthanolides from Xanthium species have been reported to have antitumor activity (14,15). Xanthatin and xanthinosin from X. strumarium L. has been reported to be a potential anticancer agent (16). Sesquiterpene lactones are the main bioactive constituents isolated from Xanthanthes species that have been reported to exhibit antioxidant and cytotoxicities against different cancer cell lines (15-17). The research aimed to investigate the effect of ethanolic extract of X. strumarium for its phytochemical constituents was assayed as well as its cytogenetic and cytotoxic potentials on breast cancer cell line MCF-7 originated from the human breast.

MATERIALS AND METHODS

Plant material

Fresh aerial fragments of Xanthium strumarium were collected between March 2020 and January 2021 from the northern region of Iraq (Erbil city). The plant material was sent to the Herbarium division of the Department of Biology of the Sciences College, Baghdad University for identification and documentation.

X. strumarium ethanol extraction

In the laboratory, the fresh plant was rinsed with distilled water following which it was cut into pieces, dried and powdered. To obtain the ethanolic extract of the plant material, about 50 grams of plant powder was mixed with 70% of ethanol and the mixture allowed to evaporate at 65°C for three hours using a
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Soxhlet apparatus. The crude extract obtained was filtered twice: once with clean Whatman no.1 filter paper and then with a muslin cloth. At a temperature between 40 and 45 °C, the filtrate was dried by evaporation. The concentrated extract obtained was stored in a plastic container at 4°C until use (18).

Estimation of total flavonoids

The plant extract was analyzed using the aluminium chloride colorimetric method to determine the concentration of the most active ingredient, which was the total flavonoid (19). In summary, the ethanolic extract (3.2 mg) was dissolved in 5 ml of 50% methanol, and then 1 ml of a 5% (w/v) sodium nitrite solution was added. 1 ml of a 10% (w/v) aluminium chloride solution was added to the mixture, and it was left undisturbed for 5 minutes. Then, 10 ml of a 10% (w/v) NaOH solution was added. The mixture was diluted to a total amount of 50 cc using distilled water and well mixed. The spectrometer was used to measure the absorbance of the mixture at 450 nm after a duration of 15 minutes. The measurement of a standard curve was conducted using rutin as a reference for flavonoids. Various concentrations (2.5, 5, 10, 20, 40, and 80 µg/ml) were employed in the procedure.

Detection of DPPH radical scavenging activity

The assessment of DPPH radical scavenging activity was conducted according to the previously reported procedure (20-21). A portion of 0.1 ml of either the plant extract or the standard (Vitamin C) at concentrations of 0.625, 0.125, 0.250, and 0.500 mg/ml, was combined with 3.9 ml of the DPPH solution. The absorbance of each solution was measured at 517 nm using a spectrophotometer after being incubated at 37 °C for 30 minutes. The effectiveness of a chemical in removing DPPH radicals was evaluated using the following equation:

\[ \text{DPPH radical scavenging activity} \% = 1 - \left( \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \right) \times 100 \]

Estimation of DPPH radical scavenging activity

The main steps of the experiment included:

1. Preparation of the DPPH solution:
   - A methanolic solution of DPPH (10 mg/ml) was prepared.

2. Preparation of the plant extract solution:
   - The X. strumarium plant extract was prepared as described in the methodology section.

3. Scavenging activity assay:
   - A 50-µl portion of the DPPH solution was added to each well of a 96-well microplate.
   - A 50-µl portion of the plant extract solution was added to each well.
   - The absorbance of each well was measured at 517 nm using a spectrophotometer after incubation at 37 °C for 30 minutes.

4. Calculation of the scavenging activity:
   - The scavenging activity was calculated using the following equation:

\[ \text{Scavenging activity} \% = \left( 1 - \frac{\text{Absorbance of control}}{\text{Absorbance of sample}} \right) \times 100 \]

Cytotoxic screening and MTT assay for X. strumarium extract on MCF-7 cancer cell line

Breast carcinoma cells MCF-7, was used for cytotoxicity screening of X. strumarium ethanol extract. Through the use of a ready kit of MTT, different plant extract concentrations ranging from 6.2, 12.5, 25, 50, 100, and 200 µg/ml were used to assess the cytotoxic effects of X. strumarium on MCF-7 breast cancer cell line in vitro. The cell line was maintained in accordance with (23,24).

In summary, MCF-7 cells were placed into 96-well microtiter plates and kept at a temperature of 37°C for 24 hours to allow them to adhere. Subsequently, varying amounts of the plant extract were applied to each well and incubated for an additional 24 hours. Subsequently, a volume of 10 mL of MTT solution was introduced into the mixture, which was then incubated once again at a temperature of 37°C and a CO₂ concentration of 5% for a duration of 4 hours. A volume of 100 µl of the stable solution from the kit was introduced, and the absorbance was then measured after 5 minutes using an ELISA microplate reader (Bio-Rad, USA) to quantify the generation of formazan at a wavelength of 570 nm.

Statistical analysis

Analysis of variance (ANOVA) was used using the computer software SPSS version 13.1 to determine differences between means. The values of the studied parameters are expressed as mean standard error.
RESULTS

The flavonoid content in the ethanolic extract of X. strumarium in this study was estimated to be 215.63 ± 5.85µg/ml. The DPPH radical scavenging ability estimated for 200, 100, 50, 25, and 12.5 mg/ml of the plant extract was 75.08±1.59, 63.23±3.742, 52.08±2.780, 39.66±4.169 and 28.70±3.108 respectively (Table 1) at an IC50 value of 50.89 µg/mL. A significant DPPH radical scavenging activity was observed at 100 and 200 µg/ml concentrations of the X. strumarium extract in comparison to Vitamin C (Table 1).

Table 1: DPPH radical scavenging activity of X. strumarium ethanolic extract and Vitamin C

<table>
<thead>
<tr>
<th>Concentration (µg/ml)</th>
<th>DPPH radical scavenging activity (Mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>X. strumarium extract</td>
<td>Vitamin C</td>
</tr>
<tr>
<td>12.5</td>
<td>28.70±3.108</td>
</tr>
<tr>
<td>25</td>
<td>39.66±4.169</td>
</tr>
<tr>
<td>50</td>
<td>52.08±2.780</td>
</tr>
<tr>
<td>100</td>
<td>63.23±3.742*</td>
</tr>
<tr>
<td>200</td>
<td>75.08±1.59*</td>
</tr>
</tbody>
</table>

*significant

Effect of X. strumarium extract on inhibiting micronucleus formation

The presence of micronucleus in breast cancer aspirate was seen (Fig. 1). The micronucleus formation in lymphocyte cultures of breast cancer patients in the presence of different concentrations of ethanolic extract is given in Table 2. The results indicated the ability of the plant to reduce micronucleus according to concentrations tested by the plant. The maximum reduction is shown at 200 µg/ml, the micronucleus formation ratio was (0.0065±0.0006, 0.0087±0.0008, 0.0124±0.0009, 0.0136±0.0008 and 0.0170±0.0007 micronucleus/cell) for (200, 100, 50, 25 and 12.5 µg/ml) respectively in comparison to culture of untreated breast cancer cell (0.0230±0.0013 micronucleus/cell).

MTT test plant extract cytotoxicity

The percentage vitality of treated cells was determined by comparing them to the normal cell line WRL-68. Table 3 showed that there was a decline in cell viability as the concentration of the plant rose. The greatest reduction in MCF-7 cell viability (%) was observed at a concentration of 200 µg/ml (50.04±3.32), while the lowest reduction was reported at a concentration of 12.5 µg/ml (86.27±0.70). Across all examined doses of the plant extract, the cell viability of MCF7 cell line cells was considerably decreased compared to normal WRL-68 cells (Table 3). The plant extract demonstrated cytotoxic action with an IC50 value of 24.02 µg/ml. From the analysis of the plant extract’s impact on the WRI-68 normal cell line, a specific IC50 value of 404.3 µg/ml was determined (Table 3, Fig2).

Table 2: Micronucleus formation in lymphocyte cultures of breast cancer patients

<table>
<thead>
<tr>
<th>Concentrations</th>
<th>Un-treated</th>
<th>12.5 µg/ml</th>
<th>25 µg/ml</th>
<th>50 µg/ml</th>
<th>100 µg/ml</th>
<th>200 µg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Micronucleus/cell (Mean± Standard Error)</td>
<td>0.0230 ± 0.0013</td>
<td>0.0170 ± 0.0007</td>
<td>0.0136 ± 0.0008</td>
<td>0.0124 ± 0.0009</td>
<td>0.0087 ± 0.0008</td>
<td>0.0065 ± 0.0006</td>
</tr>
</tbody>
</table>

Table 3: Cytotoxicity effect of plant extract on MCF-7 and WRI-68 cells

<table>
<thead>
<tr>
<th>Plant extract concentration s µg/ml</th>
<th>Viable cell count of</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MCF7 cell line Mean± S.D.</td>
</tr>
<tr>
<td>12.5</td>
<td>86.27±0.70</td>
</tr>
<tr>
<td>25</td>
<td>72.65±1.71</td>
</tr>
<tr>
<td>50</td>
<td>62.00±1.49</td>
</tr>
<tr>
<td>100</td>
<td>54.28±3.89</td>
</tr>
<tr>
<td>200</td>
<td>50.04±3.32</td>
</tr>
</tbody>
</table>

Fig.1: Micronucleus formation in breast cancer patients (100X).
DISCUSSION

In recent years, phytochemicals extracted from plants have been widely used in traditional medicine for the prevention and treatment of various health problems. Phytochemical analysis of X. strumarium ethanolic extract in the present showed the plant extract to be rich in flavonoids with a high scavenging ability. This is in line with previous studies wherein both leaves and stems of X. strumarium have been reported to contain flavonoids as one of its phytochemical components and well thought-out as sources of antioxidants and scavenging activity (25, 26). A MTT assay study for the anti-cancer activity of the ethanolic extract in this study, revealed the extract to exhibit cytotoxic activity on MCF-7 breast cancer cells with an IC₅₀ rate of 24.02 μg/ml. In addition, the extract also reduced micronucleus formation in breast cancer cells which indicates that X. strumarium ethanolic extract possesses the potential to reduce DNA damage caused at the chromosome level. Our results have shown X. strumarium ethanolic extract to have anti-proliferative and antioxidant effects which assumes significance as anti-proliferative activity has been linked to an increase in apoptosis (27). There are many attempts for cancer treated with alternative treatment such as using genetically engineered gene transfer systems (28) or by applied oncolytic viruses (29) or by applied oncolytic viruses (30, 31). However, on the basis of these results, more research on X. strumarium with anticancer activity potential required due to its role as source of phytochemicals for cancer therapies could be based on these findings (32, 33).

CONCLUSION

This study showed that X. strumarium's ethanolic extract can induce apoptosis in the MCF-7 breast cancer cell line and exhibit antioxidant and anti-cancer properties.

ACKNOWLEDGEMENT

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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