EFFECT OF HYPERTHERMIA AND COMBINATION TREATMENT
7.1. INTRODUCTION

Hyperthermia has been shown to enhance the antitumour activity of Chemotherapeutic drugs (233, 247, 255). Surgery, radiation and chemotherapy are the conventional modalities to treat the neoplastic disease, however, many clinical trials conducted in various laboratories have revealed that elevated temperature could be an additional modality in the clinical management of cancer (306, 307). This approach of combining hyperthermia along with antineoplastic agents affords a measure of targeting and selective cytotoxicity unknown earlier.

Cisplatin and cyclophosphamide are most active anticancer agents and have broad clinical application especially for, testicular, ovarian, head and neck, bladder and lung cancers (296, 300). Hyperthermia is found to potentiate the action of several antitumour agents including cisplatin (258) and cyclophosphamide (259). However the major limitations to the use of these drugs is their dose limiting toxicity (297, 300).

The extract of 'Ashoka' (Saraca asoca) showed potential antitumour activity and chemoprotective effects on toxicities induced by cisplatin and cyclophosphamide. These findings prompted us to investigate more extensively the combined effect of using 'Ashoka' extract and hyperthermia (42°C) along with cisplatin or cyclophosphamide both in vitro and in vivo.
Materials and Methods:

Drugs: Ashoka extracts (bark and flower) was obtained and purified as described in Chapter-2.

Cisplatin was purchased from Biochem Pharmaceuticals, Bombay, Cyclophosphamide (Endoxan Asta) from Khandelwal Laboratories, Bombay. These were stored at 4°C.

Thermo couple: - (IT-21) was gifted by Seven-Seas Engineers, Bombay.

Tumour: Sarcoma-180 (S-180) tumour was obtained from Cancer Research Institute, Bombay.

Mice: Inbred male Swiss albino weighing 18-20 g were used.

7.2.1. Determination of combined effect of Saraca asoca (Ashoka) extracts and hyperthermia along with cisplatin or cyclophosphamide.

S-180 cells were aspirated from Swiss albino tumour-bearing mice and washed thrice with sterile normal saline. 5x10⁴ cells were added to culture bottles containing 5 ml MEM with 10% FCS. Aliquots of the drug were added one hour after the addition of the cells. The controls did not contain the drugs. Two sets of experiments were conducted. One set of culture bottles were incubated at 37°C and
another set at 42°C for hyperthermia studies for twenty four hours in 5% CO₂ atmosphere.

After the incubation, the cells were harvested by centrifugation (1200 rpm), and counted. All the experiments were done in duplicates. The concentration of the drugs added and other details are shown in Fig 20.

7.2.2. Tumour Inoculation:

All groups of mice were injected subcutaneously with \(1 \times 10^6\) Sarcoma-180 tumour cells on the right hind leg and randomized into two mice per cage.

7.2.3. Localised Tumour Hyperthermia:

Seven days after the tumour transplantation, mice were anaesthetized with pento barbital and the tumour limb extended. Mice were placed on rack and only the tumour bearing limb was immersed into 42.5 ± 0.1°C water bath. The body of the mouse was placed on a slanted plexiglas so that it remained out of water. The tumour limb was immersed in the water bath maintained at 42.5 ± 0.1°C for 30 minutes. A type IT-21 thermocouple microprobe was passed completely through the tumour, exiting into the water bath. The prob was then slowly pulled through the tumour while continuous measurements were
made. The temperature at the site of tumour remained uniform and entirely homogenous.

7.2.4. Ashoka extract treatments in combination with cisplatin and Hyperthermia (in vivo):

Cisplatin was dissolved in sterile distilled water. The extract of 'Ashoka' bark or flower was reconstituted in 200 µl of sterile saline. Drug treatment and hyperthermia commenced on the 7th day after tumour inoculation, on alternate days i.e. (7th, 9th, 11th, 13th)

Hyperthermia was given one hour after drug administration. To the first group of mice, cisplatin was given (3 mg/kg i.p) and hyperthermia for 30 min. The second group was given cisplatin (3 mg/kg i.p) without hyperthermia. The third group was administered 'Ashoka' bark extract (50 mg/kg i.p), 30 min. before cisplatin (3 mg/kg i.p) administration and hyperthermia subsequently as above. The fourth group received 'Ashoka' bark extract and cisplatin as above but without hyperthermia. The fifth group was given Ashoka flower extract (50 mg/kg i.p) 30 min. before cisplatin (3 mg/kg i.p) and hyperthermia subsequently. The sixth group was given Ashoka flower and cisplatin as above without hyperthermia. Group seven and eight were the controls which received sterile saline (100 µl) and hyperthermia and sterile saline respectively.
7.2.5. 'Ashoka extract' treatment in combination with cyclophosphamide and hyperthermia (in vivo):

Cyclophosphamide (2 mg) was dissolved in 200 µl of sterile distilled water. Tumour inoculation, drug treatment, and hyperthermia were given using the same protocol as mentioned in 7.2.2. and 7.2.3. To the first group of mice cyclophosphamide was given (100 mg/kg i.p) and hyperthermia for 30 minutes. The second group received cyclophosphamide (100 mg/kg i.p) without hyperthermia. The third group was administered Ashoka bark extract (50 mg/kg i.p) 30 minutes before cyclophosphamide (100 mg/kg i.p) administration and hyperthermia, subsequently as above. The fourth group received Ashoka bark extract and cyclophosphamide as above both without hyperthermia. The fifth group was given Ashoka flower extract (50 mg/kg i.p) thirty minutes before cyclophosphamide (100 mg/kg i.p) and hyperthermia subsequently. The sixth group was given Ashoka flower and cyclophosphamide as above without hyperthermia. Group seven and eight were the controls which received sterile saline (100 µl) and hyperthermia and sterile saline respectively.

On the 25th day all the animals were sacrificed by cervical dislocation. The tumours on the hind leg were cut, blotted dry and weighed.
7.2.6. Determination of Biochemical and haematological studies:

Peripheral blood for various studies was collected from the caudal vein at different intervals. The total leucocyte counts were performed using a haemocytometer. Blood Urea Nitrogen (BUN) Serum alkaline phosphatase (SAKP) and Serum glutamate pyruvate transaminase (SGPT) were estimated.

7.3. RESULTS

7.3.1. Effect of hyperthermia and Ashoka extracts along with Cisplatin or Cyclophosphamide on S-180 tumour cells (in vitro):

In presence of 0.1 µg/ml of cisplatin 56.9% cells survived at 37°C. While at 42°C the cell survival was 39.5±7.5%. 'Ashoka' bark extract alone at a concentration of 0.5 µg/ml showed cell survival of 76.4±10.5 at 37°C and 64.1±11.12% at 42°C. However, the combination of cisplatin (0.1 µg/ml) and Ashoka bark extract (0.5 µg/ml) reduced cell survival to 44.3±8.2% (37°C) and 24.12±4.2% at 42°C.

In the case of flower extract alone at a concentration of 0.5 µg/ml showed a cell survival of 82.4±9.8% at 37°C and 70±8.5% at
Fig. 19: Enhancement of Cytotoxic effect of Cisplatin in presence of Saraca asoca bark or flower extract and hyperthermia (42°C) to S-180 tumour cells in vitro.

- Control (without drug)
- Saraca asoca bark (0.5ug/ml)
- Saraca asoca flower (0.5 ug/ml)
- Cisplatin (0.1 ug/ml)
- Saraca asoca flower (0.5ug/ml) + Cisplatin (0.1 ug/ml)
- Saraca asoca bark (0.5ug/ml) + Cisplatin (0.1 ug/ml)
But the combination of cisplatin (0.1 μg/ml) and flower extract (0.5 μg/ml) reduced cell survival to 48.2±8.4% (37°C) and 34.22±6.8% at 42°C (Fig. 19).

Similarly in the case of cyclophosphamide (CTX) treated cells, a concentration of 1 μg/ml showed cell survival of 42.98±6.25% at 37°C and 35.36±3.53% at 42°C. In combination ie., CTX (1μg/ml) Ashoka bark or flower extract (0.5 μg/ml) the cell survival was reduced to 24.12±4.4% and 34.14±2.8% at 37°C respectively and 14.5±2.5% and 18.4±6.14% at 42°C respectively (Fig. 20).

These studies indicate a lower cell survival (increased cell kill) when 'Ashoka' bark or flower extract is combined with cisplatin or cyclophosphamide along with hyperthermia.

7.3.2. Effect of 'Ashoka' extracts and cisplatin on Hyperthermia.

Table-46 depicts the inhibitory effect of 'Ashoka' extracts in combination with cisplatin and hyperthermia in seven day old Sarcoma-180 tumour in mice. Combination treatment with cisplatin and 'Ashoka' bark or flower extract in the absence of hyperthermia restricted the tumour growth by 68% and 58%. While animals received trimodality treatment schedule consisting of 'Ashoka' bark or flower extract, cisplatin and hyperthermia effectively inhibited the growth of S-180 tumour by 93.3% and 83% respectively (p < 0.001) (Table-48).
Fig. 20: Enhancement Cytotoxic effect of Cyclophosphamide in presence of *Saraca asoca* bark or flower extract and hyperthermia (42°C) to S-180 tumour cells *in vitro*.

- Control without drug.
- *Saraca asoca* flower (0.5 ug/ml)
- *Saraca asoca* bark (0.5 ug/ml)
- Cyclophosphamide (CTX) (1 ug/ml)
- *Saraca asoca* flower (0.5 ug/ml) + CTX (1ug/ml)
- *Saraca asoca* bark (0.5 ug/ml) + CTX (1ug/ml)
Table 48: Effect of *Saraca asoca* bark and flower extracts on the growth of Sarcoma-180 tumours in mice treated with cisplatin and hyperthermia

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose Regimen</th>
<th>Mean tumour weight (g)</th>
<th>Tumour growth inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S-180 + Cisplatin + Hyperthermia</td>
<td>3 mg/kg i.p.</td>
<td>0.77 ±0.01</td>
<td>36</td>
</tr>
<tr>
<td>S-180 + Cisplatin</td>
<td>3 mg/kg i.p.</td>
<td>0.823±0.08</td>
<td>31</td>
</tr>
<tr>
<td>S-180 + Cisplatin + <em>Saraca asoca</em> bark + hyperthermia</td>
<td>3 mg/kg + 50 mg/kg i.p.</td>
<td>0.08 ±0.01 ***</td>
<td>93</td>
</tr>
<tr>
<td>S-180 + Cisplatin + <em>Saraca asoca</em> bark</td>
<td>3 mg/kg + 50 mg/kg i.p.</td>
<td>0.38 ±0.07 **</td>
<td>68</td>
</tr>
<tr>
<td>S-180 + Cisplatin + <em>Saraca asoca</em> flower + hyperthermia</td>
<td>3 mg/kg + 50 mg/kg i.p.</td>
<td>0.21 ±0.03 ***</td>
<td>83</td>
</tr>
<tr>
<td>S-180 + Cisplatin + <em>Saraca asoca</em> flower</td>
<td>3 mg/kg + 50 mg/kg i.p.</td>
<td>0.50 ±0.05</td>
<td>58</td>
</tr>
<tr>
<td>S-180 + hyperthermia</td>
<td>-</td>
<td>1.1±0.23</td>
<td>08</td>
</tr>
<tr>
<td>S-180 Control mice (Sterile saline)</td>
<td>-</td>
<td>1.2±0.17</td>
<td>-</td>
</tr>
</tbody>
</table>

Tabular values represent mean ± SD of seven mice/group for three separate experiments (N=21). Values significantly different from mice treated with cisplatin and hyperthermia. Tumour growth inhibition (%) was calculated with respect to S-180 control mice.

*** P \(\leq 0.001\)

** P \(\leq 0.005\)
Leucocyte counts and the haemoglobin levels were significantly reduced in mice which received cisplatin 3 mg/kg in conjunction with hyperthermia or without hyperthermia respectively. The leucocyte counts and the haemoglobin levels appeared normal in both the Saraca asoca bark and flower treated groups receiving trimodality therapy (Fig.21,22).

Serum chemistry studies indicated that the serum glutamate pyruvate transaminase (SGPT) levels were increased to almost two fold in the group treated with cisplatin and hyperthermia. The administration of Saraca asoca bark extract significantly protected the groups receiving trimodality therapy from a sharp rise in SGPT levels (Table-49). Saraca asoca flower extracts did not show this protective effect. The blood urea nitrogen levels were also predictably altered in the cisplatin treated groups receiving hyperthermia. However the blood urea nitrogen were maintained in the near normal range in mice which received Saraca asoca bark or flower along with cisplatin and hyperthermia.

7.3.3. Effect of 'Ashoka' extract and Cyclophosphamide (CTX) on hyperthermia:

The effect of 'Ashoka' extracts in inhibiting tumour growth in combination with CTX and hyperthermia is shown in Table-50. Trimodality treatment consisting of the extracts of Saraca asoca bark or flower and Cyclophosphamide along with hyperthermia could
Fig. 21: Effect of *Saraca asoca* bark (50 mg/kg) or flower (50 mg/kg) extract on total leucocyte counts of Sarcoma - 180 tumour bearing mice treated with Cisplatin (3 mg/kg) and hyperthermia.

(☐☐) S - 180 + Cisplatin
(■■■) S - 180 + Cisplatin + Hyperthermia.
(○○○) S - 180 + Cisplatin + *Saraca asoca* bark + Hyperthermia.
(●●●) S - 180 + Cisplatin + *Saraca asoca* flower + Hyperthermia.
(▲▲▲) S - 180 + Cisplatin + *Saraca asoca* flower.
(△△△) S - 180 + Hyperthermia.
(◇◇◇) S - 180 + Normal saline.
Fig. 22: Effect of *Saraca asoca* bark 50mg/kg or flower 50mg/kg extract on haemoglobin levels of Sarcoma - 180 (S-180) tumour bearing mice treated with cisplatin (3mg/kg) and hyperthermia.

- **S - 180 + Cisplatin**
- **S - 180 + Cisplatin + Hyperthermia**
- **S - 180 + Cisplatin + *Saraca asoca* bark + Hyperthermia**
- **S - 180 + Cisplatin + *Saraca asoca* flower + Hyperthermia**
- **S - 180 + Hyperthermia**
- **S - 180 + Normal saline**
Table 49: Blood Urea Nitrogen and glutamine pyruvate transaminase levels of Sarcoma-180 tumour bearing mice treated with *Saraca asoca* along with cisplatin and hyperthermia

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Serum glutamate Pyruvate transaminase levels (IU/L)</th>
<th>Blood Urea Nitrogen (mg/100 ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 3</td>
<td>Day 14</td>
</tr>
<tr>
<td>S-180 + Cisplatin (3 mg/kg) + Hyperthermia</td>
<td>4.6 ± 0.2</td>
<td>8.6 ± 0.45</td>
</tr>
<tr>
<td>S-180 + Cisplatin (3 mg/kg)</td>
<td>4.7 ± 0.4</td>
<td>9.1 ± 0.5</td>
</tr>
<tr>
<td>S-180 + Cisplatin (3 mg/kg) + <em>Saraca asoca</em> bark (50 mg/kg) + Hyperthermia</td>
<td>5.2 ± 1.1</td>
<td>5.2 ± 0.3***</td>
</tr>
<tr>
<td>S-180 + Cisplatin (3 mg/kg) + <em>Saraca asoca</em> bark (50 mg/kg)</td>
<td>6.0 ± 1.0</td>
<td>7.7 ± 0.65</td>
</tr>
<tr>
<td>S-180 + Cisplatin (3 mg/kg) + <em>Saraca asoca</em> flower (50 mg/kg) + Hyperthermia</td>
<td>5.3 ± 0.6</td>
<td>7.6 ± 0.77</td>
</tr>
<tr>
<td>S-180 + Cisplatin (3 mg/kg) + <em>Saraca asoca</em> flower (50 mg/kg)</td>
<td>5.7 ± 0.7</td>
<td>8.1 ± 1.05</td>
</tr>
<tr>
<td>S-180 + Hyperthermia (Sterile saline)</td>
<td>4.1 ± 0.2</td>
<td>4.1 ± 0.32</td>
</tr>
<tr>
<td>S-180 + Control mice (Sterile saline)</td>
<td>4.0 ± 0.5</td>
<td>4.0 ± 0.25</td>
</tr>
</tbody>
</table>

Values represent the mean ± SD of seven mice used per group for three separate experiments (N = 21). Significantly different from mice treated with cisplatin and hyperthermia.

* P ≤ 0.05, ** P ≤ 0.02, *** P ≤ 0.001
effectively inhibit the growth of Sarcoma-180 tumour to 89% and 80% respectively. The same treatment schedule in the absence of hyperthermia could inhibit the tumour growth only to 71% and 72%. In contrast cyclophosphamide alone with or without hyperthermia inhibited tumour growth by 61% and 47% respectively.

Leucocyte counts and the haemoglobin levels were significantly reduced in mice which received cyclophosphamide (100 mg/kg) in conjugation with hyperthermia (Fig.23). The leucocyte counts and the haemoglobin levels appeared normal in both Saraca asoca bark and flower treated group receiving trimodality therapy (Fig.23 and Fig.24).

Serum Chemistry studies indicated that the serum glutamate pyruvate transaminase (SGPT) levels were increased to almost two fold in the group treated with cyclophosphamide and hyperthermia (normal values 4.00 ± 0.25 IU/l) and Saraca asoca bark or flower extracts, significantly protected the groups receiving trimodality therapy from a sharp rise in SGPT levels (Table 51).

The serum alkaline phosphatase levels on day 8 and 14 in cyclophosphamide treated group were also predictably altered. However the serum alkaline levels were almost near normal range in mice which received Ashoka bark or flower extracts along with cyclophosphamide and hyperthermia (Table 51).
Table 50: Effect of *Saraca asoca* bark and flower extracts on the growth of Sarcoma-180 tumours in mice treated with cyclophosphamide and hyperthermia

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose Regimen</th>
<th>Mean tumour weight (g)</th>
<th>Tumour growth inhibition %</th>
</tr>
</thead>
<tbody>
<tr>
<td>S-180 + Cyclophosphamide + Hyperthermia</td>
<td>100 mg/kg</td>
<td>0.55 ±0.03</td>
<td>61</td>
</tr>
<tr>
<td>S-180 + Cyclophosphamide</td>
<td>100 mg/kg</td>
<td>0.765±0.035</td>
<td>47</td>
</tr>
<tr>
<td>S-180 + Cyclophosphamide + <em>Saraca asoca</em> bark + Hyperthermia</td>
<td>100 mg/kg + 50 mg/kg i.p</td>
<td>0.16 ±0.02***</td>
<td>89</td>
</tr>
<tr>
<td>S-180 + Cyclophosphamide + <em>Saraca asoca</em> bark</td>
<td>100 mg/kg + 50 mg/kg i.p</td>
<td>0.4 ±0.03</td>
<td>72</td>
</tr>
<tr>
<td>S-180 + Cyclophosphamide + <em>Saraca asoca</em> flower + Hyperthermia</td>
<td>100 mg/kg + 50 mg/kg i.p</td>
<td>0.23 ±0.05***</td>
<td>84</td>
</tr>
<tr>
<td>S-180 + Cyclophosphamide + <em>Saraca asoca</em> flower</td>
<td>100 mg/kg + 50 mg/kg i.p</td>
<td>0.42±0.04</td>
<td>71</td>
</tr>
<tr>
<td>S-180 + Hyperthermia</td>
<td>-</td>
<td>1.30±0.14</td>
<td>10</td>
</tr>
<tr>
<td>S-180 Control mice (Sterile saline)</td>
<td>-</td>
<td>1.44±0.07</td>
<td>-</td>
</tr>
</tbody>
</table>

Tabular values represent mean ± SD of seven mice/group for three separate experiments (N=21)

Values significantly different from mice treated with cyclophosphamide and hyperthermia, Tumour growth inhibition (%) was calculated with respect to S-180 Control mice.

*** P < 0.001
Fig. 23: Effect of *Saraca asoca* bark (50mg/kg) or flower (50mg/kg) extract on total leucocyte counts of Sarcoma - 180 tumour bearing mice treated with Cyclophosphamide (100mg/kg) and hyperthermia.

- (○—○) S - 180 + Cyclophosphamide (CTX)
- (■—■) S - 180 + CTX + Hyperthermia.
- (○—○) S - 180 + CTX + *Saraca asoca* bark + Hyperthermia
- (○—○) S - 180 + CTX + *Saraca asoca* bark.
- (●—●) S - 180 + CTX + *Saraca asoca* flower + Hyperthermia
- (△—△) S - 180 + CTX + *Saraca asoca* flower.
- (▲—▲) S - 180 + Hyperthermia.
- (▲—▲) S - 180 + Normal Saline.
Fig. 24: Effect of *Saraca asoca* bark (50mg/kg) or flower (50mg/kg) extract on haemoglobin levels of Sarcoma-180 tumour bearing mice treated with Cyclophosphamide (CTX) 100mg/kg and hyperthermia.

(□ □) S-180 + CTX
(■ ■) S-180 + CTX + Hyperthermia.
(○ ○) S-180 + CTX + *Saraca asoca* bark + Hyperthermia
(□ □) S-180 + CTX + *Saraca asoca* bark.
(● ●) S-180 + CTX + *Saraca asoca* flower + Hyperthermia
(○ ○) S-180 + CTX + *Saraca asoca* flower
(△ △) S-180 + Hyperthermia
(▲ ▲) S-180 + Normal Saline.
Table 51: Serum glutamate pyruvate transaminase and Serum alkaline Phosphatase levels of Sarcoma-180

tumour bearing mice treated with Saraca asoca along with cyclophosphamide and hyperthermia

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Serum glutamate pyruvate (KA Units)</th>
<th>Serum alkaline phosphatase (KA Units)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 8</td>
<td>Day 14</td>
</tr>
<tr>
<td>S-180 + Cyclophosphamide (100 mg/kg + Hyperthermia)</td>
<td>6.3±0.7</td>
<td>9.6±0.6</td>
</tr>
<tr>
<td>S-180 + Cyclophosphamide (100 mg/kg)</td>
<td>5.1±0.4</td>
<td>9.1±0.6</td>
</tr>
<tr>
<td>S-180 + Cyclophosphamide (100 mg/kg) + Saraca asoca bark (50 mg/kg) + Hyperthermia</td>
<td>4.9±0.6</td>
<td>5.9±0.35***</td>
</tr>
<tr>
<td>S-180 + Cyclophosphamide (100 mg/kg) + Saraca asoca bark (50 mg/kg)</td>
<td>5.4±0.9</td>
<td>6.1±0.7</td>
</tr>
<tr>
<td>S-180 + Cyclophosphamide (100 mg/kg) + Saraca asoca flower (50 mg/kg) + Hyperthermia</td>
<td>4.6±0.7</td>
<td>5.2±0.6***</td>
</tr>
<tr>
<td>S-180 + Cyclophosphamide (100 mg/kg) + Saraca asoca flower (50 mg/kg)</td>
<td>4.9±0.6</td>
<td>4.9±0.7</td>
</tr>
<tr>
<td>S-180 + Hyperthermia</td>
<td>4.1±0.2</td>
<td>4.1±0.32</td>
</tr>
<tr>
<td>S-180 (Control Mice) (Sterile Saline)</td>
<td>4.0±0.25</td>
<td>4.1±0.25</td>
</tr>
</tbody>
</table>

Values represent the mean ± SD mice used per group for three separate experiments (N=21)

Significantly different from mice treated with cyclophosphamide and hyperthermia

** P ≈ 0.005  *** P ≈ 0.001  * P ≈ 0.01
7.4. DISCUSSION:

The selective action of supra normal temperature on malignant cells and its benefical combination with anticancer drugs on transplantable animal tumours as well as in some clinical cancer have been reported (225,308). The selective toxicity of hyperthermia against cancer cells is well documented (254). Enhancement of cytotoxicity of cisplatin (258) and cyclophosphamide (259) have been demonstrated using a wide variety of tumour models. The enhanced cytotoxicity of cisplatin may be due to increased drug mediated cross-linking of DNA (309) but other factors such as increased cellular accumulation of platinum decreased repair of potentially lethal damage or kinetic changes may also play a very significant role. While DNA is the main target for cyclophosphamide, hyperthermia may inactivate cells by several targets including membrane (310). Thus the differing modes of action may explain the enhanced effect of combined cyclophosphamide and hyperthermia in S-180 tumours. Other factors like cell cycle parameters, blood flow and the micro environment (pH hypoxia) nutrition in tumours may also be involved.

Despite of these enhanced effect the utility of hyperthermia in the treatment of human malignancy remains limited. A number of morphological abnormalities including subtle changes in DNA structure, damage to cell membrane and sub cellular organelles have
been found following exposure to heat (311). Additionally hyperthermia has been shown to affect a number of cellular functions including metabolism, macromolecular synthesis, membrane functions and the integrity of the cytoskeleton (312). Hyperthermia and Ashoka extract if used together with cisplatin or cyclophosphamide resulted in enhancement of the cytotoxicity to cultured S-180 tumour cells was observed. The trimodality treatment schedule with the Ashoka bark or flower extracts, hyperthermia and cisplatin or cyclophosphamide significantly inhibited the growth of subcutaneously transplanted Sarcoma-180 solid tumours in mice. This effect indicated the ability of *Saraca asoca* (Ashoka) extracts of bark or flower and hyperthermia to improve the antitumour efficacy of cisplatin or cyclophosphamide. Severe haematological toxicities like fall in haemoglobin levels and leucopenia after treatment with cisplatin or cyclophosphamide were significantly protected by the administration of Ashoka bark or flower with hyperthermia. The elevation in the BUN, SGPT and SAKP levels were also prevented by trimodality treatment indicating protection against renal damage and liver necrosis. Thus these studies indicate the efficacy of the Ashoka bark and flower extracts to enhance the antitumour activity of cyclophosphamide or cisplatin in combination with hyperthermia. The mechanism(s) responsible for the Ashoka/Cisplatin or Cyclophosphamide/hyperthermia interaction have not been fully investigated. Some of the possibilities which have been proposed are:

1. Increased levels of drugs in the cells
(2) Increase cell membrane permeability to drugs

(3) Membrane transport of drugs

(4) Alters cellular metabolism

(5) Overall pharmacokinetics may change, with heat affecting drug metabolism and excretion.

We conclude from the above observation that the use of Ashoka extracts along with cisplatin or cyclophosphamide and hyperthermia can enhance the antitumour activity and that this modality may be considered as prospective clinical application.