CHAPTER 3

ANTITUMOR, ANTIINFLAMMATORY AND ANTIOXIDANT ACTIVITIES OF *ANDROGRAPHIS PANICULATA* AND ANDROGRAPHOLIDE
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\textbf{3.4. DISCUSSION}
3.1. INTRODUCTION

Cancer, arises due to fundamental aberrations in cellular behavior, is characterized by uncontrolled proliferation and survival. Chronic inflammation in tumor initiation and growth is well established (Philip et al, 2004). The neoplastic transformation and subsequent tumor invasion may require a microenvironment of activated inflammatory or stromal elements (O’Byrne and Dalgleish, 2001). Inflammation is a defense response to acute tissue damage, whether resulting from physical injury, ischemic injury, infection, exposure to toxins, or other types of trauma. But more often, it appears to stimulate tumor development. Epidemiologic and clinical research indicates an increased risk of certain cancers is associated with chronic inflammation. Tumor cells produce various cytokines and chemokines that attract leukocytes, which in turn produce cytokines and chemokines that stimulate further tumor cell proliferation. In addition, tumor inflammatory microenvironment facilitates the breakage of basement membrane, a process required for invasion and migration of tumor cells during metastasis.

Reactive oxygen species (ROS) generated endogenously or exogenously are associated with pathogenesis of various disorders such as artherosclerosis, arthritis, cancer and other inflammatory diseases (Halliwell and Gutteridge, 1999). Inflammatory cells enhance the genomic instability of the tumor through the production of reactive oxygen and nitrogen species, which can form peroxynitrate, a DNA damaging agent. In combination, DNA damage and proliferative signals create a circumstance conducive to the development of cancer (Lu et al, 2006). Hence use of agents which can scavenge the free radicals and reduce the production of inflammatory mediators are expected to improve these disorders and may be effective in cancer therapy as well as prevention.

Plants are known to produce plethora of secondary metabolites showing wide array of pharmacological properties including anticancer activity by interfering various steps of tumorigenesis (Boik, 2001). In this chapter we have analyzed antitumor, antiinflammatory and antioxidant
activities of *A. paniculata* and Andrographolide.

### 3.2. MATERIALS AND METHODS

#### 3.2.1. Animals

Inbred strains of male Swiss albino mice and BALB/c mice were used for this study.

#### 3.2.2. Cell lines

L929, B16F-10 melanoma cells, CHO, Vero, HeLa, EL4, K562, Dalton’s lymphoma ascites (DLA) cells and Ehrlich ascites carcinoma (EAC) cells and HPBL were used for this study.

#### 3.2.3. Drug dose and route of administration

Animals were treated with *A. paniculata* (10mg/dose/animal) and Andrographolide (500μg/dose/animal) intraperitoneally (i.p) for 10 consecutive days for antitumor studies and 5 consecutive days with same concentration for antiinflammatory and antioxidant studies.

#### 3.2.4. Determination of *in vitro* cytotoxic activity of *A. paniculata* and Andrographolide towards DLA, EAC and Human peripheral blood lymphocytes

Dalton’s lymphoma ascites (DLA) cells, Ehrlich ascites carcinoma (EAC) cells and human peripheral blood lymphocyte (HPBL) (1X10^6 cells) were incubated with various concentrations *A. paniculata* (5-500μg/ml) and Andrographolide (1-250μg/ml) in a final volume of 1ml for 3h at 37°C. After incubation the viability of the cells were determined by trypan blue dye exclusion method as described in chapter 2.

#### 3.2.5. Determination of cytotoxicity of *A. paniculata* and Andrographolide to different cell lines using MTT assay

Cytotoxicity of *A. paniculata* and Andrographolide was determined using L929, B16F-10, CHO, Vero, HeLa, EL4 and K562 cells in culture. Cells were seeded in 96-well titre plate (5000 cells/well) and different concentrations of *A. paniculata* and (10-250μg/ml) Andrographolide (1-100μg/ml) were added and incubated at 37°C in 5% CO_2_ atmosphere for 48h and MMT assay was performed as described in chapter 2.
3.2.6. Determination of the effect of *A. paniculata* and Andrographolide on DLA induced solid tumor development

Solid tumor was induced by injecting DLA cells (1x10⁶ cells/animal) subcutaneously to the right hind limbs of four groups (8 animals/group) of Swiss albino mice. Group I animals were kept as untreated tumor bearing control. Group II animals received 10 doses of 1% gum acacia and served as vehicle control. Group III and IV animals were treated with 10 doses of *A. paniculata* and Andrographolide respectively. The radii of developing tumor were measured using vernier calipers at 3 days intervals for one month and tumor volume was calculated using the formula \( V = 0.4ab^2 \) where ‘a’ and ‘b’ represents the major and minor diameter respectively (Atia et al., 1966). This was compared with untreated control.

3.2.7. Determination of the effect of *A. paniculata* and Andrographolide on the survival of ascites tumor bearing animals

Four groups of Swiss albino mice were used for the study (8 animals/group). Ascites tumor was induced by injecting EAC cells (1x10⁶ cells/animal) into the peritoneal cavity of all the experimental animals. Group I was inoculated with EAC cells alone and kept as untreated tumor bearing control. Group II animals were treated with 10 doses 1% gum acacia and served as vehicle control. Group III and IV received 10 doses of *A. paniculata* and Andrographolide respectively. The death pattern of animals due to tumor burden was noted everyday and the percentage of increase in lifespan (%ILS) was calculated using the formula \( \frac{T-C}{C} \times 100 \) where ‘T’ and ‘C’ represent the number of days that treated and control animals survived respectively (Kuttan et al., 1985).

3.2.8. Determination of antiinflammatory activity of *A. paniculata* and Andrographolide

Antiinflammatory activity of *A. paniculata* and Andrographolide was evaluated by paw edema method (Winter et al., 1962). The experimental mice were divided into four groups (6 animals/group). Group I was kept as untreated normal control. Group II received 1% gum acacia for 5 consecutive days and served as vehicle control. Group III and IV were
treated with 5 doses of *A. paniculata* and Andrographolide respectively. One hour after the last dose of drug administration, paw oedema was induced to all animals, by injecting carrageenin (30µg/animal) subcutaneously on its left paw. The thickness of paw was measured using vernier calipers just before and after carrageenin injection and continued for 8h at 30 minutes intervals.

### 3.2.9 Determination of the effect of *A. paniculata* and Andrographolide on the production of NO and TNF-α by LPS stimulated macrophages

BALB/c mice were divided into four groups. Group I animals were treated with single dose of LPS (250µg/100µl PBS/animal, i.p.). Group II and III animals were treated with 5 doses of *A. paniculata* and Andrographolide respectively. Group IV animals were kept as normal animals without any treatment. Six hours after LPS administration which was given along with last dose of *A. paniculata* and Andrographolide, animals were sacrificed, blood was collected, serum separated, and nitrite level was determined by Griess reaction as described in chapter 2. Serum TNF-α level was estimated using ELISA kit according to manufactures protocol. The serum TNF-α level was also determined by the Bio-assay using TNF-α sensitive L929 cells as described in chapter 2.

### 3.2.10 Determination of the effect of *A. paniculata* and Andrographolide on PMA induced super oxide radical generation.

BALB/c mice were divided into three groups (6 animals/group). All animals were injected with sodium caesinate (5%, i.p) to elicit macrophages. Group I served as a control without any treatment. Group II and III were treated with five doses of *A. paniculata* (10mg/dose/animal) and Andrographolide (500µg/dose/animal) respectively. On the fifth day one hour after drug administration, peritoneal macrophages were activated by injecting PMA (100ng/animal) and harvested three hours later. The superoxide generated by the macrophages was measured by the method of Dwivedi et al (Dwivedi et al, 1992) as described in chapter 2. Percentage of inhibition was determined by comparing the absorbance
value of control and treated animal.

3.2.11. Determination of the effect of A. paniculata and Andrographolide on lipid peroxidation and hydroxyl radical production (in vitro)

A. paniculata and Andrographolide were dissolved in minimum quantity of DMSO and further diluted in PBS. Various concentrations of A. paniculata (25μg/ml-500μg/ml) and Andrographolide (1μg/ml-100g/ml) were tested for in vitro generation of lipid peroxides and hydroxyl radicals as described in chapter 2.

3.3. RESULTS

3.3.1. Cytotoxicity of A. paniculata and Andrographolide towards Daltons lymphoma ascites (DLA), Ehrlich ascites carcinoma (EAC) cells and human peripheral blood lymphocyte (HBPL)

Table 3.1 and 3.2 shows the effect of A. paniculata and Andrographolide on cytotoxicity towards DLA and EAC cells. A. paniculata and Andrographolide showed 100% cytotoxicity towards DLA and EAC cells at a concentration of 500μg/ml and 100μg/ml respectively. A. paniculata and Andrographolide was 100% non-toxic to HBPL even at higher concentrations which were toxic to EAC and DLA cells (Data not shown).

MTT assay also showed that A. paniculata and Andrographolide was cytotoxic to L929, B16F-10, Vero, HeLa, CHO, EL4, K562, cell lines (Fig.3.1). Treatment with A. paniculata at concentration of 100μg/ml showed 67.7%, 70.16% 42.21% 75.68%, 70.03% toxicity to L929, B16F-10, Vero, HeLa and CHO cells respectively. Similarly, the same concentration of A. paniculata produced 68.23% cell death in K562 and 71.23% cell death in EL4 cells in in vitro condition.

Andrographolide was also cytotoxic to these cells lines. L929, B16F-10, Vero, HeLa, CHO cells showed 56.71% 62.51%, 28.31%, 67.86% and 47.6% cytotoxicity respectively, when they were treated with 100μg/ml Andrographolide. It produced 58.02% toxicity to K562 cells and 61.99% toxicity to EL4 cells at a concentration of 100μg/ml (Fig.3.1).
Table 3.1. Cytotoxicity of *A. paniculata* and Andrographolide to Dalton’s lymphoma ascites (DLA) cells (*in vitro*)

<table>
<thead>
<tr>
<th>Concentrations (μg/ml)</th>
<th>Percentage of cell death</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>A. paniculata</em></td>
<td>Andrographolide</td>
</tr>
<tr>
<td>500</td>
<td>100</td>
<td>ND</td>
</tr>
<tr>
<td>250</td>
<td>73</td>
<td>ND</td>
</tr>
<tr>
<td>100</td>
<td>34</td>
<td>100</td>
</tr>
<tr>
<td>50</td>
<td>20</td>
<td>85</td>
</tr>
<tr>
<td>25</td>
<td>5</td>
<td>44</td>
</tr>
<tr>
<td>10</td>
<td>0</td>
<td>16</td>
</tr>
<tr>
<td>5</td>
<td>ND</td>
<td>6</td>
</tr>
<tr>
<td>1</td>
<td>ND</td>
<td>0</td>
</tr>
</tbody>
</table>

Dalton's lymphoma cells (DLA) (1x10^6 cells) were incubated with different concentrations of *A. paniculata* and Andrographolide for 3h and percentage cell death was determined by trypan blue exclusion method.

ND- Not determined
**Table 3.2a. Cytotoxicity of *A.paniculata* and Andrographolide to Ehrlich ascites carcinoma (EAC) cells *(in vitro)***

<table>
<thead>
<tr>
<th>Concentrations (μg/ml)</th>
<th>Percentage of cell death</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>A.paniculata</em></td>
<td>Andrographolide</td>
</tr>
<tr>
<td>500</td>
<td>100</td>
<td>ND</td>
</tr>
<tr>
<td>250</td>
<td>86</td>
<td>ND</td>
</tr>
<tr>
<td>100</td>
<td>50</td>
<td>100</td>
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<tr>
<td>50</td>
<td>27</td>
<td>77</td>
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<tr>
<td>25</td>
<td>6</td>
<td>50</td>
</tr>
<tr>
<td>10</td>
<td>0</td>
<td>16</td>
</tr>
<tr>
<td>5</td>
<td>ND</td>
<td>3</td>
</tr>
<tr>
<td>1</td>
<td>ND</td>
<td>0</td>
</tr>
</tbody>
</table>

Ehrlich ascites carcinoma cells (EAC) (1X10^6 cells) were incubated with different concentrations of *A.paniculata* and Andrographolide for 3h and percentage cell death was determined by trypan blue exclusion method. ND-Not determined.

**Table 3.2b. Cytotoxicity of *A.paniculata* and Andrographolide to Human periphaeral blood lymphocyte (HBPL)**

<table>
<thead>
<tr>
<th>Concentrations (μg/ml)</th>
<th>Percentage of cell death</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>A.paniculata</em></td>
<td>Andrographolide</td>
</tr>
<tr>
<td>500</td>
<td>Non toxic</td>
<td>ND</td>
</tr>
<tr>
<td>250</td>
<td>Non toxic</td>
<td>ND</td>
</tr>
<tr>
<td>100</td>
<td>Non toxic</td>
<td>Non toxic</td>
</tr>
<tr>
<td>50</td>
<td>Non toxic</td>
<td>Non toxic</td>
</tr>
<tr>
<td>25</td>
<td>Non toxic</td>
<td>Non toxic</td>
</tr>
<tr>
<td>10</td>
<td>Non toxic</td>
<td>Non toxic</td>
</tr>
<tr>
<td>5</td>
<td>ND</td>
<td>Non toxic</td>
</tr>
<tr>
<td>1</td>
<td>ND</td>
<td>Non toxic</td>
</tr>
</tbody>
</table>

Human peripheral blood lymphocytes (1X10^6 cells) were incubated with different concentration of *A.paniculata* and Andrographolide for 3hr. Percentage of cell death was determined by trypan exclusion method. ND-Not determined.
Fig. 3.1. Effect of *A. paniculata* and Andrographolide on cytotoxicity towards different cell lines.
3.3.2. Effect of A.paniculata and Andrographolide on DLA induced solid tumor development

Administration of A.paniculata and Andrographolide significantly inhibited solid tumor development in mice (Fig.3.2). Tumor volume of untreated and vehicle treated control animals were 2.67±0.21cm³ and 2.59±0.17cm³ respectively on 31st day after tumor induction. Treatment with A.paniculata reduced the solid tumor development as observed from the tumor volume of 0.83±0.18cm³ on 31st day while Andrographolide reduced the tumor volume to 1.18±0.14cm³ on the same day.

3.3.3. Effect of A.paniculata and Andrographolide on the survival of Ehrlich ascites tumor bearing animals

The effect of A.paniculata and Andrographolide on the survival of Ehrlich ascites tumor bearing animal and the increase in life span is presented in Table 3.3. There was 31.58% increase in the life span of Ehrlich ascites tumor bearing mice when treated with A.paniculata but administration of Andrographolide produced only 15.79% enhancement in the lifespan of Ehrlich ascites tumor bearing animals.

3.3.4. Effect of A.paniculata and Andrographolide on carrageenan induced inflammation

Intraperitoneal administration of A.paniculata and Andrographolide was found to significantly inhibit the carrageenan induced paw oedema formation (Fig.3.3). A.paniculata treated group of animals did not produce paw oedema after carrageenan injection compared to untreated control animals in which paw edema was formed and was normalized only after 360 min. Andrographolide treated group of animals showed slight inflammation after carrageenan injection and was normalized after 60 min. Vehicle treated animals also showed similar result as that of untreated control animals.

3.3.5. Effect of A.paniculata and Andrographolide on serum nitrite level in LPS stimulated animals

Effect of A.paniculata and Andrographolide on serum nitrite level is shown in Table 3.4. Treatment with LPS drastically enhanced the serum
Fig. 3.2. Effect of *A. paniculata* and Andrographolide on solid tumor development

Tumor volume (cm³)

- Untreated control
- *A. paniculata*
- Vehicle control
- Andrographolide

Days after tumor induction

- 0
- 0.5
- 1
- 1.5
- 2
- 2.5
- 3
- 7 10 13 15 17 19 22 25 28 31

Tumor volume (cm³)

[Graph showing the comparison of tumor volume over time for different treatments.]
Table 3.3. Effect of *A.paniculata* and Andrographolide on the life span of ascites tumor bearing animals

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Mean survival days</th>
<th>% increase in life span (%ILS)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tumor alone</td>
<td>14.25±1.20</td>
<td></td>
</tr>
<tr>
<td>Vehicle control</td>
<td>14.98±1.01</td>
<td></td>
</tr>
<tr>
<td>Tumor+A. <em>paniculata</em></td>
<td>18.75±1.30***</td>
<td>31.58</td>
</tr>
<tr>
<td>Tumor+Andrographolide</td>
<td>16.50±3.04</td>
<td>15.79</td>
</tr>
</tbody>
</table>

All the animals were injected with Ehrlich ascites carcinoma (EAC) cells (1X10^6 cells/animal). Vehicle control received 10 doses of 1% gum acacia. Treated animals received 10 doses of *A.paniculata* 10mg/dose/animal and Andrographolide (500μg/dose/animal). The death pattern of animals due to tumor burden was noted and percentage increase in life span (%ILS) was calculated. Values are expressed as mean ±SD. ***$P<0.001$ compared with tumor alone control animals.
Fig. 3.3. Effect of *A. paniculata* and Andrographolide on carrageenan induced paw edema
nitrite level (81.68±0.96μmol) and it was reduced to 23.71±0.86μmol (71.04% inhibition) and 26.36±0.92μmol (67.79% inhibition) by A.paniculata and Andrographolide administration respectively, which was closer to that of normal serum nitrite level (21.76±0.41μmol).

3.3.6. Effect of A.paniculata and Andrographolide on serum TNF-α level in LPS stimulated animals

Effect of A.paniculata and Andrographolide on serum TNF-α level is shown in Table 3.5. Drastically enhanced level of serum TNF-α (668±21.6pg/ml) in LPS alone treated control animals were significantly reduced by A.paniculata (116.71±6.2pg/ml) and Andrographolide (168.72±7.4pg/ml) treatment.

Bioassay using TNF-α sensitive L929 cells is given in Fig.3.4. Serum collected from LPS alone treated control animals was highly cytotoxic to the TNF-α (Fig.3.4b). Cytotoxicity to the L929 cells was significantly reduced by A.paniculata and Andrographolide administration (Fig.3.4c and Fig.3.4d) and grown similar to normal (Fig. 3.4a), which indicate that both A.paniculata and Andrographolide could inhibit the production of TNF-α in LPS stimulated animals.

3.3.7. Effect of A.paniculata and Andrographolide on PMA induced super oxide radical generation

Superoxide radicals generated during activation with PMA in sodium caesinate injected macrophages were scavenged after the intraperitoneal administration of A.paniculata and Andrographolide. The percentage inhibition of superoxide radical generation was 34.09% and 31.80% by A.paniculata and Andrographolide treatment respectively (Table 3.6).

3.3.8. Effect of A.paniculata and Andrographolide on hydroxyl radical generation

A.paniculata and Andrographolide could significantly inhibit the hydroxyl radical generation in in vitro in a dose dependent manner. A.paniculata (500μg/ml) produced 80% inhibition where as Andrographolide showed 60% inhibition in hydroxyl radical generation at a concentration of 10μg/ml (Fig.3.5).
Table 3.4. Effect of *A. paniculata* and Andrographolide on serum nitrite level of LPS treated animals

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Concentration of nitrite (μmol)</th>
<th>% inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>21.76±0.41</td>
<td></td>
</tr>
<tr>
<td>LPS (250μg/100μl PBS)</td>
<td>81.86±0.96</td>
<td></td>
</tr>
<tr>
<td>LPS+A. <em>paniculata</em></td>
<td>23.71±0.86***</td>
<td>71.04</td>
</tr>
<tr>
<td>LPS+Andrographolide</td>
<td>26.36±0.92***</td>
<td>67.79</td>
</tr>
</tbody>
</table>

Animals were treated with five doses of *A. paniculata* (10mg/dose/animal) and Andrographolide (500μg/dose/animal). Six hours after the stimulation with LPS (250μg/100μl PBS, i.p.), animals were sacrificed, blood was collected and serum separated. Serum nitrite was estimated by Griess reaction. Values are expressed as mean ±SD. ***P< 0.001 with respect to LPS alone treated group.
Table 3.5. Effect of *A. paniculata* and Andrographolide on serum TNF-α level of LPS treated animals

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Concentration of TNF-α (pg/ml)</th>
<th>% inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>20.32±3.20</td>
<td></td>
</tr>
<tr>
<td>LPS (250μg/100μL PBS)</td>
<td>668±21.6</td>
<td></td>
</tr>
<tr>
<td>LPS+A. <em>paniculata</em></td>
<td>16.71±6.2***</td>
<td>82.52</td>
</tr>
<tr>
<td>LPS+Andrographolide</td>
<td>168.72±7.4***</td>
<td>74.74</td>
</tr>
</tbody>
</table>

Animals were treated with five doses of *A. paniculata* (10mg/dose/animal) and Andrographolide (500μg/dose/animal). Six hours after the stimulation with LPS (250μg/100μl PBS i.p.) animals were sacrificed, blood was collected and serum separated and used for TNF-α estimation. Values are expressed as mean ±SD.

***P< 0.001** with respect to LPS alone treated group.
Table 3.6. Effect of *A.paniculata* and *Andrographolide* on PMA induced super oxide generation by peritoneal macrophages

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Optical Density at 515 nm</th>
<th>% of inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.44 ± 0.03</td>
<td></td>
</tr>
<tr>
<td><em>A.paniculata</em></td>
<td>0.29 ± 0.004***</td>
<td>34.09 ± 1.80</td>
</tr>
<tr>
<td><em>Andrographolide</em></td>
<td>0.30 ± 0.01***</td>
<td>31.8 ± 0.77</td>
</tr>
</tbody>
</table>

Macrophages were elicited by injecting 0.2ml of 5% sodium caesinate on first day. Treated animals were received 5 doses *A.paniculata* (10mg/dose/animal, i.p) and *Andrographolide* (500μg/dose/animal, i.p). 1h before 5th dose of drug administration, PMA was injected to activate macrophages in control as well as treated animals. Animals were sacrificed and macrophages were isolated and used to estimate super oxide level. Values are expressed as mean ±SD.

*** p <0.001
Fig. 3.4. Effect of *A. paniculata* and Andrographolide on serum TNF-α (Bio-assay) level of LPS treated animals

(a) Normal L929 cells
(b) L929 cells incubated with serum from LPS alone treated animal
(c) L929 cells incubated with serum from LPS+*A. paniculata* treated animal
(d) L929 cells incubated with serum from LPS+Andrographolide treated animal
Fig. 3.5. Effect of *A. paniculata* and Andrographolide on hydroxyl radical generation

% inhibition

Concentration (micro g/ml)

- ■ *A. paniculata*
- ▲ Andrographolide
Fig. 3.6. Effect of *A. paniculata* and Andrographolide on lipid peroxidation.
3.3.9. Effect of *A. paniculata* and Andrographolide on lipid peroxidation

*A. paniculata* and Andrographolide was found to significantly inhibit the lipid peroxidation of liver homogenate. *A. paniculata* at a concentration of 500μg/ml showed 77% inhibition and Andrographolide (10μg/ml) was found to produce 67.4% inhibition in the lipid peroxidation in *in vitro* (Fig.3.6).

3.4. DISCUSSION

One of the most promising novel strategy has been the so called targeted anticancer therapy based on compounds that interferes with cellular target directly connected with pathogenic events. In the present study *A. paniculata* and Andrographolide was found to be cytotoxic towards various cancer cell lines *in vitro*. These compounds are less cytotoxic to nontransformed cell Vero, while nontoxic to normal human peripheral blood lymphocytes. This result indicates that the cytotoxicity of *A. paniculata* and Andrographolide is tumor cell specific. Inhibition of DLA induced solid tumor development as well as increased life span of EAC bearing animals by the administration of *A. paniculata* and Andrographolide strongly support the antitumor activity of *A. paniculata* and Andrographolide.

Investigations on various tumor models, demonstrated that proliferation of cells alone does not cause cancer. But the sustained cell proliferation in an environment rich in inflammatory cells, growth factors, activated stroma and DNA-damage-promoting agents, certainly potentiates and promotes neoplastic risk (Coussens and Werb, 2002). Carrageenin induced acute inflammation in animals is one of the suitable test used to screen antiinflammatory drug. Administration of *A. paniculata* and Andrographolide could inhibit the carrageenin induced paw edema in BALB/c mice suggest the antiinflammatory activity of these compounds.

Macrophages and other phagocytic cells are the inflammatory component of a developing neoplasam, all of which are capable of producing an assorted array of cytokines (Philip et al, 2004).
Proinflammatory cytokine TNF-α (tumor necrosis factor-α) controls inflammatory cell populations as well as mediate other aspects of the inflammatory process. TNF-α was found to be involved in the activation of iNOS in macrophages. During inflammation, NO and its metabolites, like peroxynitrite, are potentially cytotoxic and capable of injuring and eliminating the pathogens and altered cells (Liaudet et al, 2000). However, indiscriminate destruction of cells and tissues by NO and its reactive nitrogen intermediates may lead to pathological condition; therefore, production of NO by iNOS may reflect the degree of inflammation and provide a measure to assess the effect of drugs on the inflammatory process. Thus, selective inhibition of the iNOS pathway is a rational approach because attenuation of inflammation and suppression of NO production may be effective therapeutic strategy for preventing inflammatory reactions and diseases (Hussain et al, 2003; Martinez-Florez et al, 2005). Administration of LPS induces inflammation in animals as well in cultured cells. Reactive oxygen species generated after LPS administration induce inflammatory cytokines (Macdonald et al, 2003). The results of the present study are in well correlation with these findings. Administration of *A.paniculata* and Andrographolide significantly reduced the drastically elevated level of serum nitrite in LPS stimulated animals. Serum TNF-α level of these animals was also significantly lowered by the administration of *A.paniculata* and Andrographolide which indicate that *A.paniculata* and Andrographolide are potent inhibitors of iNOS and TNF-α. This inhibitory effect may be the reason for the inhibition of carrageenan induced paw edema formation by *A.paniculata* and Andrographolide.

Oxidation and production of free radicals are integral part of metabolism. Due to their high reactivity, ROS readily combine and oxidize biomolecules making them inactive with subsequent damage to cells by inducing DNA modification and chromosomal aberrations. Involvement of free radicals like superoxide, hydroxyl radical and lipid peroxide in carcinogenesis and inflammatory process is well recognized (Ohshima and Bartsch, 1994). At high concentration free radicals can also initiate lipid
peroxidation. Increased lipid peroxidation impairs membrane functions by altering membrane fluidity, activity of membrane bound enzymes and receptors. *A. paniculata* and Andrographolide could inhibit the *in vitro* hydroxyl radical generation and lipid peroxidation process; indicates *A. paniculata* and Andrographolide possesses free radical scavenging activity and regulates the level of metabolites which are capable of signaling and communicating important information to the cellular genetic machinery. Moreover, *A. paniculata* and Andrographolide could also inhibit PMA induced superoxide radical formation strongly supports their antioxidant activity. Hence the present investigation suggests that *A. paniculata* and Andrographolide have antiinflammatory and antioxidant activity, this may contribute to the inhibition of tumor growth and offer therapeutic potential in cancer development.