Chapter 4. Investigation of the anti-cancer activity of *Bauhinia tomentosa* during ascites and solid tumour development.

4.1 Introduction

Cancer is one of the leading causes of death among humans and is characterized by uncontrolled growth and spread to distant site. Scientific investigations are making best efforts to combat this disease, but the sure-shot, perfect cure is yet to be brought into world medicine. There are several external factors (tobacco, chemicals and radiation) and internal factors (inherited mutations, hormones etc.) leading in the initiation or promotion of carcinogenesis (Bruce et al., 1995). There are several therapies available to treat cancer and the major one among them is radiotherapy and chemotherapy. Even though these therapies have shown promising results, some of the major drawbacks of this therapy are toxic side effects and suppression of the immune system. (Diwanay et al., 2004).

Cyclophosphamide (CTX) belongs to a group of alkylating agents used widely to treat several types of cancer and autoimmune disorders (Haque et al., 2003). CTX has been reported to disturb fundamental mechanisms concerned with cell growth differentiation and function (Alenzi et al., 2010). This chemotherapeutic has also been reported to induce several toxic side effects like nausea, fatigue, hair loss, and to cause reduction in host levels of immune cells (Triozzi and Laszlo, 1987). Due to the toxic side effects of CTX, the need for new drugs effective against solid tumors is an important and necessary strategy to improve the arsenal of agents that could be used during chemotherapy. There has been growing interest in alternative therapies and therapeutic use of natural products, especially those derived from plants (Schwartsmann et al., 2002). Of the latter class, only a small percentage (10%) has been investigated phytochemically
and an even smaller percentage properly studied in terms of pharmacologic properties (Rates, 2001). Natural products represent over 50% of all drugs in clinical use and about 85% of traditional medicine involves use of plant extracts; many have been reported to possess several pharmacologically important components (Sakthivel and Guruvayoorappan, 2012).

*Bauhinia tomentosa* is a medicinal plant widely distributed in tropical/subtropical regions. Many *Bauhinia* species are rich in β-sitosterol, lupeol, ascorbate (Vitamin C), campferol, flavonone, and quercetin, etc. and have been shown to impart anti-tumor, anti-bacterial, and anti-fungal effects (Rajkapoor, 2006). *Bauhinia tomentosa* has been reported to impart anti-oxidant (Mannangatti et al., 2010), -diabetes (Devaki et al., 2011), and -microbial effects (Gopalakrishnan and Vadivel, 2011). Based on those findings, the present study to evaluate potential anti-tumor effects of an extract of *B. tomentosa* against Dalton Lymphoma Ascites (DLA)-induced ascites and solid tumor formation.

### 4.2 Materials and Methods

#### 4.2.1 Preparation and administration of plant extract

*B. tomentosa* leaves were collected from Coimbatore, India. The plant was identified and authenticated at the Botanical Survey of India, Coimbatore (No: BSI/SRC/5/23/2011-12/Tech-756). Air-dried whole plants of *B. tomentosa* were powdered and extracted (10 g) overnight in 100 ml of 70% methanol. Supernatant was collected after centrifuging at 5,000 rpm for 10 min. Methanol was removed by evaporation and yield of the extract was 12% (w/v). For *in vivo* studies, a fixed amount of the dried extract was suspended in 1% gum acacia to provide a solution that would yield a dosage of 10 mg/kg body weight via intraperitoneal injection (100 μl/dose).
4.2.2 Chemicals

Cyclophosphamide (CTX; LEDOXAN™) was obtained from Dabur Pharma Ltd. (New Delhi, India). DTNB (5-5’-dithiobis-2-nitrobenzoic acid) was purchased from SISCO Research Labs (Mumbai, India). All other chemicals used in the study were of analytical reagent grade. γ-glutamyl transpeptidase kit was purchased from Merck, Mumbai, India. Highly specific quantitative sandwich ELISA kits specific for evaluating the levels of mouse interleukin (IL)-1β, IL-6, IL-2, GM-CSF, VGEF, and interferon (IFN)-γ were procured from Koma Biotech™, (Seoul, Korea). Kits for measuring tumor necrosis factor (TNF)-α and iNOS were bought from USCN Life Sciences (Hubei, China).

4.2.3 Animals

Adult male Balb/c mice (4-6-wk-of-age, 23-25g) were purchased from Kerala Veterinary and Animal Sciences University (Mannuthy, India). The animals were kept under specific pathogen-free conditions in facilities maintained at 24 [± 2]°C and a 50% relative humidity and with a 12-hr light: dark cycle. All mice were provided ad libitum access to normal mouse chow (Sai Durga Feeds, Bangalore, India.) and filtered water. All animal experiments were performed according to the CPSCEA guidelines, Government of India.

4.2.4 Tumor cells

Dalton Lymphoma Ascites (DLA) cells were obtained from the Amala Cancer Research Institute Thrissur, India. The DLA cells were maintained in vivo in normal Balb/c mice by intra- peritoneal (IP) inoculation of 10^6 cells into a new mouse after every 10 days.
4.2.5 Assessment of effect of *B. tomentosa* extract on DLA-induced ascites tumor formation

The mice were randomly allocated into four groups (n = 15/group). Mice in Group I were to serve as a normal control and to be treated with saline. Mice in Group II were to serve as DLA only controls. Mice in Group III were to be treated with the DLA and also with the *B. tomentosa* extract (10 mg/kg B.wt). Mice in Group IV were to be treated with DLA and also with methotrexate (at 3.5 mg/kg B.wt). DLA cells were collected from donor mice (on Day 9 after initial injection), suspended in sterile saline, and their viability and concentration determined via trypan blue exclusion. Except for mice in Group I, all mice were injected IP with 1x 10^6 DLA cells; this was designated as Day 0. The respective treatments were to continue daily up to 10 days post-injection of the DLA cells. Body weights of the mice were recorded at the beginning of the experiment (Day 0) and every 5 days over the course of the treatment period.

On Days 11 and 15, subsets of mice (n = 6) from each group were euthanized by cervical dislocation and ascites fluid was recovered; blood was also collected (by cardiac puncture) to permit estimation white blood cell (WBC) levels. Other samples of the blood were processed to yield serum for subsequent analyses of various parameters. The remaining three mice in each group were maintained to monitor lifespan. Mean survival times across the treated groups were compared with the DLA control group using the calculation Mean Survival Time (MST) = (Day of first death + Day of last death)/2. The Percentage Increase in Lifespan (% ILS), a reflection of anti-tumor efficacy of a given treatment, was calculated as 100 x [(MST of treated group) – (MST of DLA control group)]/MST of DLA control group (Huang et al., 1984).
4.2.6 Determination of the effect of *B. tomentosa* on γ-Glutamyl Transpeptidase (GGT), Nitric oxide (NO) and Glutathione (GSH)

The serum and ascites tumor samples were collected from above described experiment. NO level was estimated by using Griess reaction according to the method described by Green et al., (1982). GSH level was determined using the standard method explained by Moron et al. (1979). GSH in serum were allowed to react with Ellman's reagent (5, 5-Dithio-2- nitrobenzoic acid) in phosphate buffer saline (PBS, pH 8.0) and the absorbance was measured at 412 nm. GSH concentration were calculated using the standard GSH. The results are expressed as nmol/g protein. γ-Glutamyl Transpeptidase (GGT) was estimated using span diagnostic kit procedure was followed as per the manufactures instruction.

4.2.7 Effect of extract against DLA-induced solid tumor and cyclophosphamide (CTX) toxicity

Another set of mice was randomly allocated into five groups (n = 18/group). Mice in Group I were to serve as a normal control and to be treated with saline, were divided in to five groups of 12 animals each. Group I served as normal untreated group. Group II- V were injected with DLA cells (1x10^6 cells) intramuscular (i.m) to induce solid tumor. Group II served as solid tumor control where as group III and IV were treated with CTX 25mg/kg B.wt. and *B.tomentosa* respectively. Group V was treated with CTX and *B.tomentosa*. As before, DLA cells were collected from donor mice, suspended in sterile saline, and their viability/concentration determined via trypan blue exclusion. Except for mice in Groups I-III, all other mice were injected IP with 1x 10^6 DLA cells on Day 0. The respective treatments were to continue daily up to 10 days post-injection of the DLA cells.
Body weights of the mice were recorded at the beginning of the experiment (Day 0) and every 5 days over the course of the treatment period. Tumor mass in each Group II-V was measured starting on Day 3 after tumor cell injection. Measures of tumor radii (performed at right angles to one another) were taken every 3 day for a period of 30 days. Volume of the tumor mass was calculated using \( V = \frac{4}{3} \pi r_1^2 r_2 \), where \( r_1 \) and \( r_2 \) are the radii of the tumor (Majumdar 1997). During this same period (i.e., prior to the extract administration and continued every third day for 30 days), blood from six mice/group was collected from the tail vain and total WBC (white blood cell) counts performed using a hemocytometer.

At time of sacrifice on Days 15 and 30 post-tumor injection, 6 mice from each group were euthanized and their femurs collected to permit analyses of bone marrow samples. Blood was also collected for WBC count. Specifically, bone marrow cells were collected from both femurs and made into single cell suspensions and aliquots placed on glass slides (and stained with hematoxylin) for evaluation of non-specific \( \alpha \)-esterase activity (using azodye coupling method; Bancroft and Cook, 1984).

4.2.8 Effect of extract on serum TNF\( \alpha \), iNOS, IL-1\( \beta \), IL-6, IL-2, IFN-\( \gamma \), GM-CSF and VEGF levels

Blood was collected from each animal on Days 15 and 30 and the corresponding serum assessed for tumor necrosis factor (TNF)-\( \alpha \), iNOS, interleukin (IL)-1\( \beta \), IL-6, IL-2, interferon (IFN)-\( \gamma \), GM-CSF and VEGF levels using highly specific quantitative sandwich ELISA kits. The kits for evaluating mouse IL-1\( \beta \), IL-6, IL-2, GM-CSF, VEGF and IFN-\( \gamma \) were procured from Koma
Biotech™ (Seoul, Korea). Kits for measuring TNFα and iNOS were from USCN Life Sciences (Hubei, China).

4.2.9 Statistical analysis

All values are expressed as mean ± SD. For each endpoint, group means were compared using a one-way analysis of variance (ANOVA) followed by a Dunnett’s post-hoc test (Instat Version 3.0 software; Graphpad, San Diego, CA). A p-value < 0.05 was considered significant.
4.3 Results

4.3.1 Changes in MST and %ILS due to *B. tomentosa* extract

Mice injected with DLA cells (control) had an MST of 16.60 [± 1.03] days. This value was significantly increased to 25.33 [± 2.07] days due to the *B. tomentosa* extract treatment. These results are almost comparable to that of cyclophosphamide (CTX), the standard drug for which the MST was 29.83 [± 1.16] days. The % ILS of treated mice increased 52% compared to that of the DLA-bearing control hosts. In addition, the body weights of DLA tumor control mice increased 39% (relative to Group 1 mice controls); this value was reduced ≈17% due to the treatment with *B. tomentosa* (Table 4.1). These changes are important as host body weight is directly reflective of increases in DLA tumor volume in the peritoneal cavity.

4.3.2 Effect of extract on WBC counts in DLA-bearing mice

Total WBC counts in DLA control mice were reduced (10.22 x 10³ cells/mm³) compared to those in with normal mice (15.72 x 10³ cells/mm³). Administration of the *B.tomentosa* extract led to increases in the WBC counts on both Days 11 and 15 (respectively, 13.25 and 15.78 x 10³ cells/mm³) compared to those seen in the DLA control hosts (Figure 4.1).
Table 4.1: Effect of *B. tomentosa* on Mean Survival Time (MST), Percentage increase in Lifespan and Body weight:

<table>
<thead>
<tr>
<th>Treatment design</th>
<th>MST (in days)</th>
<th>Percentage increase in Bodyweight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>&gt;50</td>
<td>10.29</td>
</tr>
<tr>
<td>DLA Tumor Control</td>
<td>16.66 ±1.03</td>
<td>38.77</td>
</tr>
<tr>
<td>Tumor + Methotrexate (3.5 mg/Kg/ B.wt)</td>
<td>29.83 ±1.16**</td>
<td>13.58</td>
</tr>
<tr>
<td>Tumor + <em>B. tomentosa</em> (10mg/Kg/ B.wt)</td>
<td>25.33 ±2.07**</td>
<td>16.70</td>
</tr>
</tbody>
</table>

The results are expressed as the mean of each group (n=6) and ** Statistically Significant different (p<0.01) from DLA tumor control animals.
Figure 4.1: Effect of *B. tomentosa* on WBC count during Tumour development.

The results are expressed as the mean of each group (n=6) and ** Statistically significant different (P<0.01) from DLA tumor control animals.
4.3.3 Effect of extract on serum γ-glutamyl-transpeptidase (GGT) and nitric oxide (NO) levels in DLA-bearing mice

GGT plays an important role in the gamma-glutamyl cycle, a pathway for the synthesis and degradation of glutathione (Siest et al., 1992). Dominici et al., (2005) indicate that GGT can also involve a prooxidant role, with regulatory effects at cellular signal transduction and cellular pathophysiology in varies level. In the present study the serum GGT level was significantly (p<.01) increased at DLA tumor bearing group in both a days. Similarly, B.tomentosa controlled GGT level and reduces significantly as like a standard drug. High levels of NO expression (for example, in the form of iNOS generated by activated macrophages) indicates the tumor formation, whereas low level activity can express delayed tumor formation. The current study result showed higher level of serum NO level in DLA tumor control animals, (28.08±0.47; 36.22±0.85) 11th and 15th day respectively, (Figure 4.2 and 4.3) where as it was condensed significantly (p<0.01) to 24.50±0.75, 26.40±0.61 after B.tomentosa treatment. This level was well intentioned when compare with a standard drug Methotrexate.

4.3.4 Effect of B.tomentosa extract on cellular glutathione (GSH) in DLA-bearing mice

Effect of B. tomentosa on DLA induced cellular GSH level was shown in Figure 4.4. The higher level of cellular GSH in the DLA tumor control group (14.93±1.31, 17.16±2.85 nmol/mg protein 11th and 15th day) was found to be significantly (p<0.01) decreased (9.12±1.32, 9.89±1.83 nmol/mg protein) after B. tomentosa treatment (10 mg/kg B.wt.).
Figure 4.2: Effect of *B. tomentosa* on serum Gamma Glutamyl Transpeptidase (GGT) level during tumour development.

The results are expressed as the mean ± SD of each group (n=6) and * (p<0.05), ** (p<0.01) significantly different from DLA tumor control animals.
Figure 4.3: Determination of effect of *B. tomentosa* on serum Nitric Oxide (NO) level during tumour development

Each group consisted of six BALB/c mice. The results are expressed as the mean ± SD of each group (n=6) ** (p<0.01) significantly different from DLA tumor control animals.
Figure 4.4: Effect of *B. tomentosa* on cellular Glutathione (GSH) level during tumor development.

Each group consisted of six BALB/c mice. The results are expressed as the mean ± SD of each group (n=6) and ** (p<0.01) significantly different from DLA tumor control animals.
4.3.5 Effect of *B. tomentosa* on body weight changes during DLA induced solid tumor

Changes in host body weight during the experimental period (recorded before and every third day of experiment - up to Day 30) are shown in Figure 4.5. The results indicate CTX-treated mice had reduced body weight gains compared to normal hosts, while DLA-injected mice had increasing weights due to tumor growth. In contrast, *B. tomentosa* extract-treated mice shows had slower body weight gains relative to those of DLA-only mice. In mice treated with both CTX and *B. tomentosa* (in conjunction with DLA), body weight changes were on par with those in normal hosts.
Figure 4.5: Effect of *Bauhinia tomentosa* on Body weight during DLA induced solid tumor.

The results are expressed as the mean ± SD of each group (n=6) and Results are given as grams (g). Value significantly different from tumor control at * p < 0.05.
4.3.6 Effect of *Bauhinia tomentosa* on Bone Marrow Cellularity and α-esterase activity during DLA induced solid tumor

The effect of the test extract on bone marrow cellularity on Day 15 are shown in Figure 4.6. By Day 30, in non-tumor cell-injected mice, those hosts that received CTX alone had an average of 38.2 ± 3.3 x 10³ cells/femur; this was significantly increased to 68.2 ± 8.3 x 10⁵ cells/femur by the co-administration of the extract. In tumor cell-injected mice, those that had the DLA had cell values of 64.0 ± 4.5 x 10³ cells/femur at both timepoints. This values was significantly reduced by CTX treatment to 27.4 ± 3.5 x 10³ cells/femur; in mice with DLA that were treated with *B. tomentosa* extract only or with the CTX-extract co-treatment, the values increased to 79.0 ± 9.5 and 70.7 ± 7.8 x 10³ cells/femur, respectively. *B. tomentosa* treatment enhance the differentiation of stem cells even co-administration of CTX. (Figure 4.7).

4.3.7 Effect of *B. tomentosa* on tumor volume during DLA induced solid tumor.

Effect of *Bauhinia tomentosa* on tumor volume during DLA induced solid tumor with CTX treatment results were shown in Figure 4.8. Tumor volume of DLA control animal was gradually increasing on 30th day this level was 2.52 ± 0.14 mm³ while CTX and *B. tomentosa* treated groups were significantly reduced to 0.88 ± 0.07 and 1.20 ± 0.10 mm³, respectively, on the same day. More over the combined treatment with *B. tomentosa* and CTX treated group resulted more reduction (0.68 ±0.06 mm³) then other group of animals.
Figure 4.6: Effect of Bauhinia tomentosa on Bone Marrow Cellularity during DLA induced solid tumor.

The results are expressed as the mean of each group (n=6) and Results are given as $10^3$ cells/mm$^3$, ** Statistically Significant different ($p<0.01$) from DLA tumor control animals
Figure 4.7. Effect of *Bauhinia tomentosa* on α-Esterase Activity during DLA induced solid tumor.

The results are expressed as the mean of each group (n=6) and Results are given as $10^3$ cells/mm$^3$. * Statistically Significant different ($p<0.05$) from DLA tumor control animals
Figure 4.8. Effect of Bauhinia tomentosa on tumor volume during DLA induced solid tumor.

The results are expressed as the mean of each group (n=6) and Results are given as $10^3$ cells/mm$^3$. Value significantly different from tumor control at *** $p < 0.001$, ** $p < 0.01$ and * $p < 0.05$. 
4.3.8. Effect of *B. tomentosa* on WBC count during DLA induced solid tumor.

The mice which were treated with CTX has the total WBC count of 3.10 ± 0.27 x 10^3 cells/mm³ on 12th day and the animals treated with *B. tomentosa* the total WBC count were 23. 4.37 ± .37cells/mm³ on same day. The total WBC increased evidently towards normal level. A reduction in the level of total WBC was seen in CTX treated control animals throughout the period of study. Whereas, WBC level in DLA solid tumor bearing group and DLA with *B. tomentosa* treated group shows gradually amplified and reach greater than a normal level (11.68 ± .35 x 10^3cells/mm³ and 12.35 ± .46 x 10^3cells/mm³) respectively, but CTX treated along with DLA and *B. tomentosa* treated group the WBC level was reach nearby normal level at end of the experiment. Results were shown in Figure 4.9.

4.3.9. Effect of *B. tomentosa* on TNF-α, IL-1β, IL-6, GMCSF, VEGF, IL-2 and IFN-γ levels in serum during DLA induced solid tumor.

Serum concentrations of select inflammatory mediators were measured by ELISA. The elevated level of serum TNFα, iNOS, IL-1β, IL-6, GM-CSF, and VEGF in tumor control mice, were reduced in hosts that also received the *B. tomentosa* extract (Figures 4.10 to 4.15). The patterns of changes in IL-2 and IFNγ levels differed from those of the other mediators, i.e., levels were lower in the tumor control hosts and then markedly elevated due to treatment with extract. Results were shown in Figure 4.16 and 4.17.
Figure 4.9. Effect of *Bauhinia tomentosa* on WBC count during DLA induced solid tumor.

![Graph showing the effect of *Bauhinia tomentosa* on WBC count during DLA induced solid tumor.](image)

The results are expressed as the mean of each group (n=6) and Results are given as $10^3$ cells/mm$^3$. Value significantly different from tumor control at $** p < 0.01$. and $* p < 0.05$. 


Figure 4.10: Effect of *B. tomentosa* on serum TNF-α levels during DLA induced solid tumor.

Values shown are means (± SD); n = 6/group. **Values are significantly different from tumor control at $p < 0.01$. 
Figure 4.11: Effect of *B. tomentosa* on serum iNOS levels during DLA induced solid tumor.

Values shown are means (± SD); n = 6/group. **Values are significantly different from tumor control at *p* < 0.01.
Figure 4.12: Effect of *B. tomentosa* on serum IL-1β levels during DLA inducted solid tumor.

Values shown are means (± SD); n = 6/group. **Values are significantly different from tumor control at p < 0.01.
Figure 4.13: Effect of *B. tomentosa* on serum IL-6 levels during DLA-inducted solid tumor.

Values shown are means (± SD); n = 6/group. **Value are significantly different from tumor control at $p < 0.01$. 

*Normal*  
*Tumour alone*  
*Tumour + *B.tomentosa*  
*Tumour + CTX*  
*Tumour + CTX + *B.tomentosa*
Figure 4.14: Effect of *B. tomentosa* on serum GM-CSF levels during DLA induced solid tumor.

Values shown are means (± SD); n = 6/group. **Values are significantly different from tumor control at *p* < 0.01.
Figure 4.15: Effect of *B. tomentosa* on serum VEGF levels during DLA-induced solid tumor.

Values shown are means (± SD); n = 6/group. **Values are significantly different from tumor control at \( p < 0.01 \).
Figure 4.16: Effect of *B. tomentosa* on serum IL-2 levels during DLA-induced solid tumor.

Values shown are means (± SD); n = 6/group. **Values are significantly different from tumor control at *p* < 0.01.
Figure 4.17: Effect of *B. tomentosa* on serum IFN-γ levels during DLA-induced solid tumor.

Values shown are means (± SD); n = 6/group. **Values are significantly different from tumor control at p < 0.01.
4.4 Discussion

Interest in alternative therapies using natural products for cancer treatment are increasing due to the wide range of adverse side effects from currently available chemotherapeutics (Jemal et al., 2009). Many plant extracts and their active compounds have been tested in vitro and in vivo cancer models (Newman and Cragg, 2012). The present study reveals the anti-tumor effect by *B. tomentosa* extract in both an ascites and solid tumor model.

Generally, ascites tumor implantation induces local inflammation, cellular migration and finally, progressive ascites fluid accumulation (Deepak et al., 2009). Here, the *B. tomentosa* extract had an increased survival rate than DLA control group. These findings suggested us, that there was either a direct cytotoxic effect on the tumor cells by the extract. CTX has pro-oxidant character and generates oxidative stress; metabolites of CTX also bind to DNA and cause damage (chromosomal breaks, micronuclei formation) and cell death (Murata et al., 2004; Sakthivel et al., 2012). Here, administration of *B. tomentosa* extract in conjunction with CTX was found to enhance total WBC counts whereas these levels were drastically reduced in mice received CTX. Bone marrow cellularity was also increased significantly in the co-treated hosts, indicating that extract appeared able to stimulate the hematopoietic system. Moreover, there was increase in presence of α-esterase marrow cells, suggesting that, the extract could also enhance differentiation of stem cells.

In DLA control group, tumor volume was increased 8-times over a 30-days period, but *B.tomentosa* treated group shows 50% reduction in tumor volume. The effect was even greater in hosts that received *B.tomentosa* extract and CTX co-treatment. These results suggested us, that the inhibitory effect related not only reduced the side effect caused by CTX but also with the systemic disturb. Similar
results were obtained by Natesan et al., (2007) using Careya arborea methanolic extract.

The role of NO levels during tumor formation is regulated by interaction between endothelial cells in the tumor and infiltrating immune cells include macrophages and T-cells (Ruttimann, 2007). The Nitric oxide synthase (iNOS) activity during tumor formation is correlated positively with tumor grade (Kolb, 2000). Taken in that context, the results of the present study concerning NO and iNOS confirmed the anti-tumor effect of the B. tomentosa extract in the mouse hosts.

Immune cells have a broad impact on tumor initiation, growth and progression; many of these effects are mediated by pro-inflammatory cytokines. Previous studies suggests that TNF-α is a key mediator of cancer, in addition with interleukins. The release of TNF-α causes polymorphonuclear neutrophil influx and release of various inflammatory mediators from multiple cell types (Manusama et al. 1996); Tenhagen et al., 2000). IL-6 is a multifunctional immunomodulatory cytokine, which activate B and T lymphocytes produced by both normal and tumor cells. The serum concentration of IL-6 can be used as a marker for progress of tumor cells (Richerd et al., 1997). IL-1β is involved in the proliferation, differentiation and apoptosis of cells. It has been implicated as a factor in tumor progression via the expression of metastatic and angiogenic genes and growth factors (Lewis et al., 2006). Granulocyte macrophage–colony stimulating factor (GM-CSF) has been shown to increase the immune response both in animal models and clinical trials. (Sakthivel and Guruvayoorappan, 2013) which was consistently induces dense CD4+ and CD8+ T-lymphocyte and plasma cell infiltrates, in metastatic lesions (Soiffer et al.,1998). The critical role of GM-CSF is not well characterized but clinical trials using GM-CSF secreting tumors cells have been reported in patients with several tumor types (Small et al., 2007;
Zarei et al., 2009). Vascular endothelial growth factor (VEGF) is a potent and specific angiogenic factor required for tumor growth. They activate endothelial cells to proliferate and migrate, subsequently resulting in new tube formation and blood flow (Verheul and Pinedo, 2000). However, the result obtained in this study indicates that *B. tomentosa* reduced the production of TNFα, iNOS, IL-1β, IL-6, GM-CSF and VEGF levels clearly express the regulatory effect of these intermediates and tumor inhibition by *B. tomentosa* extract.

Interleukin (IL)-2 is produced by T-cells and also reported that IL-2 receptor deficient mice exhibit lethal autoimmunity (Malek, 2003, Gaften and Liu, 2004). Ouyang et al. (2006) confirmed that IL-2 augmented activation of (TAM) would play the main role in induction of the MHC class I molecule through secretion of IFN-γ, and would contribute to the IFN-γ-mediated apoptosis induction in tumor cells. IFN-γ, an important immunoregulatory molecule against tumor cells, appears capable of driving novel cellular and molecular inflammatory mechanisms that may underline tumor initiation, invasion and survival (Blankenstein and Qin, 2003).

In conclusion, *B. tomentosa* extract exhibited a strong inhibitory effect on the proliferation of both solid and ascites tumor cells. The activity of the extract could be attributed to immune system activation, and blocking (or induction) of key immune system regulatory products (including TNF-α, IL-2, IL-6, IL-1β, GM-CSF or IFN-γ) also protects the bone marrow cells. It is well known that, that can impact on tumor cells during proliferation, cell cycle regulation, signal transduction, or even the motility and invasiveness of the cancer cells.