Chapter 5: Investigation of the protective effect of *Bauhinia tomentosa* in the inhibition of tumour metastasis.

5.1 Introduction

Metastasis is a phenomenon which involves spread of cancer cells from the primary tumor site to distant site through the circulation to form secondary tumor at a distant site, followed by extravasation to secondary site for tumorogenesis. Metastasis becomes the great challenge to face the cancer treatment because of side effects of the treatment and is the major reason for cancer related death (Liotta, 1991; Condeelis and Segall, 2003). All type of cancers are characterized by the multiple step deregulation of cell signaling pathways. Currently using anticancer chemotherapies involves the modulation of a single target and they are ineffectiveness, lack of safety and high expensive. In additional chemotherapy is the only treatment that may be recommended to prevent the spread of tumor cells but severe side effect occurs. The chemotherapeutic drug not only kill the cancer cell but also kills normal immune cells (Diwanay et al., 2004) which may cause side effect and reduce the immunity. As a result pharmaceutically needed to develop plant based drugs, however, it have been achieve multi targeting naturally and are inexpensive and safe.

More than 50% of drugs in clinical use are natural products and their derivatives. Almost 70% of drugs approved by FDA used for cancer treatment are natural phytomedicine or natural origin (Sitranga et al., 2011). There are several bioactive compounds of plant origin that has been reported for their immunomodulatory, anti-cancer and apoptotic effect both *in vitro* and *in vivo*, some of these compounds include curcumin, resveratrol, noscapine, parthenolide, piceatannol, chrysin and proanthocyanidin. Bioactivities of phenolic compounds are responsible for their immunomodulation properties (antioxidant,
and also contribute to apoptosis by arresting cell cycle and oncogene expression, inhibiting, cell migration, adhesion and proliferation (Moriai et al., 2012; Wiczak et al., 2012; Winzer et al., 2012). Bauhinia species are trees that belong to the family Fabaceae. This is a tree widely distributed in India that has been reported to possess medicinal value (Rajkapoor et al., 2006). Among these, *Bauhinia tomentosa* is prevalent in south India which exhibit medicinal properties such as anti-microbial and anti-diabetic activity (Devaki et al., 2011; Dugasani et al., 2010).

In the present study we had studied the protective effect of *B.tomentosa* on the inhibition of metastasis and the possible mechanism of action. The effect of *B.tomentosa* on the production of proinflammatory cytokines as well as the activation and nuclear translocation of transcription factor Nuclear Factor κB (NF-κB) were also studied.

### 5.2 Materials and methods

#### 5.2.1. Preparation and administration of plant extract

*B.tomentosa* fresh leaves were collected from the Herbal garden, Department of Biotechnology, Karunya University, Coimbatore. The plant specimen was authenticated by Botanical Survey India (BSI) Coimbatore. *B.tomentosa* leaves were shade-dried and powdered using a pulverizer. The leaf powder (10g) was extracted using with 70% methanol (100ml) using soxhlet apparatus. The yield of the extract was found to be 8%. For *in vivo* studies, the extract was re-suspended in 1% gum acacia and administered at a concentration of 10 mg/Kg Body weight.
5.2.2. Animals

Male C57BL/6 mice (20-25g) were purchased from National Institute of Nutrition (Hyderabad, India). The animals were maintained in ventilated cages, fed with normal mouse chow (Sai Feeds, Mumbai, India) and given water *ad libitum*. All the animal experiments were performed after obtaining approval from the Institutional Animal Ethics Committee, Karunya University (Approval No. IAEC/KU/BT/13/08).

5.2.3. Cell line

The B16F-10 melanoma cells were procured from National Centre for Cell Sciences, Pune, India. The cells were maintained in DMEM supplemented with 10% FCS and antibiotics (100 Unit/ml Penicillin and 100 Unit/ml Streptomycin).

5.2.4. Chemicals

MTT (3-(4, 5- dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide), hydroxyproline and glucuronic acid lactone were procured from Sigma Chemicals (St. Louis, USA). N-acetyl neuraminic acid and papain were purchased from Sisco Research Laboratory (Mumbai, India). Dulbecco’s Modified Eagle’s Medium (DMEM) was purchased from HiMedia (Mumbai, India). All other reagents were of analytical reagent grade.
5.2.5. ELISA kits

Highly specific quantitative sandwich ELISA kits for mouse Interleukin-1β (IL-1β), Interleukin-6 (IL-6), Tumor Necrosis Factor-α (TNF-α), Granulocyte Monocyte Colony stimulating Factor (GM-CSF) and Interleukin -2 (IL-2) were procured from Koma Biotech™, Gangseo-gu Seoul, Korea. Gamma glutamyl transpeptidase kit was purchased from Merck™, Mumbai, India. iNOS and COX-2 kits were purchased from USCN Life science Inc™, Houston, USA and Bluegene Biotech™, Shangai, China respectively. Transcription factor profiling kit for NF-κB p50 and NF-κBp65 was procured from Cayman™, Michigan, USA.

5.2.6 Determination of the effect of *B.tomentosa* on *in vivo* inhibition of experimental lung tumor metastasis and survival rate.

Male C57BL/6 mice (20-25g) (n=28) were divided in to two groups (n=14/group). Group I and II were induced metastasis by injecting B16-F10 cells (1 x 10⁶ cells) (i.v.). Group I served as metastasis control group and group II was treated with *B.tomentosa* simultaneously for 10 consecutive days. Eight animals from each group were sacrificed by cervical dislocation after day 21. Blood was collected by cardiac puncture and the serum was separated by centrifugation (3000 rpm for 10 minutes at 4°C. The lungs were dissected out, washed with ice-cold Phosphate Buffered Saline (PBS, pH7.4) and used quantification of tumour nodules. A portion of the lung was subjected for histopatological studies. The remaining six animals in each group were kept for monitoring the survival rate.
5.2.7 Determination of the effect of *B.tomentosa* on the lung hydroxyproline, uronic acid and hexosamine content during metastasis

The lung hydroxyproline content was measured according to the procedure described by Bergman and Loxley (1970). The lung hexosamine content was estimated by the method described by Elson and Morgen (1933). The lung uronic acid content in the lungs were estimated according to the protocol explained by Bitter and Muir (1962).

5.2.8 Determination of the effect of *B.tomentosa* on serum sialic acid and gamma glutamyl transpeptidase level during metastasis

The serum sialic acid level was determined by thiobarbituric acid assay method (Bhavanandan et al., 1981). Serum gamma glutamyl transpeptidase level was estimated using the highly specific quantitative sandwich ELISA kit (Merck\textsuperscript{TM}, Mumbai, India) as per the manufacturers’ instructions.

5.2.9. Determination of the effect of *B.tomentosa* on the lung iNOS and COX-2 level during metastasis

The lung tissue homogenate were used for the measurement of iNOS and COX-2 level according to the manufacturer’s instructions (USCN Life Science Inc, Houston, USA). The values were expressed as ng/g tissue (for iNOS and COX-2 respectively).

5.2.10. Determination of the effect of *B.tomentosa* on the serum TNF-α, IL-1β, IL-6, GM-CSF and IL-2 during metastasis.

Serum TNF-α, IL-1β, IL-6, GM-CSF and IL-2 levels were quantified using ELISA kits according to the manufacturers’ instructions.
5.2.11. Preparation of Nuclear Extracts

Nuclear extracts were prepared by slightly modified previously published method (Bhavanandan et al., 1981). B16F-10 cells were grown in 25 cm$^2$ flask. When the cells had become sub confluent, they were treated with *B. tomentosa* (10 μg/ml) and incubated for 2 hours at 37°C and 5% CO$_2$. The cells were washed with phosphate buffered saline (PBS) twice and incubated with TNF-α (10 pg/ml) for 30 minutes at 37°C in 5% CO$_2$. Cells were washed with PBS, dislodged with a cell scraper, and collected by centrifugation at 5000 rpm. The cell pellet was resuspended in ice cold cell lysis buffer containing HEPES (10mM), MgCl$_2$ (1.5 mM), KCl (10mM), phenylmethyl sulfonyl fluoride (1mM), dithiothreitol (DTT) (1mM), Nonidet P40 (0.5%) and EGTA (1mM) followed by centrifugation at 5000 rpm for 15 min. The cell pellet was resuspended in double the volume of lysis buffer and the cells were disrupted by repeated single rapid stroke using a sterile syringe. The nuclear pellet was resuspended in extraction buffer (0.2 ml) containing HEPES (20mM), glycerol (25%), MgCl$_2$ (1.5mM), NaCl (420mM), PMSF (0.1mM) and DTT (1mM) and incubated in ice for 30 min. The nuclear suspension was centrifuged at 10,000 rpm for 15 min at 4°C and the supernatant was collected as nuclear extract and used for the transcription factor profiling. A portion of the nuclear extract was used for the estimation of protein concentration by Bradford method.

5.2.12. Transcription factor profiling

Transcription factor profiling was done using the Cayman™ transcription factor kit (Cayman™, Michigan, USA). The kit provided rapid, high-throughput detection of specific transcription factors, namely subunits of NF-kB such as p65 and p50. Using an ELISA based format, the transcription kit detected the DNA bound transcription factors. Bound transcription factors in the DNA were detected by specific primary antibodies towards NF-kB p65 and NF-kB p50 subunits. A
horse radish peroxidase-conjugated secondary antibody was then used to detect the primary antibody. The enzymatic product was then measured using an ELISA reader and the percentage inhibition was calculated using the formula: 100 – ([Optical density of *B. tomentosa* treated/ optical density of control] x 100).

5.2.13. Histopathology analysis

The lung specimens were collected from each group of animals and were fixed in 10% formalin, dehydrated, embedded in paraffin and cut into 4 μm sections. The paraffin sections were deparafinized with xylene, hydrated and stained with hematoxylin and eosin for studying mucosal damage assessment. The images were captured at 10x magnification.

5.2.14. Statistical analysis

The results are expressed as mean ± S.D. Statistical analysis was carried out using one-way analysis of variance followed by Dunnet's test using GraphPad Instat (version 3.0 for Windows 95; Graph Pad Software, San Diego, CA). p values less than 0.05 were considered as statistically significant. All the experiments were performed at least three times.
5.3 Results

5.3.1 Effect of *B.tomentosa* on the Lung tumour nodule formation and survival rate of metastatic tumour-bearing animals

Effect of *B.tomentosa* on lung tumour nodule formation were shown in table 5.1. Treatment with *B.tomentosa* reduced pulmonary metastasis formation of B16F-10 melanoma cells. In control animals developed substantial number of countable colonies were 250±25, whereas simultaneously treated with *B.tomentosa* group shown significant (p<0.01) reduction in the nodule formation. The percentage inhibition of tumour nodules formation of *B.tomentosa* is 87.5% when treated group compared with control group. All animals which received *B.tomentosa* extract survived even after 90 days of experimental regimen.

5.3.2 Effect of *B.tomentosa* on the lung hydroxyproline, uronic acid and hexosamine content during metastasis

Effect of *B.tomentosa* on the lung biochemical parameters were shown in Figure 5.1. The lung collagen hydroxyproline content was drastically elevated in the control group (24.4 ± 1.4μg/mg protein) when compared with normal group (0.98 ± 0.08μg/mg protein) representing the fibrosis of lung tissue. This higher level was significantly reduced (8.2 ± 1.4μg/mg protein) in the animals treated with *B.tomentosa* indicating the decreased lung fibrosis.
Table 5.1: Effect of *B.tomentosa* on lung tumour nodule formation and survival rate of metastatic tumour bearing animals.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of animals per group</th>
<th>No. of nodules per lung</th>
<th>No. of animals Survived after 90 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>14</td>
<td>250 ± 25</td>
<td>0/6</td>
</tr>
<tr>
<td><em>B.tomentosa</em></td>
<td>14</td>
<td>35 ± 4.2**</td>
<td>6/6</td>
</tr>
</tbody>
</table>

B16F-10 (10⁶ cells/animal) were injected via tail vein. *B.tomentosa* was administered intraperitoneally for 10 consecutive days. Controls were treated with the vehicle-gum acacia. 8 animals from the each group were sacrificed at 21st day and the tumour nodules counted. 6 animals from each group were kept for survival studies. **Significant compared to untreated controls, p<0.01.
Figure 5.1 Effect of *B.tomentosa* on lung hydroxyproline levels.

The results are expressed as mean ± SD of each group (n=6) ** (*p*<0.01.) Significantly different from metastasis control animals.
Tumor bearing control animals shown significant increase in lung uronic acid content \((318 \pm 14.8 \mu g/100mg \text{ tissue})\) when compared with normal \((35 \pm 2.6 \mu g/100mg \text{ tissue})\). Treatment with \textit{B.tomentosa} at a dose of 10 mg/kg body weight significantly reduced \((67.0 \pm 6.0 \mu g/100mg \text{ tissue})\) these increased levels in lung tissue. The lung uronic acid content were shown in Figure 5.2.

The lung hexosamine content in the control metastatic tumor bearing animals was found to be \(5.2\pm 0.4 \text{ mg/100mg tissue}\) when compared with normal animals \((0.5 \pm 0.04 \text{ mg/100mg tissue})\). Treatment with \textit{B.tomentosa} significantly reduced \((1.4 \pm 0.3 \text{ mg/100mg tissue})\) the elevated hexosamine content. The lung hexosamine content were shown in Figure 5.3.

### 5.3.3 Effect of \textit{B.tomentosa} on the serum sialic acid and GGT level during metastasis

The effect of \textit{B.tomentosa} on serum sialic acid and GGT level in metastasis tumor bearing animals is shown in figure 5.4 and 5.5. The serum sialic acid level was drastically increased \((116.2 \pm 5.6\mu g/ml)\) in the control group, when compared with normal group \((26.2 \pm 6.4\mu g/ml)\). \textit{B.tomentosa} treatment showed a significant reduction in the sialic acid content \((44.4 \pm 8.0 \mu g/ml)\). Similarly GGT activity in the metastasis control animals shown higher level \((110.4 \pm 8.2 \text{nmol p-Nitroaniline/ml serum})\) when compared with normal animals \((25.6 \pm 2.8 \text{nmol p-Nitroaniline/ml serum})\). Whereas, GGT level in \textit{B.tomentosa} treated group shown significant \((p< .001)\) reduction towards normal level \((45.7 \pm 5.7 \text{nmol p-Nitroaniline/ml serum})\).
Figure 5.2 Effect of *B. tomentosa* on the lung Uronic acid content during metastasis.

The results are expressed as mean ± SD of each group (n=6) ** (p<0.01.) Significantly different from metastasis control animals.
Figure 5.3 Effect of *B. tomentosa* on lung Hexosamine levels.

The results are expressed as mean ± SD of each group (n=6) ** (p<0.01.) Significantly different from metastasis control animals.
Figure 5.4 Effect of *B.tomentosa* on serum sialic acid.

The results are expressed as mean ± SD of each group (n=6) ** (*p*<0.01.) Significantly different from metastasis control animals.
Figure 5.5 Effect of *B. tomentosa* on serum γ-glutamyl transpeptidase.

The results are expressed as mean ± SD of each group (n=6) ** (p<0.01.)
Significantly different from metastasis control animals.
5.3.4 Effect of *B.tomentosa* on the lung iNOS and COX-2 levels in metastasis tumor bearing animals

The effect of *B.tomentosa* on lung iNOS and COX-2 levels in metastasis tumor bearing animals is shown in Figure 5.6 and 5.7, respectively. The administration of *B.tomentosa* significantly (P<0.01) reduced the lung iNOS level (37.4 ± 2.8ng/g tissue) on 21st day when compared with metastasis control (65.6 ± 2.6 ng/g tissue) on the same day. The serum nitrite level after the treatment of *B.tomentosa* was also found to be decreased from 40.2 to 30 μmol compared with metastatic control group (Data not shown). The treatment with *B.tomentosa* also significantly decreased the lung COX-2 level (46.6 ± 4.0ng/g tissue) on 21st day when compared with metastasis control (74.6 ± 5.6).
Figure 5.6 Effect of *B.tomentosa* on the lung iNOS expression of tumour-bearing mice

The results are expressed as mean ± SD of each group (n=6) ** (*p*<0.01.) Significantly different from metastasis control animals.
Figure 5.7 Effect of *B. tomentosa* on the lung COX-2 expression of tumour-bearing mice

The results are expressed as mean ± SD of each group (n=6) ** (*p*<0.01.) Significantly different from metastasis control animals.
5.3.5 Effect of *B.tomentosa* on proinflammatory cytokine profile of metastasis-induced animals

Effect of *B.tomentosa* on proinflammatory cytokine profile is shown in Figure 5.8 to 5.12. The elevated level of serum TNF-α, iNOS, IL-1β, IL-6 and GM-CSF in metastatic control animals was observed whereas these levels were found to be decreased after *B.tomentosa* treatement. The decreased serum IL-2 observed in metastatic control group was markedly elevated after *B.tomentosa* treatment.

5.3.6 Effect of *B.tomentosa* on the activation and nuclear translocation of NF-κB transcription factors

The transcription factor NF-κB p65 and p50 subunit level was quantified by ELISA method. The administration of *B.tomentosa* significantly inhibited the activation and nuclear translocation of NF-κB subunits p65 and p50 (Table 5.2).

5.3.7 Effect of *B.tomentosa* on the lung histopathology

The tissue specimen from metastatic control animal showed prominent tumour nodules around the terminal bronchiole. A clear area of necrosis has been observed. The alveolar passage could not be distinguished because of massive infiltration of neoplastic cells (Figure 5.13 a). Tumour mass has been decreased in *B.tomentosa* treated animals. The alveoli and pleura were tumour free and the alveolar passage was lined with healthy columnar epithelial cells (Figure 5.13 b).
Figure 5.8 Effect of *Bauhinia tomentosa* on TNF-α levels in serum during B16F-10 induced lung metastasis in C57BL/6 mice.

The results are expressed as mean ± SD of each group (n=6) ** (*p*<0.01.) Significantly different from metastasis control animals.
Figure 5.9 Effect of *Bauhinia tomentosa* on IL-1β levels in serum during B16F-10 induced lung metastasis in C57BL/6 mice.

The results are expressed as mean ± SD of each group (n=6) ** (p<0.01.) Significantly different from metastasis control animals.
Figure 5.10 Effect of *Bauhinia tomentosa* on IL-6 levels in serum during B16F-10 induced lung metastasis in C57BL/6 mice.

The results are expressed as mean ± SD of each group (n=6) ** (p<0.01.) Significantly different from metastasis control animals.
Figure 5.11 Effect of *Bauhinia tomentosa* on GM-CSF levels in serum during B16F-10 induced lung metastasis in C57BL/6 mice.

The results are expressed as mean ± SD of each group (n=6) ** (*p*<0.01.) Significantly different from metastasis control animals.
Figure 5.12 Effect of *Bauhinia tomentosa* on IL-2 levels in serum during B16F-10 induced lung metastasis in C57BL/6 mice.

The results are expressed as mean ± SD of each group (n=6)  * (p<0.05.) Significantly different from metastasis control animals.
Table 5.2 Effect of *B.tomentosa* on the activation and nuclear translocation of transcription factors

<table>
<thead>
<tr>
<th>Transcription Factor</th>
<th>Optical Density of the Control (B16F-10 alone)</th>
<th>Optical Density after <em>B.tomentosa</em> treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>NF-κB p65</td>
<td>0.552 ± 0.026</td>
<td>0.175 ± 0.020*</td>
</tr>
<tr>
<td>NF-κB p50</td>
<td>0.645 ± 0.042</td>
<td>0.206 ± 0.025**</td>
</tr>
</tbody>
</table>

NF= nuclear factor. B16F-10 cells were cultured in a 25 cm² culture flask and treated with *B.tomentosa* (10μg/ml) and incubated for 2 hours at 37°C in 5% CO₂. Cells were washed with phosphate buffered saline and incubated with TNF-α (10pg/ml) for 30 minutes at 37°C in 5% CO₂. Nuclear extracts were prepared separately and subjected for transcription assay. Values are mean ± SD. *p<0.05, **p<0.01 compared with untreated controls.
Figure: 5.13 Effect of *B.tomentosa* on the lung histopathological analysis of lung metastasis tumor

Figure 5 (a and b) shows the histopathological analysis of lung metastasis tumor bearing animals. The metastatic control animal sections shows substantial tumor growth around the bronchiole and infiltration of metastatic colonies. This colonies were composed of polygonal tumor cells. Furthermore it shows increased fibrosis leads to reduces alveolar space, which may leads to vital capacity of the lung (Figure 5 a). This lung architecture were reversed and reduced tumor mass shown in the *B.tomentosa* treated animals, the alveoli and pleura were normal and alveolar passage was covered with normal epithelial cells (Figure 5 b).
5.4 Discussion

The cancer cells migrate from a primary tumor to all other sites of the body involves many distinct, successive steps termed as metastatic cascade (Dignam et al., 1983) During metastasis, there will be an aberrant regulation of specific gene expression, which leads to remarkable changes in inflammatory mediators, growth factors and biochemical productions. In the present study, we had evaluated the inhibition of lung metastasis by *B. tomentosa* as evidenced by decrease in lung tumor nodule formation. Administration of *B. tomentosa*, showed 87.5% inhibition of lung tumor nodule formation compared with metastasis control group. An increase the life span of was also observed after the treatment with *B. tomentosa*.

Sialic acid, an acetylated derivative of neuraminic acid, which are typically found at the outer most end of glycan chain of all cell types, involved in cellular adhesion and recognition during invading tumor cells (Nicolson, 1988; Varki, 2007; Angata and Varkai, 2002; Schauer, 2000; Ilver, 2003). Progression from tumorigenic to metastatic phenotype in both rodent and human cancers has been associated with equivalent up regulation of sialic acid content (Lehmann et al., 2006; Fernandes, 1991). The amount of sialic acid content on the surface of the malignant cells is directly relevant with the metastasis ability of the tumor cells (Varki and Varki, 2007).

Hydroxyproline is synthesized by hydroxylation of the aminoacid proline using prolylhydroxylase. It is a major component of the protein collagen and play key roles for collagen stability (Nelson and Cox, 2005; Brinckmann et al., 2005). During the lung metastasis collagen is deposited massively in the alveoli of lungs. Quantification of hydroxyproline, serves a direct marker of lung fibrosis (Kotch et al., 2005). In the present study *B. tomentosa* significantly reduced the lung hydroxyproline. These findings correlated with the reduction in the number of lung tumor nodules in the treated group.
Hexosamine and uronic acid (glucuronic acid) are produced from the acid and base modification of mono saccharides. Hexosamine and uronic acid are the basic structure of sialic acid. Malignant cells yield uronic acid by the oxidation of monosaccarides, this action leads to the glucronic acid formation. Which is an essential form of uronic acid. The excess hexosamine level is directly correlated with the active growth and proliferation of malignant cells (Pradeep and Kuttan, 2002; Vote and Vote, 1995; Stern, 2004; Carretero et al., 1999). In the present study B.tomentosa administration significantly reduced in lung hexosamine and uronic acid level. B.tomentosa decrease the excess synthesis of hexosamine, thereby reducing lung fibrosis and preventing malignant cells metastasis.

In the present study, elevated levels of GGT, a marker of cellular proliferation, was increased in the tumor bearing animals when compared to normal animals. γ- glutamyl-cysteine peptidase is enzyme used to cleaves the γ-glutamyl-cysteine peptide bond in GSH. GSH is produced an intra-cellular matrix, which supply energy to the tumor cells through gamma glutamyl cycle. GGT expression is associated with modulation of metastatic properties several tumor (Carretero et al., 1999; Raphael and Kuttan., 2003).

The aberrant expression of Cyclooxygenase-2 (COX-2) that occurs in majority of tumors is through to play a crucial role during tumor development (Sinicrope and Gill 2004; Greenhough et al., 2009). COX-2 may involve different steps of cancer progression by increasing proliferation of mutated cells thus favoring tumor promotion as well as escape from programmed cell death finally implicated in metastasis formation. (Choi et al., 2005; Grossmann 2002; Sobolewski et al., 2010). COX-2 is the inducible isoform, which is regulated by the growth factors and different cytokines such as IL1β, IL-6 and TNF-α. (Ramsay et al., 2002).
The transcription factor NF-κB family involved mainly in stress-induced inflammatory and immune response constitutively active NF-κB has been shown to contribute to the development of many cancers (Mori et al., 2002). NF-κB can be activated as during cellular transformation by a various cytokines including IL-1 and TNF-α. Among its multiple effects it also induces anti apoptotic proteins and blocks caspase activation (Dixit and Mak 2002; Strozyk et al., 2006). NF-κB plays an important role in the interaction between melanoma cells and tumor vasculature also required for development of tumor invasiveness (Rayet and Gelas, 1999; Sharma and Narayan, 1996). Among the transcription factor NF-κB family prototypical NF-κB is a heterodimer composed of most frequent components of active NF-κB they are p50 and p65 subunits (Neurath et al., 1998).

It is commonly accepted that cancer cells interact with host cells via cytokines and growth factors to create a micro environment favouring metastasis, same time they act as mediators of immune response and regulation of inflammatory responses, (Sosnoski et al., 2013; Cermi, 1992). TNF-α is a multifunctional cytokines which plays an important pathogentic processes such as chronic inflammation apoptosis autoimmunity and TNF-α contributes to all stages of the malignant process (Balkwill, 2006; Esposite and Cuzzocrea, 2009; Ilyasova, 2005; Lin and Karin, 2007). IL-6 is a multifunctional immunomodulatory cytokines, was activates B and T lymphocytes, which was produced by both normal and tumor cells serum concentration of IL-6 can used to predict tumor progression. It also stimulate VGEF (Hoeben et al., 2004; Chechlinska et al., 2008). As well as IL-1β 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, (Slingluff et al, 2003) which was consistently induces dense CD4 and CD8 T-lymphocyte in metastatic lesions (Small et al.,
The critical role of GM-CSF is not well characterized but clinical trials GM-CSF involved in angiogenesis for differentiation of angioblasts into endothelial cells, their migration and proliferation in several tumor types (Nemunaitis et al., 2004; Small et al., 2007; Zarei et al., 2009; Kuhlmann et al., 2007). Vascular endothelial growth factor (VEGF) is a well-known angiogenic factor, which activate endothelial cells to proliferate and migrate, subsequently resulting in new tube formation for blood flow (Verheul and Pinedo, 2000). However, in this study indicates that B.tomentosa reduced the production of TNF-α, iNOS, IL-1β, IL-6, GM-CSF, and VEGF levels clearly shows that the regulatory effect of this intermediates and metastatic tumor inhibition of B.tomentosa. IL-2 has been considered a key growth factor for T lymphocytes IL-2 and IL-2 receptor deficient mice exhibit lethal autoimmunity and also stimulate natural killer cells (Boyman et al., 2012; Gaffen and Liu, 2004; Ouyang et al., 2006). In the present study, treatment with B.tomentosa could significantly enhance the IL-2 level, which may involve to the stimulation of the natural immune system and control the metastasis formation.

The present study concludes that B.tomentosa shown anti-metastatic ability. Histopathological analysis confirms and also shows a distinct difference in the lung architecture these data suggest that transcription factor and cytokines may be a good target for treatment designed for metastasis this inhibition may be due to the interference with signaling cascade, may inhibit the expression of several proinflammatory cytokines. Phytochemical analysis of B.tomentosa showed the presence of many pharmacologically active ingredients, include Kaempferol-3-O-rhamnoside, Kaempferol-3-O-rutinoside, Quercetin 3-O-glucoside and quercetin 3-O-rutinoside. Further studies have to be conducted to evaluate the mechanism of action of B.tomentosa and its phytochemical compounds in the inhibition of tumor metastasis and explore how it can be used in cancer therapy.