Summary and Conclusions

*Bacopa monniera has anti-inflammatory property*: Screening of different extracts like methanol, ethanol and aqueous fractions of whole plant and roots alone of *Bacopa monniera* for anti-inflammatory activity showed that methanolic extract of the whole plant of *Bacopa monniera* (BME) possessed potential anti-edematogenic effects as evident by decreased carrageenan induced hind paw edema. Time kinetics study revealed that BME (100 mg/kg) exhibited anti-edematogenic effects at 3rd and 5th hour post carrageenan induction. The effect of BME lasted for up to 24 hours. BME significantly inhibited carrageenan induced 5-LOX and 15-LOX activities in rat mononuclear cells, COX-2 and MPO activities in paw tissue and NOS activity and nitrite levels in paw exudates. BME significantly inhibited granuloma formation in cotton pellet test and also down regulated LPS induced TNF-α indicating its ability to alleviate inflammation.

*BME has anti-arthritic effects*: Using adjuvant induced and Type II collagen induced arthritic models, it was confirmed that BME possessed anti-arthritic effects. BME (100 mg/kg) significantly decreased edema formation during the disease progression in rats. Indomethacin (3 mg/kg) was used as the reference drug. It was found that preventive regimen was more effective in alleviating arthritis in rats compared to treatment regimen. BME decreased polyarthritic index and inhibited pro-inflammatory mediators like 5-LOX activity in rat mononuclear cells, COX-2 and MPO activities in paw tissue and PGE$_2$ levels in articular cartilage. BME was also effective in decreasing splenic hypertrophy and serum ALP, SGPT and SGOT activities compared to arthritic rats. BME decreased lipid peroxidation and improved the antioxidant status of arthritic rats. BME effectively inhibited β-glucuronidase and β-hexosaminidase activities in liver, spleen (lysosome fraction) and cartilage as well as collagenase, hyaluronidase and cathepsin activities in articular cartilage compared to arthritic rats. BME also effectively protected against GAG loss in the articular cartilage of arthritic rats. Moreover BME was effective in decreasing serum anti-collagen
antibodies like IgG and IgM levels indicating decreased disease progression. The macroscopic and biochemical observations were confirmed with histopathological data. Joint sections of BME supplemented rats showed minimal evidence of inflammation or joint destruction. These results denote the anti-arthritic effects of BME.

**Chloroform fraction of BME is superior in anti-inflammatory activity:** Bioactivity guided fractionation of BME showed that chloroform fraction (CF) has superior anti-edematogenic compared to ethyl acetate, butanol, aqueous and bacoside enriched fractions. Further evaluation of CF for anti-arthritic effects showed that CF was indeed more effective in alleviating inflammation during arthritis than BME. CF (25 mg / kg) exhibited 89% paw edema inhibition in arthritic rats on day 30. CF significantly inhibited 5-LOX activity in rat mononuclear cells, COX-2 activity and PGE\(_2\) levels in paw tissue and articular cartilage as well as protected against adjuvant induced GAG loss in articular cartilage. CF also significantly down regulated IL-6 expression in rat mononuclear cells of arthritic rats. Another characteristic feature of CF (not found in BME) was that CF inhibited nitrite levels and NOS activity in synovial effusate of arthritic rats. CF also inhibited nitrite production in LPS activated hPBMCs indicating that constituent(s) in CF possess nitrite inhibiting property. The histopathological study of paws obtained from the arthritic rats showed characteristic signs of severe inflammation with massive mixed infiltration of neutrophils. Further purification of CF using silica gel column chromatography using gradient solvent system showed that fraction 2 (hexane: ethyl acetate 75:25) exhibited potent anti-inflammatory activity as evident by carrageenan induced paw edema assay. This fraction on purification with TLC, comparison with authentic sample and H\(^1\) NMR analysis showed the presence of betulinic acid. This suggested that betulinic acid, a triterpenoid in CF was responsible for its anti-inflammatory activity.

**Betulinic acid has anti-inflammatory activity:** Betulinic acid isolated from chloroform fraction of BME significantly inhibited LPS induced IL-6 production and PGE\(_2\) production in hPBMCs in a dose dependent manner. Downregulation of IL-6 expression and decreased PGE\(_2\) production was also observed in the paw
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tissue of betulinic acid pre-treated carrageenan challenged rats. This data indicates that betulinic acid is an effective anti-inflammatory agent in vivo. Betulinic acid also inhibited nuclear translocation of p65 and also inhibited LPS induced activation of p38 and ERK pathways but not JNK pathway. It was observed that p38 (SB203580) and ERK1/2 (PD98059) inhibitors blocked IL-6 production in hPBMCs whereas pre-treatment with JNK inhibitor (SP600125) had no effect.

To demonstrate the effect of MAPK inhibitors on nuclear translocation of NF-κB, cells were pre-treated with SB203580 and PD98059 prior to LPS activation. PD98059 and SB203580 significantly prevented nuclear translocation of p65 NF-κB indicating the involvement of NF-κB and MAPK in LPS activated inflammatory response in hPBMCs. Addition of each of these inhibitors to cell cultures along with betulinic acid caused significant inhibition of p65 NF-κB nuclear translocation. The inhibitory effect of both betulinic acid and the inhibitors was higher than that of cells treated with inhibitors alone. These results suggest
that activation and translocation of NF-κB had occurred on LPS induction and betulinic acid prevented NF-κB activation. There is a possibility that the prevention of p65 nuclear translocation may involve p38 and ERK MAPKs as cross talks occur between MAPK and NF-κB pathways.

Another significant finding of the present study is that betulinic acid protected mice from LPS-induced lethal toxicity in vivo. The increased survival of mice pre-treated with betulinic acid correlated with our in vitro experiments using human primary monocytes in which betulinic acid downregulated LPS induced IL-6 production. The protective effects of betulinic acid in LPS induced mice were confirmed using histopathological data that showed reduced infiltration of leukocytes in lungs and around the portal vein in the liver. These findings are in line with our observations on the protective effects of this triterpenoid and strengthen our hypothesis that triterpenoids like betulinic acid can be used to block the cascades triggered by endotoxin in vivo.

This study gives an insight into the anti-inflammatory activity of *Bacopa monniera* and use of its active constituent for the treatment of inflammatory diseases like arthritis.

**Future prospects:**

- Further evaluate the molecular mechanisms of betulinic acid.
- Evaluate the bioavailability of betulinic acid and its chemically modified forms in higher animal model systems.
- Encourage clinical trials in humans to evaluate the efficacy of betulinic acid as an anti-arthritic agent. A combination therapy trial using betulinic acid and other pharmacological agents in optimal ratio is also encouraged.