GENERAL CONSIDERATIONS

The significant prevalence of acute and chronic pain worldwide has great impact on socio-economic life as well as it is a costly health issue in terms of lost productivity and income. Due to its impact on the society and economy worldwide, discovery and development of novel analgesics is the subject of extensive research in both academia and industry. Therefore, an urgent need was felt to study the possibilities of alternate strategies for better pain management. Pain, the unpleasant physical sensation, can be broadly classified into three types, namely nociceptive pain, inflammatory pain and neuropathic pain.

Nociceptive pain being acute in nature is resolved easily with removal of the stimulus causing it. It is basically an alarm system for further possible damage and hence, crucial for survival. Primary afferent Aδ-fibers (fast conducting and myelinated) and C- fibers (slow-conducting and unmyelinated) gets activated in response to external stimuli. Activation of these fibers leads to activation of supra-spinally projecting dorsal horn neurons, finally resulting in nociceptive pain.

Inflammatory pain could be acute or chronic in nature. Inflammatory pain, which occurs due to tissue injury arising from ongoing exposure to high intensity stimulus, is a pain sensation which continues beyond the removal of originating stimulus. It actually resolves as a function of healing and, in parallel, to the resolution of the inflammation. But during the course of disease, at the peripheral terminal, injury/inflammation leads to an innate immune cascade such as release of active factors from blood, local and migrating inflammatory cells and injured cells which sensitizes C-fibers (Xu and Yaksh, 2011). At spinal dorsal horn level, afferent activation leads to initiation of a robust facilitation of dorsal horn. This facilitation includes enhancement of NMDA receptor, increased trafficking of AMPA receptor subunits, activation of kinases, activation of caudal mid-line raphe-spinal serotonergic neurons and loss of inhibitory control (GABAergic and gycinergic interneurons) leading to allodynic condition (Yaksh, 1989; Huang, 1992; Galan, 2004; Svensson, 2006; Ji *et al.*, 2009). Local non-neuronal cells such as spinal microglia and astrocytes get activated during peripheral inflammation and release a variety of pro-excitatory neurohormones including cytokines, chemokines, ATP and PAR agonists (Milligan and Watkins, 2009).

Neuropathic pain - a form of chronic pain - arises due to injury to the peripheral nerves. This injury to nerve could be due to tumors, diabetic neuropathy, herpes zoster, complex regional pain syndrome, AIDS, multiple sclerosis, hypoxia and stroke (Mika, 2008) thus, making neuropathic pain heterogenous in nature. It is one of the more challenging syndromes for clinicians worldwide due to its complex nature and diverse etiology. Activation of small afferent fibers arising from the distal sprouting of the injured axons and from the DRG of the injured axon leads to increased spontaneous activity. The peripheral and central mechanisms of neuropathic pain involves ectopic impulse generation development as a result of abnormal expression of sodium channels, development of chemical sensitivities in damaged primary sensory neurons and degenerative and the regenerative changes in the spinal cord leading to aberrant connectivity and irreversible state (Scadding, 2003). Apart from above mechanism, peripheral nerve injury also provokes a reaction from the immune system at various anatomical locations including the injured nerve, the dorsal root ganglia (DRG), the spinal cord and supraspinal sites associated with pain pathways (Austin and Moalem-Taylor, 2010). Emerging lines of evidence have revealed that changes also occur in spinal microglia, the immune cells of the central nervous system (Inoue and Tsuda, 2009). Activation of microglia is a major feature of neuropathic pain and growing evidence suggests that microglia have a causal role in pathogenesis of persistent neuropathic pain.

Available Therapeutic Options to Mitigate Pain

Even though inflammatory pain and neuropathic pain share common mechanisms, they might differ with respect to time course and their contribution. Inflammatory pain may be sensitive to agents such as NSAIDs and opiates but that is not the case with neuropathic pain. Treating neuropathic pain is often considered as a trial and error exercise due to its heterogenous nature. Available therapeutic options for neuropathic pain include NMDA antagonists (e.g. ketamine, methadone), Mu-opiates (e.g. morphine), gabapentinoid drugs (e.g. Gabapentin, pregabalin), anticonvulsants (e.g. carbamazepine, diazepam, midazolam) and TNFα blockers (Xu and Yaksh, 2011). Many of the above mentioned agents have either limited efficacy or are efficacious at very high dose levels, which may be accompanied by severe side effects. Moreover, opiates which are found to be effective at high dose levels pose a threat of addiction. Most of these medications aimed at mechanisms of neuropathic pain may provide complete or partial relief in only about half of the patients (Stillman, 2006). This leaves room for improved and efficacious therapeutic agents.

Alternative therapies and pain management

Owing to the lack of good efficacious agents and severe side effects among the therapeutic options available currently, people look for alternate treatment for relief from chronic and debilitating pain. Even in the era of new therapeutic agents, plants still remain to be the major possible source of new drugs and chemicals. They continue to be the source of lead structures for synthetic modifications and optimization of bioactivity. Due to limited efficacy and severe side effects associated with currently available agents, medicinal products derived from plants, marine organisms, etc. are preferred and are fast becoming part of integrative health care systems in industrialized nations (Qadrie *et al.*, 2009). There are reports of increase in the number of patients opting for complementary and alternative medicine and consuming extracts from biological compounds from folklore medicine (Smith and Mills, 2001). Along with mechanisms of action being broader than that of currently available options such as NSAIDs, analgesics, opiates etc., herbal medicinal products are believed have lesser side effects. Even when exact mechanism of action of herbal medicinal product remains elusive, it is for sure that most of them exhibit their efficacy/potency through several pathways which include inhibition of cyclooxygenase (COX) and /or lipoxygenase (LOX), inhibition of cytokine release, inhibition of elastase or hyaluronidase, and also induction of anti-oxidative activity (Cameron *et al.*, 2009a, b).

Rationale for selecting BIRM

As is widely known, prostaglandins, which are thought to play important role in pain transmission at peripheral sites and in the spinal cord (Malmberg and Yaksh, 1992; Yamamoto and Nozaki-Taguchi, 1996; Vane *et al.*, 1998), are synthesized in tissues by cyclooxygenase, an enzyme involved in the metabolism of arachidonic acid into PGs. COX-2, an isoform of COX, is highly inducible in response to cytokines, growth factors or other inflammatory stimuli (Vane *et al.*, 1998). There are reports indicating that COX-2 inhibitors are effective in producing an anti-nociceptive effect in rat inflammatory pain models thus providing the much needed proof that COX-2 has a major role in nociceptive transmission in both the spinal cord and at the peripheral sites (Yamamoto and Nozaki-Taguchi, 1996; Yamamoto and Sakashita, 1998). COX-2, being an inducible enzyme, increases in the peripheral and central nervous system post injury or inflammation (Seibert *et al.*, 1994; Zhao *et al.*, 2000) and plays an important role in neuropathology. Jean *et al.* (2009) observed overexpression of COX-2 in injured nerve in rats following CCI, partial sciatic nerve ligation,

spinal nerve ligation and complete sciatic nerve transaction intervention. These studies provide enough proof of involvement of COX-2 in inflammatory as well as neuropathic pain. Microglial cells as mentioned elsewhere are the resident immune cells of the central nervous system (CNS). They act as the main form of active immune defense in the CNS and upon getting activated following any insult to the nervous tissue, they become the main source of inflammatory mediators (*viz*., IL-1β, IL-6, TNF-α, PGE2, NO, BDNF, etc.) in the nervous system (Hung *et al.*, 2009). Nerve injury induces extensive proliferation of spinal microglia and related gene expression. They become activated and adopt the immunological functions of the tissue following the damage (Mika, 2008). The release of inflammatory mediators is responsible for further amplifying the expression of activated microglia.

Hence, in the case of peripheral nerve injury, increased activation of microglia will lead to release of prostaglandins. As mentioned earlier, prostaglandins are synthesized by COX enzymes. Therefore, the questions of interest are 'If COX is inhibited in this cycle, leading to reduction in PG synthesis, will it have any effect on microglia expression?' Or 'Will any alteration to COX-2 expression lead to amelioration of neuropathic pain condition?'

We came across an herbal medicinal product marketed as Biological Immune Response Modulator (BIRM) that is thought to exert its potential efficacy through inhibition of COX in therapeutic area of pain and inflammation. Jaggi and co-workers (2004) have studied mother tincture of *Solanum dulcamara* (source of BIRM) through *in vitro* test systems and found that it inhibits the activity of COX-1 and COX-2 but does not inhibit the production of leukotriene LTB_4 by 5-LOX. BIRM is an oral solution extracted from Amazonian plant formulated by a physician (Edwin Cevallos A). Based on the local folklore of the Ecuadorian native population, it is promoted as a natural herbal medicine in South America. BIRM is considered to be a natural remedy for various diseases such as cancer, HIV-1-infection and so on (Cevallos, 1994; Cevallos, 1996). Dandekar *et al*. (2003) have shown through their *in vitro* and *in vivo* studies that BIRM has anti-proliferative property against prostate cancer cells. However, even though the COX inhibitory property of BIRM is known since a while now, systematic studies on the efficacy of this drug in ameliorating pain are yet to be initiated. Hence, we decided to study BIRM in a systematic way using *in vivo* models of pain and inflammation to evaluate its anti-nociceptive and anti-inflammatory properties.

The aims of the current study

- To assess the anti-nociceptive and analgesic properties of BIRM in animal models of nociceptive pain
- To study the peripheral and central acting mechanisms of BIRM in animal models of inflammatory pain
- To study the neuroprotective effect of BIRM in animal models of neuropathic pain and role of microglia and cytokines in maintaining the pathological environment.

In the current study, in order to ascertain BIRM's peripheral analgesic activity and its anti-inflammatory property we employed the acetic acid-induced writhing test and carrageenan induced paw edema test, respectively. Moreover, formalin-induced paw licking test was used to confirm whether BIRM's anti-nociceptive property is mediated through central or peripheral nervous system. Secondly to test its efficacy in chronic inflammatory pain, we studied monosodium iodoacetate-induced osteoarthritis model in rats. Lastly to assess the efficacy of BIRM and role of microglia in neuropathic pain we studied two models of peripheral neuropathic pain namely: chronic constriction injury (peripheral nerve injury model) and streptozotocin-induced diabetic neuropathy (disease model).

Acetic acid-induced writhing test:

Acetic acid-induced writhing test was mainly performed to assess the peripheral analgesic activity of the compound in question. In general, acetic acid causes spontaneous pain by causing secretion of endogenous substances such as serotonin, histamine, prostaglandins, bradykinins and substance P. Derardt *et al.* (1980) have shown increased presence of Prostaglandin E_2 in the peritoneal fluid post acetic acid administration. Prostaglandins along with local peritoneal receptors are thought to be responsible for abdominal constriction and activation and sensitization of the peripheral chemo-sensitive nociceptors (Bentley *et al.*, 1983; Dirig *et al.*, 1998) and causing inflammatory pain (Bley *et al*, 1998). BIRM was administered orally for seven days to male Swiss albino mice prior to administration of acetic acid (0.6% v/v in distilled water, 10 ml/kg, i.p). BIRM significantly reduced the frequency of the writhing in mice subjected to intraperitoneal acetic acid administration, similar to the conventional NSAID diclofenac sodium. BIRM as standalone treatment was found to be equally efficacious as compared to standard Diclofenac but BIRM when administered as combination therapy along with Diclofenac was found to be more effective in terms of inhibition of writhes as compared to BIRM or Diclofenac alone.

Carrageenan-induced paw edema

Carrageenan induced inflammation model is used extensively in the development of NSAIDs and selective COX-2 inhibitors and in assessing the contribution of mediators involved in vascular changes associated with acute inflammation (Necas and Bartosikova, 2013). Carrageenan-induced paw edema test, commonly used as an experimental model for acute inflammation, is observed to be biphasic. Acute inflammation observed in both phases, leads to leakage of plasma elements from blood vessels to the inflamed tissue and the infiltration of neutrophils (Zhou *et al.*, 2006; Thakare *et al.*, 2010). Histamine, serotonin, bradykinin, prostaglandins, hydrogen sulfide and nitric oxide are some of the inflammatory mediators which play role in this model (Necas and Bartosikova, 2013). BIRM was administered orally for seven days prior to intraplantar injection of carrageenan (100 µl of 1% carrageenan diluted in saline). BIRM administration significantly reduced the carrageenan induced paw edema at all time points of the study. The observed reduction was comparable to the reference drug, diclofenac, used in this study.

Formalin test

As we know, formalin test is capable of discriminating between neurogenic pain (early phase which is considered to be CNS modulated and non-inflammatory) and inflammatory pain (chronic and peripheral pain). The neurogenic pain caused due to direct chemical stimulation of nociceptive afferent fibers (predominantly C fibers) can be attenuated by opiates like morphine (do Amaral *et al.*, 2007), whereas inflammatory pain caused by the sensitization of spinal cord mediated through activation of NMDA receptors and release of inflammatory mediators like histamine, prostaglandins, bradykinin, serotonin in the peripheral tissue can be attenuated by opiates, NSAIDs etc. (Dalal *et al.*, 1999). BIRM was administered orally for seven days prior to administration of formalin (50 µl of 2.5% concentration, s.c. in plantar surface of hind paw). Repeated treatment of BIRM was able to inhibit both neurogenic as well as inflammatory pain significantly indicating it's centrally as well as peripherally acting analgesic activity.

Monosodium iodoacetate (MIA) induced osteoarthritis (OA):

Osteoarthritis being a chronic condition is widely prevalent in elderly population. It is believed that subchondral bone, periosteum, synovium, ligaments and the joint capsule are richly innervated and contain nerve endings which may be the sources for nociceptive stimuli (Heppelmann, 1997; Mach *et al.*, 2002). In addition to this, Schaible *et al.* (2002) have reported occurrence of peripheral and central pain sensitization in OA. MIA-induced OA

model reflects the different pain states observed in clinical conditions. In the initial duration of the MIA-induced OA model implies a transient synovial inflammation involving role of macrophages (Haywood *et al.*, 2003). Inflammation normally gets resolved in the joints at 1 week post MIA injection and biomechanical forces affecting the articular cartilage and subchondral bone are the reason behind pain sensation. There are many proinflammatory cytokines, oxidants and other factors exerting action in initiation and development of OA.

We carefully characterized the widely used MIA model of OA in relation to development of pain related behavior and investigated effects of BIRM on the clinical and behavioral changes associates with MIA-induced OA. To summarize, single dose of MIA induced osteoarthritis in animals accompanied with pain behavior. Swelling in the knee joint and limping gait was observed in the first week post MIA injection. Abnormally increased response to non-noxious mechanical stimulus (tactile allodynia) and heightened sensitivity to noxious radiant heat source was observed in animals injected with MIA. We also observed that MIA treated animals reacted more profoundly when they were subjected to normal movement such as flexion and extension of knee joint. BIRM was administered orally from day 15 of OA induction and continued till day 28. Repeated oral administration of BIRM not only reduced the swelling in affected joint but also significantly reversed the nociception-related behavior as assessed by the knee bend test at day 21 and day 28. Although it could not lower the score comparable to saline treated animals but the effect produced by BIRM was much similar to that of the standard drug, celecoxib. It was also able to inhibit tactile allodynia by increasing the paw withdrawal threshold significantly on day 28. Although increased response to the noxious thermal stimulus was not consistent by MIA-induced animals throughout the study, BIRM showed improvement in paw withdrawal latency thus inhibiting thermal hyperalgesia to some extent. Our observation with respect to inconsistent hypersensitivity to noxious thermal stimulus throughout the study duration in the MIA-induced OA animals is in line with the findings of the other studies conducted in rats (Combe *et al.*, 2004; Vonsy, 2009) as well as with clinical observations. These results suggest that BIRM may have the potential to be used as therapeutic for OA.

Chronic constriction nerve injury model– Neuropathic pain

This model produces allodynia and hyperalgesia – salient features of peripheral neuropathic pain (Bennett and Xie, 1988). It simulates the clinical condition of chronic nerve compression such as that occurring in nerve entrapment neuropathy or spinal root irritation by lumbar disk herniation (Kumar *et al.*, 2014). The nerve injury is created by tying loosely constrictive

ligatures around the rat's sciatic nerve. The ligatures evoke intraneural edema; the swelling is opposed by the ligatures leading to strangulation of nerves. Pain behavior such as allodynia and hyperalgesia develops in 10-14 days post nerve injury and lasts for 2 months. Spontaneous pain and evoked pain sensation observed in CCI rats are similar to those observed in patients with painful peripheral neuropathies.

Mechanical allodynia and thermal hyperalgesia - considered as salient features of peripheral neuropathic pain – were observed in animals subjected to CCI from day 13 onwards. Repeated oral administration of BIRM for 14 days significantly inhibited thermal hyperalgesia and mechanical allodynia.

The present study demonstrates that microglia cells are useful for evaluating the effects of anti-neuroinflammatory effects of novel compounds. The immunohistochemical results showed CCI-induced microglia activation, which is evident from their morphology in CCI-Vehicle control group (CCI-VC). The western blot results showed increased expression of Iba-1 protein in lumbar spinal cord in CCI induced neuropathic pain, which supports the immunohistochemical data. Increased expression of Iba-1 protein under neuropathic condition indicates activation of microglia cells in the spinal cord. Repeated administration of BIRM orally, improved pathological conditions in animal model of neuropathic pain in the spinal cord by reducing the expression of Iba-1 protein and the proportion of activated microglia cells along with significant inhibition of neuropathic pain symptom, thermal hyperalgesia and mechanical allodynia.

Further, the gene expression analysis of COX-2 and pro-inflammatory cytokines (TNF-α) showed significant fold increase in their mRNA levels in lumbar spinal cord tissue of rats from CCI-induced vehicle control group as compared to normal control rats. Repeated oral administration of BIRM not only inhibited the neuropathic pain symptom namely thermal hyperalgesia and mechanical allodynia but also prevented CCI-induced changes in spinal cord by lowering fold increase of mRNA levels of inflammatory mediators like COX-2 and TNF-α in lumbar spinal cord tissue. At the same time, we were able to observe significant fold increase in mRNA levels of anti-inflammatory cytokine IL-10 in lumbar spinal cord tissue of CCI-BIRM treated rats as compared to CCI-VC rats.

Streptozotocin induced diabetic neuropathy

Neuropathic pain being one of the major complications of diabetes mellitus, STZ-induced diabetes rat model was used to study chronic neuropathic pain. It develops behavioral

abnormalities like allodynia and hyperalgesia during the course of disease which is believed to be having correlation with pain symptoms under clinical conditions.

Development of diabetic condition was confirmed by hyperglycemic state accompanied with loss of body weight in animals treated with Streptozotocin. Progression of diabetes gradually led to development of diabetic neuropathy, which was confirmed by changes in nociception. Changes in nociception were evident by decreased paw withdrawal latency (thermal hyperalgesia), decreased paw withdrawal threshold to mechanical (von Frey) and increased response to chemical allodynic stimulation (acetone test). This behavioral observation is in confirmation with earlier findings by Courteix *et al.* (1998), indicating that hyperglycemia induced by diabetes in rats alters pain sensitivity by producing both allodynia and hyperalgesia. Repeated oral administration of BIRM for over a period of 14 days does improve blood glucose levels and shows reduction in body weight loss in animals treated with STZ. These results point towards a blood glucose lowering potential of BIRM in diabetic conditions. It also exhibits improvement in threshold level to mechanical and chemical allodynic stimulus and increases paw withdrawal latency to thermal stimulus as compared to STZ treated animals (STZ-VC group).

Earlier studies have shown involvement of Cyclooxygenase (COX) system in transmission of pain stimuli in STZ induced diabetic neuropathy (Bujalska *et al.*, 2008). In line with this above observation, in our present study, fold increase in mRNA levels of COX-2 in lumbar spinal cord was observed in STZ treated animals (STZ-VC) as compared to normal rats (NC), indicating its role in generating neuropathic pain. After repeated administration of BIRM (4 ml/kg) daily for two weeks in STZ treated animals (STZ-BIRM), significant reduction in fold increase in mRNA levels of COX-2 was observed as compared to STZ-VC group. These results support the earlier findings of Jaggi *et al.* (2004) demonstrating inhibitory effect of mother tincture *Solanum dulcamara* on PGE_2 production via COX-1 and COX-2 *in vitro* and our hypothesis that BIRM might exhibit its pain ameliorating effects through inhibiting COX-2

Studies conducted using various animal models emphasize microglia getting activated in spinal cord under circumstances like cancer pain (Mantyh *et al.*, 2002), nerve injury (Winkelstein *et al.*, 2001) and diabetes induced neuropathic pain (Tsuda *et al.*, 2003; Daulhac *et al.*, 2006; Wodarski *et al.*, 2009; Morgado *et al.*, 2011; Graeber and Christie, 2012; Kim *et al.*, 2012,) and microglia inhibition through glia inhibitors like minocycline helping in

amelioration of neuropathic pain (Raghvendra *et al.*, 2003). Supporting the above observations, we noticed significant upregulation of Iba-1 protein, a marker of activated microglia cells in lumbar spinal cord tissue of STZ treated animals (STZ-VC) as compared to normal rats (NC). Similarly, through immmunohistochemical studies, we could observe significant increase in number of activated Spinal microglia cells (identified through their altered morphology). BIRM, when administered daily for 14 days, showed reduction in Iba-1 protein expression in lumbar spinal cord as compared to STZ-VC. With respect to morphological aspects of microglia cells, we observed a shift from activated to resting state of microglia cells in BIRM treated group (STZ-BIRM).

Inflammatory mediators such as IL-1β, IL-6 and TNF-α released through activated microglia has the potential to modulate spinal cord synaptic transmission, leading to increased excitability of dorsal horns neurons, partially through suppression of inhibitory synaptic transmission (Wen *et al.*, 2011). In our present study, we observed fold increase in mRNA levels of TNF-α in dorsal spinal cord in fifth week post STZ treatment (STZ-VC) as compared to normal control animals (NC).There are several studies showing implications of spinal proinflammatory cytokines in pain modulation by blocking or disrupting their actions, which in turn block/improve the exaggerated pain state (Milligan *et al.*, 2000; Sweitzer *et al.*, 2001a; Watkins *et al.*, 2001b). Similarly, repeated treatment with BIRM for 14 days was able to reduce significantly the fold increase in mRNA levels of TNF-α in dorsal spinal cord as compared to STZ-VC group. This was reflected in terms of improved paw withdrawal threshold to mechanical and chemical stimuli and increased paw withdrawal latency to thermal stimuli. This observation also indicates direct interaction of BIRM with immune cells in the central nervous system. Also supporting our observations, studies by De Leo *et al.* (1996) and Winkelstein and De Leo, (2002) have reported increased expression levels of TNF-α, IL-1 and/or IL-6 in spinal cord in animal models of neuropathic pain.

It is a known fact that the creation of imbalance in the pro-inflammatory and anti-inflammatory cytokines is also one of the reasons for pain development. Anti-inflammatory cytokines such as IL-1α, IL-4 or IL-10 are believed to inhibit development of neuropathic pain by playing crucial role in nocicepetion (Zychowska *et al.*, 2013) through suppressing the production and release of pro-inflammatory cytokines (TNF- α, IL-1, IL-6) (Moore *et al.*, 1995; Milligan *et al.*, 2005). In our study we observed that there was significant fold increase in mRNA levels of IL-10 in spinal cord of animals treated with BIRM (STZ-BIRM) as compared to STZ-VC. However, Rojewska *et al.* (2014) have made the observation in their

study where significant upregulation of mRNA IL-10 was observed in spinal cord and dorsal root ganglion (DRG) in neuropathic rats (CCI-induced neuropathic pain) as compared to normal rats but treatment with Minocycline reduced the mRNA IL-10 levels in spinal cord as well as in DRG.

In a nutshell, we observed repeated administration of BIRM to be effective in attenuating pain and inflammation occurring due to CNS activation and peripheral inflammatory mediators thus showcasing its anti-nociceptive and anti-inflammatory role as peripherally and centrally acting compound. Secondly, we observed that BIRM when administered in combination with conventional NSAIDs is found to be more efficacious in attenuating pain thus rendering BIRM to be used as standalone or in combination to conventional therapy. The anti-inflammatory and anti-nociceptive properties exhibited by BIRM in above animal models could be attributed to its inhibitory action on COX-2 and thereby hampering the production of PGE_2 - the major mediator of inflammation or BIRM having the possible ability of hindering the endogenous synthesis or release of inflammatory mediators such as prostaglandins, histamine, serotonin, bradykinin and leukotrienes. The latter mechanism of amelioration of pain by BRIM however, remains to be tested. BIRM seems to manifests its anti-nociceptive effect in MIA-induced OA model through inhibiting mechanical allodynia (primary and secondary) and thermal hyperalgesia. Although the exact mechanism of anti-nociceptive/anti-inflammatory action of BIRM in OA remains unknown, BIRM's inhibitory effect on COX-2 may play a crucial role in exerting its therapeutic potential in MIA-induced OA model. BIRM shows inhibition of microglia activation in the CNS and reduction in fold increase of mRNA levels of pro-inflammatory cytokines TNF-α and COX-2 in animal models of neuropathic pain. It also showed fold increase in expression of anti-inflammatory cytokine IL-10. Thus, as the name suggests, BIRM shows its action of modulating the immune system in showcasing its potential to ameliorate the pain condition. BIRM attenuates the development of hyperalgesia and allodynia in a rat model of both nerve injury-induced and disease-induced neuropathic pain. Overall, this study not only demonstrates the effectiveness of BIRM in improving pathological conditions of nerve injury induced and disease induced neuropathic pain but also showed the important role played by microglia and cytokines in regulating the induction of a chronic pain state induced by peripheral nerve injury.

Based on the results obtained from the preclinical evaluation of BIRM on relevant models of pain namely nociceptive pain, inflammatory pain and neuropathic pain, it could be summarily

deduced that the selected botanical, which is available in the market for quite some time as a general immune stimulator tonic, has all the potential to ameliorate the pain condition and possibly even reduce the progression of diabetic neuropathy when administered as a standalone therapeutic agent or more safely as adjuvant to standard therapy. Nevertheless, a follow-up regulated clinical evaluation is essential for the clinical acceptance of the present finding which however is beyond the scope of our academic ambit.