2. REVIEW OF LITERATURE

A plant containing active chemical constituents in any of its part or parts like root, stem, leaves, bark, fruit, and seed which produces a definite curing physiological response in the treatment of various ailments in humans and other animals is termed as medicinal plant. The various chemicals work together to reach equilibrium in the body as they do in plants, and so produce gentle progressive healing within the body tissues. Among plants of economic importance medicinal and aromatic plants have played an important role in alleviating human sufferings (Bacquar, 2001). Plants are used as therapeutic agents since time immemorial in Folk, Tribal, and Native form (Girach et al., 2003). The healing properties of many herbal medicines have been recognized in many ancient cultures. Early herbalists believed that the plant part resembling any part of the human body was considered useful for cure of those parts and there is no part of the body without its corresponding herb, a hypothesis known as the “Doctrine of Signature” (Bacquar, 2001). In the past few decades there has been a resurgence of interest in the study and use of medicinal plants in health care and in recognition of the medicinal plants to the health system (Lewington, 1993; Mendelsohn and Balick, 1994; Hoareau and Da Silva, 1999). This awakening has led to a sudden rise in demand for herbal medicines, followed by a belated growth in international awareness about dwindling supply of the world’s medicinal plants (Bodeker, 2002). In modern medicine, plants are used as sources of direct therapeutic agents, as models for new synthetic compounds, and as a taxonomic marker for discovery of new compounds. They serve raw material base for the elaboration of more complex semi-synthetic chemical compounds (Akerele, 1992).

According to world Health Organization report (2002), 70% of world’s population use medicinal plants for curing diseases through their traditional
practitioners. In Indian sub-continent, plant oriented drugs are used extensively and from a very long-time. According to a survey conducted by W.H.O., traditional healers treated 65% patients in Sri Lanka, 60% in Indonesia, 75% in Nepal, 85% in Myanmar, 80% in India and 90% in Bangalore. In Pakistan, 60% of the population, especially in villages get health care by traditional practitioners (Hakims), who prescribe herbal preparation (HaQ, 1983). India has more than one fourth (8000) of the world’s known medicinal plant species (30,000) of which 90% are found in forest habitats (Kumar and Katakam, 2002). Cultivation of medicinal and aromatic plants is constrained due to lack of suitable technology which has led to low yield and poor quality. Consequently medicinal herbs are predominantly harvested in sufficient quantities from the world in an unregulated manner (Shabbir et al., 2003). Also there is a growing tendency all over the world, to shift from synthetic to natural based products including medicinal and aromatic plants. So, it is the time to consider neglected and little known medicinal and aromatic plants at global as well as regional level for unfolding the medicinal cure preserved in them.

The present study was carried out on two medicinally important plants viz: *Gentiana kurroo* Royle and *Artemisia amygdalina* Decne. These two plants belonging to two different plant families were studied for anti-inflammatory potential. The research work reported earlier on these plants is mentioned as under:

*Gentiana kurroo* Royle belongs to the family Gentianaceae and is a critically endangered (CR) medicinal plant species, endemic to the north-western Himalayas. The generic name of Gentiana has been derived from 'Gentius', a king of Illyria (Europe), who is believed to have discovered medicinal value of the gentian root. In fact, the specific name of *Gentiana kurroo* Royle is from local name for the root of this plant, 'Karu" meaning bitter. The dried roots contain 20% of a yellow, transparent, and brittle
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resin (Coventry, 1927). The drug (rootstock) of this plant is administered in fevers and urinary complaints, also used as a bitter tonic, antiperiodic, expectorant, antibilious, astringent, stomachic, antihelminthic, blood purifier, and carminative (Kirtikar and Basu, 1935). The methanolic root extract of this plant contains tannins, alkaloids, saponins, cardiac glycosides, terpenes, flavonoids, phenolics, and carbohydrates. The root extract of this plant has been found to have an analgesic activity (Wani et al., 2011). The ethanolic extract of flower tops of this plant contains alkaloids, flavonoids, glycosides, free phenols, and sterols/terpenes and thus has been found to show an anti-inflammatory activity (Latif et al., 2006).

*Artemisia amygdalina* Decne is an endemic medicinal plant of Kashmir valley belonging to the family Asteraceae and grows in subalpine region of Kashmir Himalaya and North-West Frontier Province of Pakistan (Dar et al., 2006). *A. amygdalina* is a widely used medicinal plant in folk medicine for anti-helminthic, anti-diabetic and anti-inflammatory activity (Ashraf et al., 2010; Sivagnanam et al., 2012). The plant has been reported to have antioxidant potential and free radical scavenging activity (Rasool et al., 2012; Rasool et al., 2013). It also has other pharmacological actions, such as protecting liver, lowering the blood pressure, eliminating fever and sedation, and is used for curing gastrointestinal ailments (Qaisar, 2006; Sivagnanam et al., 2012). The anti-diabetic potential of *A. amygdalina* has been evaluated through experimentation (Ghazanfar et al., 2014). The hexane root fraction of this plant has been reported with potent cytotoxic activity and six cytotoxic constituents, namely, ergostadien-3-ol (1), ludartin (2), 5-hydroxy-6, 7, 3, 4-tetramethoxyflavone (3) (from shoot) and trans-matricaria ester (4), diacetylenic spiroenol ether (5), and cis-matricaria ester (6) have been isolated (Lone et al., 2013). The plant has been cultured under *in vitro* conditions for conservation of its germplasm (Mubashir et al., 2014). The active principles of this
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plant using GC-FID and GC-MS found were the terpenes, p-cymene, and 1, 8-cineole (Rather et al., 2012).

Since the literature survey finds less work documented on these plants, although a lot of work is required for complete exploration of medicinal potential of these two plants. So the current review reports the different medicinal plants (regional, national and international level) evaluated for treatment of inflammatory diseases. The experiments have been carried out using different plant parts for preparation of extracts. The studies have been carried out both under in vivo and in vitro conditions using different methods and these plant extracts have also been worked out for their active principles and mechanisms at the molecular level (pro- and anti-inflammatory molecules). To begin with the study on Lactuca scariola and Artemisia absinthium, seeds and samples of stems respectively extracted in absolute methanol were used to determine analgesic and anti-inflammatory activity. The drugs were administered orally at the doses of 300, 500 and 1000 mg/kg with acetylsalicylic acid (300 mg/kg) used as standard drug. The anti-inflammatory activity was estimated by measuring the mean increase in hind paw volume and showed that Lactuca had potent analgesic activity and Artemisia had significant analgesic and anti-inflammatory activity (Fayyaz et al., 1992). In a similar type of study on Vitex leucoxylon, the ethanolic extract of leaf was found to decrease significantly the edema formation and thus inhibit the inflammatory response in carrageenin paw edema and granuloma tissue formation in rats (Makwana et al., 1994). Iso-orientin a known C-glycosyl flavonoid was studied for anti-inflammatory potential in carrageenin animal model. The drug at 15 mg/kg and 30 mg/kg doses possessed significant anti-inflammatory activity, with no apparent acute toxicity and gastric damage (Esra et al., 2004). The aqueous suspension of dried latex of Calotropis procera (Arka) was examined for anti-inflammatory property using
carrageenin and formalin induced rat paw edema models. The aqueous suspension of this plant showed inhibition of inflammation in both the models (Kumar and Basu, 1994). The anti-inflammatory effect of _swertia chirata_ was investigated in carrageenin-induced paw edema and formalin-induced paw edema animal models. The results showed that higher dose of SC-I significantly reduced acute (57\%) and chronic (58\%) inflammatory response with the exudate volume decreased to 35\% (Shivaji et al., 2000).

The roots and leaves of _Butea frondosa_ (Palash) were evaluated for ocular anti-inflammatory activity in rabbits. The results showed reduction in intra-ocular pressure, decreased leucocytosis and mitosis and were comparable to flubiprofen gel (Mengi et al., 1995). The leaf aqueous extract of _Gymnema sylvestre_ was reported to show significant anti-inflammatory activity in carrageenin induced rat paw edema and mouse peritoneal ascitis models. Further the extract was found safe in use even at high doses as it had no effect in the integrity of gastric mucosa, thus appeared to be a less gastro-toxic anti-inflammatory agent as compared to other NSAIDS (Diwan et al., 1995). Sandhika is an ayurvedic drug used in the treatment of rheumatoid arthritis. This drug was tested against carrageenin induced paw edema and cotton pellet granuloma. The results showed significant anti-inflammatory activity and the possible mechanism postulated was free radical scavenging activity (Chaurasia et al., 1995).

In the study of _Ocimum sanctum_ (Tulsi), oil instead of plant extract was used as test drug. The triglyceride fraction of this oil offered higher protection against carrageenin induced paw edema in rats and acetic acid induced writhing in mice, as compared to the fixed oil (Singh et al., 1996). In another similar anti-inflammatory study on fatty acids of fixed oil of _Ocimum sanctum_, linolenic acid possessed significant anti-inflammatory activity against PGE2, leukotriene and arachidonic acid induced paw
oedema. The anti-inflammatory activity was due to blockade of cyclooxygenase and lipo-oxygenase pathways of arachidonic acid metabolism (Singh and Majumdar, 1997). Rat paw edema and cotton pellet granuloma models were used for studying effect of alcoholic extract of Ochna obtusata stem bark in inflammation. The observations demonstrated potent anti-inflammatory effect of the plant (Sivaprakasam et al., 1996). The ethanolic and aqueous extracts of the root of Pongamia pinnata decreased inflammation in PGE1 induced edema models by inhibition of prostaglandins. While butanolic extract was effective against carrageenin induced inflammation. The anti-inflammatory property in these extracts was due to intermediate polar constituents but not due to lipophilic or extremely polar constituents. Further petroleum ether and chloroform extract from seeds of this plant showed potent acute anti-inflammatory effect and aqueous extract showed pro-inflammatory effect (Singh and Pandey, 1996a; Singh and Pandey, 1996b). The study on anti-inflammatory effects was extended to Abies pindrow. Different animal models of inflammation such as carrageenin induced paw edema; granuloma pouch and Freund’s adjuvant arthritis were used. The leaf extracts of this plant showed anti-inflammatory effect with inhibition in the synthesis of prostaglandins and decrease in capillary permeability (Singh and Pandey, 1997). Treatment with Ease, a polyherbal formulation, reduced Freund’s adjuvant-induced non-established and established arthritis in rats. The polyherbal drug also protected protein denaturation and RBC membrane damage and exhibited significant proteinase inhibitory action in vitro, thus potentiating its possible use as an anti-arthritic drug (Chatterjee and Das, 1996). In a similar type of study on another polyherbal formulation, Jigrine, against acute and subacute inflammation, carrageenin and cotton pellet granuloma models respectively were used. The drug exhibited anti-inflammatory activity against acute inflammation but not against
subacute inflammation. The anti-inflammatory effect was due to antioxidant and membrane stabilising effect of the polyherbal drug (Karunakar et al., 1997).

The effect against subacute inflammation was observed in methanolic extracts of flowers of Michelia champaca Linn. (Champaka), Ixora brachiata Roxb (Rasna) and Rhynchosia cana Willd. Cotton pellet induced subacute inflammatory model results inferred that methanolic extracts from latter two plants showed higher activity as compared to Michelia champaca. The extracts had significant inhibitory effect on biochemical parameters like protein content, acid phosphatase, SGPT and SGOT activities in liver and serum. These properties were attributed to the presence of flavonoids in the flower extracts of these plants (Vimala et al., 1997). In a similar study on methanolic extract of aerial parts of Sida rhombifolia (Atibala), significant suppression of edema formation was observed in the carrageenin induced paw edema model. The anti-inflammatory activity of this plant was attributed to inhibition in release of mediators of inflammation such as histamine, 5-hydroxytryptamine, bradykinin etc. (Rao and Mishra, 1997). The biochemical modes of action of Gmelina asiatica root powder were observed in carrageenin and cotton pellet granuloma models. The root powder was effective in reducing edema in acute inflammation. But in chronic inflammation, it not only reduced weight of granuloma but also lipid peroxide content of granuloma exudate and liver. There was decrease in gamma-glutamyl transpeptidase in the granuloma while serum albumin and serum acid and alkaline phosphatase levels remained normal (Ismail et al., 1997). The study of various biochemical parameters in cotton pellet exudate obtained from cotton pellet granuloma assay in rats showed decreased concentration of lipid peroxide, acid phosphatase and alkaline phosphatase. The drug used for this study was water soluble part of alcoholic extract of Azadirachta indica. Such type of study suggested the role of these biochemical parameters in
inflammation (Chattopadhyay, 1998). The anti-inflammatory and chronic toxicity effects of leaf extract of Ageratum conyzoides were observed in vivo. Although these animals were treated with daily doses of 250 or 500 mg/kg extract for 90 days, but the treatment related abnormalities were not seen in biochemical or haematological parameters towards toxicity (Moura et al., 2005). Anti-inflammatory study was carried out on alcoholic extract of roots of Clerodendron serratum against the carrageenin induced paw edema and cotton pellet granuloma models. The extract had significant effect in the inhibition of inflammation in both models (Narayanan et al., 1999). Again carrageenin and 5-hydroxytryptamine induced rat paw edema models were used for elucidating the anti-inflammatory effect of methanolic extract of Nelumbo nucifera rhizome as well as the steroidal triterpenoid (betulinic acid) isolated from it. The results had significant anti-inflammatory potential which was almost comparable to that of phenylbutazone and dexamethasone (Mukherjee et al., 1997).

Odontuya along with his colleagues studied structure-activity relationship of luteolin and its derived glycosides for anti-inflammatory potential and demonstrated positive relationship between the reactivity of luteolin and its related glycosides with their molecular structures against arachidonic acid synthesis and hydrogen peroxide scavenging. The presence of functional groups like ortho-dihydroxy in B ring and -OH substitution at C-5 position of A ring significantly contributed to the anti-inflammatory and antioxidant activities of these flavonoids (Odontuya et al., 2005). Similarly, the crude water and ethanolic extracts from five herbal remedies were investigated for their ability to inhibit COX-1 and COX-2 catalysed prostaglandin biosynthesis. COX-1 catalysed prostaglandin biosynthesis was inhibited actively by ethanolic extracts and only ethanolic extract of Epilobium parviflorum inhibited both COX-1 and -2 catalysed prostaglandin biosynthesis (Steenkamp et al., 2006).
Yucca schidigera being an important medicinal plant is a rich source of polyphenolics. These phenolics have anti-inflammatory activity. These polyphenolics include resveratrol and a number of other stilbenes (yuccaols A, B, C, D and E). Yucca phenolics aid in suppressing reactive oxygen species which stimulate inflammatory responses and thus also act as anti-oxidants and free-radical scavengers (Cheeke et al., 2006).

The carrageenin-induced edema model was used for evaluating anti-inflammatory properties of defatted methanolic extract of Dioscorea esculenta tuber. Different doses were tested in the range of 100-200mg/kg bw of rats. The extract showed dose dependent inhibition of edema formation with significant initial effect after 1 h and 2 h at doses of 100 mg/kg and 150 mg/kg, respectively. The reference drug used in this study was acetylsalicylic acid (150mg/kg bw) (Olayemi and Ajaiyeoba, 2007). In other similar type of study bark of Xeromphis spinosa was extracted with petroleum ether, ethyl acetate and methanol in equal proportions. These extracts were used orally at doses of 200 and 400 mg/ kg body weight in carrageenin induced paw edema model and were found to exhibit anti-inflammatory activity by inhibiting the edema formation significantly as compared to control (Biswa et al., 2009). Again the same carrageenin-induced paw edema model was used for study of inflammation. Here ethanolic extract of leaves of Mitragyna parvifolia (MPEE) was used at various dose levels and showed maximum anti-inflammatory effect at 300 mg/kg and this effect was equivalent to phenylbutazone (PBZ) (80 mg/kg, orally; p<0.05) used as standard drug (Vikas et al., 2009). Many other studies have been carried out on different plant extracts for their role in inflammation using same carrageenin model, some of them include the petroleum ether, ethyl acetate and methanolic extracts of bark of Albizia lebbeck Benth. were found to inhibit the paw edema volume after 4 h of carrageenin
injection by 36.68% at 400 mg/kg dose level (Achinto and Muniruddin, 2009). In
other study, the ethanolic extract of male flowers (inflorescences) of Borassus
flabellifer were administered orally at doses of 150 and 300 mg/kg bw. The extract
produced anti-inflammatory effect in both acute as well as chronic (cotton pellet
granuloma) inflammation in a dose dependent manner (Mahesh et al., 2009). Further
the study on methanolic extract of berries of Solanum nigrum Linn. at a dose of 375
mg/kg bw revealed significant decrease in acute inflammatory response (Ravi et al.,
2009). The seed extracts of Cordia dichotoma forst prepared in ethanol and water at oral
doses of 250 mg/kg and 500 mg/kg produced inhibitory effects against acute
inflammation. Diclofenac sodium (10mg/kg bw) was used as the standard drug. The
results revealed anti-inflammatory potential of this plant (Sharma et al., 2010).
Different fractions of Pterospermum acerifolium were evaluated for anti-inflammatory
activity by using in-vitro and in vivo models i.e., thermally induced protein denaturation
and carrageenan induced inflammation respectively. Out of the different fractions ethyl
acetate fraction showed significant anti-inflammatory activity in both in-vivo and in-
vitro models. Further these extracts were observed to have antioxidant potential with
ethyl acetate fraction again showing maximum effect and hence the role against
inflammation (Sannigrahi et al., 2010). Preliminary screening of fruits of Coriandrum
sativum, leaves of Datura stramonium and Azadirachta indica for anti-inflammatory
activity was carried out in albino rats using carrageenin induced rat paw edema model.
All extracts were prepared in ethanol with diclofenac sodium as the standard drug. The
extracts of all these plants exhibited significant anti-inflammatory activity. However
after every hour this effect was seen to be maximum in the leaves of Azadirachta indica
(Sonika et al., 2010).
Again, in the study of *Kigelia pinnata* for anti-inflammatory activity carrageenin-induced paw edema and cotton pellet-induced granuloma methods were used. The leaf extract was given at a dose of 200mg/kg & 400mg/kg and standard drug indomethacin at a dose of 10mg/kg i.p. Both doses of leaf extract showed decrease in paw edema and reduction in the weight of cotton pellet granuloma of Wistar rats in carrageenin induced and cotton pellet induced granuloma models respectively, in a dose dependent manner (Namita et al., 2012). Four fractions of *Vetiveria zizaniodes* were prepared in n-hexane, chloroform, ethyl acetate and butanol. These fractions were tested for anti-inflammatory activity at a dose of 200mg/kg using carrageenin induced paw edema and cotton pellet induced granuloma rat models. The maximum anti-inflammatory potential was shown by ethyl acetate and chloroform fractions in both models. This activity was further substantiated by biochemical findings, catalase & GSH levels in blood and also free radical suppression. It was concluded that anti-inflammatory potential of this plant was due to its anti-oxidant effect (Kamble et al., 2013). The anti-inflammatory activity of ethanolic extract obtained from roots of *K. reticulata* was checked by carrageenin induced paw edema in Wistar rats. The extract was used orally at dose levels of 200 and 300mg/kg. The ethanolic extract showed significant anti-inflammatory activity at 300mg/kg dose level as compared to control (Soni et al., 2014). The anti-inflammatory potential of two plants namely- *Moringa oleifera* and *Ocimum gratissimum* was investigated by carrageenin induced hind paw edema method. For this study leaves of both plants were taken and extracts were prepared separately in methanol and water. The extracts of *Moringa oleifera* showed maximum reduction in edema formation at oral doses of 500mg/kg bw with methanolic extract showing higher activity than the aqueous extract. Similar results were reported in *Ocimum gratissimum*, where again the methanolic extract had higher effect than
aqueous extract at oral administration of 100 mg/kg bw. The overall study concluded that *Ocimum gratissimum* had higher anti-inflammatory potential than *Moringa oleifera*, as the anti-inflammatory effect produced by the latter plant at the dose of 500mg/kg bw was produced by former plant at only 100mg/kg bw. In both experiments diclofenac sodium at 100 mg/kg bw had been used as the standard (*Yadav and Shah, 2014*).

Similarly, *Cynodon dactylon* Linn. was evaluated for anti-inflammatory activity in carrageenin induced paw edema model. The hydroalcoholic extract of whole plant at two different doses 200 mg/kg and 400 mg/kg with some flavonoids i.e. quercertin, kaempferol and epicatechin each at dose of 100mg/kg and diclofenac sodium at dose of 12.5 mg/kg as standard drug were administered to rats. The results showed that plant extracts at these respective doses had higher anti-inflammatory potential than the flavonoids, thus signifying the potential of this plant against the inflammatory disorders (*Amit and Rana, 2014*). Again, whole plant of *Asystasia travancorica* was screened for anti-inflammatory activity using carrageenin paw edema in albino rats. The whole plant material was extracted with ethanol. The ethanolic extract exhibited significant anti-inflammatory activity at a dose of 500 mg/kg after 3hr oral dose administration (*Komalavalli et al., 2014*). The methanolic extract of flowers of *Salvia officinalis* Linn. (MESO) also showed maximum edema inhibition at 3rd hour post carrageenin injection in carrageenin induced rat paw edema animal model. MESO was given in different doses to the test groups and edema inhibition was found to be dose dependent. Further the extract was also reported to show antioxidant potential (*Shamnas et al., 2014*).

In other type of study, hexane, methanolic and aqueous whole plant extracts of *Phyllanthus amarus* were investigated for their inhibitory role on pro-inflammatory cytokines and NO production from different cell lines. The extracts (100µg/ml) had
significant (p<0.001) effect on LPS stimulated mouse macrophage cells and RAW 264.7 against the production of different pro-inflammatory cytokines, interleukin 1β (IL-1β), tumor necrosis factor α (TNF-α) and nitric oxide (NO). Further interleukin 2 (IL-2) production in EL 4 lymphoma cells stimulated with 4µg/ml of Concavalin A (Con-A) was also found to be inhibited (p<0.001) with addition of extracts (Kanwar and Bhutani, 2007). Again, the study on pro-inflammatory and anti-inflammatory mediators was carried out for assessment of anti-inflammatory potential of M. suaveolens Ledeb. The ethanolic extract of this plant lead to down-regulation of pro-inflammatory mediators such as TNF-α, IL-1β, COX-2 and iNOS gene expression through suppression of NF-κB activation. While on the other hand, the ethanolic extract increased anti-inflammatory molecules HO-1 and IL-10. The basis for anti-inflammatory potential of this plant was presence of coumarin in this extract as observed from HPLC fingerprint (Anshu et al., 2012). In a similar study on RAW 264.7 macrophages challenged with LPS, the effect of aqueous extract of A. millefolium L. inflorescences on pro-inflammatory molecules was observed. The extract (25 - 300µg/ml) showed significant effect in the inhibition of LPS induced NO production without any change in cell viability. The extract had dose dependent effect on NO production which could be correlated with the down regulation of iNOS protein expression. Further the extract was found to be ineffective against the PGE2 synthesis and protein COX-2 levels (David et al., 2010). Similar anti-inflammatory study was carried out using methanolic extract of I. obscura (10 mg/kg bw). Inflammation was induced in mice by carrageenin, dextran, and formalin. The methanolic extract was administered intraperitonially in mice before inducing inflammation. The extract showed significant inhibition in paw edema of animals in all the inflammatory models with maximum effect in formalin induced model. This extract also showed potent inhibition of lipopolysaccharide (LPS)-induced
pro-inflammatory molecules in peritoneal macrophages like NO, CRP and TNF-α (Hamsa and Girija, 2011). *In vitro* anti-inflammatory study was carried out on *Tecoma stans* extracts prepared in ethanol, methanol and water. The extracts were found efficient for anti-inflammatory action as they inhibited the heat induced albumin denaturation and heat induced hemolysis and thus helped in red blood cells membrane stabilization. This activity was observed to be higher in the order of water, ethanol and methanol extracts respectively (Govindappa et al., 2011). Similar type of study was carried out on HP-4 which is a herbal preparation from *Aloe vera*, *Bacopa monnieri*, *Moringa oleifera* and rhizome of *Zingiber officinale* in 80% alcohol. The anti-inflammatory activities were observed under *in-vitro* conditions on RBC’s membrane stabilization and protein denaturation activity caused by hypotonic solutions. The standard drug acetylsalicylic acid had anti-inflammatory effect almost similar to that of control. It was found that HP-4 caused dose dependent inhibition of protein denaturation activity and RBC’s membrane stabilization (Padmanabhan and Jangle, 2012). The different extracts of roots of *Berberis aristata* prepared in hexane, chloroform, ethanol and water were screened for anti-inflammatory potential using *in vitro* procedures viz. HRBC membrane stabilization, inhibition of albumin denaturation and inhibition of heat induced hemolysis and *in vivo* carrageenin induced paw edema model. The results showed increased anti-inflammatory potential of polar extracts than non-polar extracts in dose dependent manner. Ethanolic extract followed by aqueous extract showed maximum activity by inhibiting paw edema and also hypotonicity induced lysis of erythrocyte membrane and thus significant membrane stabilization. Berberine was isolated and confirmed by High-performance liquid chromatography (HPLC) and FT-IR spectra results in potent ethanolic extract (Gupta et al., 2014).
The flavonoids were isolated from *Artemisia herba alba* and were added in different doses to cell culture containing peripheral blood mononuclear cells isolated from Algerian ABD patients and healthy controls. The supernatants collected were investigated for the presence of cytokines and NO produced by means of ELISA assays and Griess modified method respectively. The flavonoids significantly reduced production of key effector of T helper 1 (Th1) cells i.e., IL-12, and NO in a dose-dependent manner in ABD patients, while the key marker of Th2 cells which is IL-4 was produced in higher concentration (*Messaoudene et al., 2011*). The anti-inflammatory effects were reported in citrus peel extracts against LPS-induced macrophage cells. The cells treated with these extracts showed slightly decreased expression of iNOS, COX-2 and about 40% inhibition in NO production. However the extract had not shown any concentration dependent change in these effects. Further the extracts also inhibited the production of pro-inflammatory cytokines with IL-6 mostly inhibited (44%) followed by TNF-α (13%) and no inhibition in IL 1 (*Kang, 2014*). The *in vitro* study was carried out on lipopolysaccharide (LPS) stimulated J774 murine macrophage cell lines for the production of pro-inflammatory mediators using *Withania somnifera* aqueous fraction (WSAF). The findings indicated a dose dependent inhibition in the production of tumor necrosis factor (TNF-α), interleukin 1 (IL 1) and nitric oxide (NO) in LPS induced murine macrophage cell lines (*Srinivasulu, 2014*). The *in vitro* studies were carried out on leaf ethanolic extract prepared from *Dictamnus dasycarpus* using LPS-stimulated RAW 264.7 cells. The extract was used at the doses of 0.5 and 1 mg/mL and production of cytokines, transcription factor NF-κB and different enzymes involved in promotion of inflammation were compared between the treated and untreated groups in LPS activated macrophages. The results showed suppression in NO production, decrease in pro-inflammatory cytokines viz. IL 1β and TNF-α, down-
regulation in expression of transcription factor NF-κB and decreased synthesis of pro-inflammatory enzymes, iNOS and COX-2 due to their attenuation in treated groups as compared to untreated groups in LPS activated macrophages. Further these effects were shown to be dose dependent (Ghosh et al., 2014). In a similar study on root bark of Lycium chinense, Lycii radicis cortex water extract at the dose range of 10 - 200μg/mL was evaluated for its effect on inflammatory mediators in LPS-activated RAW 264.7 mouse macrophages. The extract was incubated for 24 h with RAW 264.7 cell culture and was found to increase cell viability, inhibit the production of granulocyte colony stimulating factor (G-CSF), NO, TNF-α, platelet derived growth factor-BB, IL-10 and (IL)-2 in LPS-activated RAW 264.7. Further the production of granulocyte macrophage colony-stimulating factor (GM-CSF) and LPS-induced CXC chemokine (LIX) was also diminished (Park, 2014).

In a different type of study, the ethanolic extract of green soybeans was irradiated with visible light. This irradiated extract was used in LPS treated human monocyte THP-1 cells. The findings indicated suppression of IL-6, IL-12 and TNF-α expression in these cells in a dose dependent manner (Tanaka et al., 2014). In vivo and in vitro anti-inflammatory studies were done on Siegesbeckia orientalis ethanol extract. In cell culture experiments, LPS stimulated RAW264.7 cells pre-treated with ethanol extract showed reduction in the production of NO, IL-6, and TNF-α. Furthermore, the extract inhibited LPS-induced NF-κB activation by blocking degradation of IκB-α. Also in vivo studies demonstrated decreased edema formation and less concentration of IL-6 in the serum of treated mice (Hong et al., 2014). The anti-inflammatory study was carried out in green fruit methanolic extract of S. integrifolium. The reported results indicated inhibition in cytotoxicity and LPS-mediated NO release in RAW264.7 macrophages. Further, significant down-regulation occurred in expression of LPS
induced pro-inflammatory genes, like iNOS, COX-2, IL-1β and IL-6 (Wang et al., 2014).

The Freund’s adjuvant-induced and collagen-induced arthritic rat models were used for the study of anti-arthritic potential of ethanolic extract of Justicia gendarussa. The results of study showed significant anti-arthritic activity statistically similar to that of aspirin which was used as standard drug (Paval et al., 2009). Euphorbia prostrata (EPA) was investigated for anti-inflammatory and anti-arthritic potential employing in vivo models - carrageenin induced inflammation and Mycobacterium adjuvant induced chronic inflammation respectively. Results of in vivo studies revealed its highly significant anti-inflammatory/anti-arthritic activity with the γ-Glutamyl transpeptidase (γ-GT) concentration reduced at the site of inflammation (Singh et al., 2011). Intragastric administration of multi-drug herbomineral formulation (100 mg/kg) and dexamethasone (2 mg/kg) was carried out in CFA induced arthritic model. The treatment was continued for 21 days and physical, biochemical, and haematological parameters were estimated. The herbomineral formulation showed anti-arthritic activity with improvement in physical parameters like paw thickness, paw edema, arthritic index. In biochemical tests reduction in inflammatory markers like serum rheumatoid factor, C-reactive protein, and erythrocyte sedimentation rate was also observed. In haematological parameters hemoglobin (%) increase, decrease in splenomegaly and improvement in thymus index were reported. Further the anti-arthritic potential of this formulation was proved by histopathological examination with the destruction of joint space, ameliorative effect of formulation in hyperplasia of synovium, and pannus formation observed (Patel and Shah, 2013).

Anti-arthritic potential was evaluated in the fractions of hydroalcoholic leaf extract of Plumeria alba L. The ethyl acetate and n-butanol fractions (100 and
200mg/kg bw) were tested against formaldehyde and complete Freund’s adjuvant (CFA) induced arthritis. At both doses, the extracts caused a significant reduction in paw swelling in both models. ESR and spleen weight decreased in arthritic rats treated with extracts. In addition to this ethyl acetate fraction also showed improvement in thymus weight of arthritic rats. While in standard group treated with diclofenac Hb level also improved. The behavioural symptoms like motor incoordination and nociceptive threshold in treated groups were also improved (Choudhary et al., 2014). The ethanolic leaf extract of C. caudata was given to arthritic rats of CFA model for 28 days. The doses were administered orally at 200 and 400 mg/kg bw with dexamethasone (1mg/kg, i.p) as standard drug. The extract showed gradual decrease in paw volumes and change in body weight recorded on 7, 14, 21 and 28 day. Analysis of blood samples collected from treated groups after 28 days showed increased RBC’s count and Hb level. Furthermore, in animals where the dose given was 400mg/kg bw showed decrease in WBC’s, serum rheumatic factor (SRF), C-reactive protein (CRP) and ESR as compared to untreated arthritic rats (Pashikanti et al., 2014). Different acute and chronic inflammatory models were used for the study of n-butanolic extract of I. stolonifera (BE-IS). The oral administration of BE-IS significantly decreased carrageenan-induced edema and exudate formation along with cellular migration in acute models. In cotton pellet model the granuloma formation was inhibited and in CFA model also improvement was observed after treatment of these animals by BE-IS. The preliminary mechanistic work demonstrated decrease in the levels of myeloperoxidase (MPO) and malondialdehyde (MDA), increased activity of anti-oxidant enzyme superoxide dismutase (SOD) in BE-IS treated animals in vivo. This extract when used in LPS-activated RAW264.7 macrophages in vitro reduced the production of nitric oxide (NO),
prostaglandin E2 (PGE2), tumor necrosis factor-α (TNF-α), interleukin (IL)-1β and IL-6 in them (Cai et al., 2014).

From the above review it is seen that a number of plants have been investigated for their role in inflammatory disorders. Animal in vivo models have been used for the study of plants against acute and chronic conditions of inflammation. The activities shown by different plants differ on the basis of plant part/s used. The mechanisms underlying the activities shown by these plants have been elucidated and it has been found that immune system has been the major target. The herbal medicines have been evaluated in cell culture under in vitro conditions also and inter-relationship with the effects produced in vivo have proved very useful for understanding the mechanism adopted by these herbal drugs for curing of diseases. Lastly, the importance in use of these herbal drugs is that they are safer and have fewer side effects as seen in the above given review. Nature keeps the remedy for every disease, the need of the hour is to search and screen natural resource and explore better ways and forms in which these herbal drugs can be used. This type of study gives us insight about the analysis of traditional drugs for their mechanistic actions and also isolation of principle active component/s from them, which may thus help in increasing the efficacy of drug/s and alleviation of noxious responses.