6. DISCUSSION

In the present study, pharmacognostical, phytochemical and pharmacological studies have been carried out to establish the authenticity of the plant based on morphological, physicochemical parameters.

Based on the ethnobotanical uses of *C. hirsutus* (Kirtikar, 1996, Chopra, 1958, Chatterjee, 1996), *B. prionitis* (Nadkarni 1994, Bhalla *et al.*1992), and *B. amarissima* (Ong *et al.*, 2004), these plants were selected for carrying out this study with the help of different animal models for treatment of inflammation, arthritis, diabetes and cancer.

**Pharmacognostical study**

To ensure the quality of plant material, the macroscopic and microscopic study of medicinal plant is the first step towards establishing it identity and purity. It revealed that the characteristics of various parts were identical to those reported earlier by Kirtikar & Basu (1981) and Wealth of India (1950).

In physiochemical studies of various parameters established, like various ashes content which showed the presence of inorganic salts by naturally occurring or adhering to it, or deliberately added to it as a form of adulteration (Khandelwal, 2003). These values are important quantitative standards. Results of extraction of MECH, MEBP and MEBA indicated extractive values were found to be 5.92, 11.16 and 23.43% respectively.

Qualitative chemical examination of various extracts of powder drug indicated the presence of alkaloids, carbohydrates, saponins, glycosides, fixed oils, fats, phytosterols, flavanoids. Alkaloids were detected by dragondroff’s reagent (Khandelwal, 2003; Geissman, 1955), phytosterols and triterpenoids were detected by libermann burchard test and salkowski reaction (Harborne, 1991, Ravishankar et al., 2002), carbohydrates by molisch’s test, fehling’s reagents and benedict’s test, (Gupta, 2005), saponin by foam test (Harborne, 1991, Ravishankar et al., 2002), flavonoids by shinoda test and Fluorescence test (Geissman et al., 1955), anthraquinones by borntrager’s test and Modified borntrager’s test Kokate, 1999,
Oguyeme et al., 1979) and phenolics by methanolic FeCl₃ and Lead acetate test (Oguyeme et al., 1979).

The phytochemical analysis of MEBP indicated the presence of alkaloids, glycosides, flavonoids, tannins terpenoids, steroids and phenolic compounds whereas MEBA and MECH was found to exhibit presence of flavonoids, carbohydrates and saponins. Jensen et al., (2007) reported the presence of irridoid glycosides in B. prionitis. Earlier investigation shows the presence of flavonoids, tannins, saponins in B. prionitis plant extracts (Chavan et al., 2010).

**Pharmacological activity**

**Antioxidant activity**

Antioxidants are a class of vitamins and nutritional ingredients that help to attenuate effects of free radicals that can cause damage to human body. Some antioxidants like BHT may be carcinogenic therefore natural antioxidants have gained importance. Antioxidants inhibit or prevent oxidation of substrates and evolve to protect cells against the damaging effects of reactive oxygen species (ROS), such as singlet oxygen, superoxide, hydroxy radical etc. An imbalance between antioxidants and ROS results in oxidative stress, which leads the cellular damage (Gulcin, 2010; Gulcin et al., 2010).

DPPH is stable free radical at room temperature and accepts an electron on hydrogen radical to become stable diamagnetic molecule. In the present study, MECH, MEBP and MEBA showed significant antioxidant activity compare to ascorbic acid by DPPH radical scavenging method and super oxide radical scavenging method.

The high content of phenolic compounds is known to have direct antioxidant property due to presence of hydroxyl groups which can function as hydrogen donor (Jayprakkash et al., 2001). Polyphenolics display important role in stabilizing lipid oxidation that associated with its antioxidant activity (Gulcin et al., 2003a). Flavonoids and phenolic compounds, alkaloids, saponins and triterpenoids are reported to possess antioxidant activity. Eg. *Nelumbo nucifera* seeds (Sujay et al., 2006). It is reasonable to expect that the presence of high antioxidant potential in
these plants to reduce free radicals in the body. In addition to, the presence of flavonoids, alkaloids and triterpenoids in alcoholic extract of *Alstonia scholaris* has been reported (khan et al., 2003)

**Acute toxicity study**

In acute toxicity studies the plant extracts did not produce any significant changes in the autonomic responses at the dose levels studied. Extracts did not exhibit any adverse effect up to 14 days which may be due its composite nature where the presence of phyto constituents could counteract its toxicity (Saurabh et al., 2009). Based on the results of this study, doses of different plant extracts were selected for various animal models.

**Screening of anticancer activity**

*Micronucleus formation in mouse bone marrow cells*

The present study revealed the anti-cancer potential of *B. Amarissima* extract in dose dependent manner. Earlier studies carried out by several researchers on the properties of *Solanum* constituents. A number of species of the genus *Solanum* have been shown to contain steroidal glycoalkaloids and steroidal saponins have significant cytotoxic and antitumour activities (Silva et al., 2007). Thus the cytotoxic activity of MEBA may be attributed, at least partially, to steroidal alkaloid and steroidal saponin substances.

The genotoxic nature of any drug can also be determined on the basis of presence of phytoconstituents. MEBA contains flavonoids which have been shown to possess anticarcinogenic activity (Sammour et al., 1992, Chattopadhyay et al., 2007, Hirano et al., 1989). The underlying mechanism behind anti-cancer activity of MEBA is still unknown.

**Ehrlich Ascites induced carcinoma in mice**

Earlier investigations carried out on in vitro antiproliferative Activity of Brucea javanica Leaves Extract indicated that the brucea amarissima L. possess anti-tumour activity. Therefore, in our present study the anti tumour activity of MEBA extract (suh et al., 1995).
The results showed that the MEBA at dose of 200mg/kg can inhibit cell growth of tumor bearing mice satisfactorily, reduce tumor growth markedly and increase life span. These parameters are important in justifying the potency of a compound in cancer chemotherapy (Hogland et al., 1982) but in cancer chemotherapy the major problems are myelosuppression and anemia (Maseki et al., 1981).

Cyclophosphamide, which is an indirect alkylating agent well-known for its genotoxic properties, was used as a positive control for the Ehrlich ascites and micro nucleus bone marrow test (Lajmanovich et al. 2005).

In this study, the extracts by a direct anticancer effect and by arresting the tumor growth, increased the life span of EAC-bearing mice. The anemia encountered in tumor bearing mice is mainly due to reduction in RBC or hemoglobin percentage and this may occur either due to iron deficiency or due to hemolytic or myelopathic conditions (Fenninger and Mider, 1954). Similar results were observed in the present study in animals of the EAC tumor control group, standard treatment and other test extracts (MECH and MEBP).

The reversal of hematological parameters indicates that the extracts may possess protective action on the hematopoietic system. This reinstates that use of herbs might be a more effective strategy in the treatment of cancer.

Flavonoids have been shown to possess antimalignant and antimutagenic effects (Brown et al, 1980., Hirano et al., 1989). Flavonoids have chemopreventive role in cancer through their effects on signal transduction in cell proliferation and angiogenesis (Weber et al., 1996, Fotsis et al., 1997). The anticancer properties of the MEBA may be due to these compounds.

**Screening of antiarthritic activity** *Freund’s adjuvant induced poly arthritis activity*

Rheumatoid arthritis is an autoimmune disease that causes chronic inflammation of the joints and characterised by progressive joint destruction, deformity, disability and premature death in most patients. Recent studies have revealed the key roles of pro inflammatory cytokines, such as tumor necrosis factor TNF—α, Interleukin 1b(IL-1b), IL-6 and IL-8 in the pathogenesis of rheumatoid arthritis (Cai et al., 2005).
In the present study MECH significantly reduced the mean percentage change in paw swelling at 21st day evaluation and the percentage protection was 58.94 and 68.40 and in a dose dependent manner at 100 and 200 mg/kg, respectively. However, the standard drug dexamethasone exhibited 71.90% ($P < 0.001$) protection as compared with the control group.

In the histopathological study when treatment was given with MECH, it showed mild vascular proliferation with focal area of mononuclear inflammation as compared to other extract. Therefore from histopathology it can be concluded that MECH and MEBP were more potent than MEBA.

**Proteoglycan induced polyarthritis in in mice**

The aetiology of rheumatoid arthritis is showed indications of the autoimmune nature of the disease but precise pathogenic mechanisms leading to the destruction of articular cartilage and bone remain unknown. Previous Studies (Glant et al., 1987) have shown valuable insights into potential mechanisms for joint disease following immunisation with exogenous and endogenous antigens. The proteoglycan induced arthritis in BALB/c mice shows many similarities to rheumatoid arthritis (Mikecz et al., 1987). MECH and MEBP showed significant activity as compared to MEBA.

**Screening of antidiabetic activity**

**Alloxan induced antidiabetic activity**

Alloxan is reported to selectively destroy insulin secretory β-cells to impair insulin secretion and function (Lenzen et al., 2008). In this study, continuous treatment with MECH, MEBP and MEBA caused significant decrease in blood glucose level (BGL) of treated rats compared to untreated diabetic rats. The observed reduction in BGL of the diabetic rats by glibenclamide portrays an insinere state of diabetes (Rajkumar et.al., 1991).

Phytochemical compounds like phenols, saponins, tannins, alkaloids, steroids, cardiac glycosides and terpenes present in this extract have been reported to exert antilipidemic activity (Tandon et. al., 2005; Bnouham et al., 2006; Kumar et al., 2011, Tiwari and Rao, 2002).
Diabetes is also associated with altered lipid levels. The most commonly observed lipid abnormalities in diabetes are hypertriglyceridemia and hypercholesterolemia (Shepherd et al., 2005; Shirwarkar et al., 2006) and contribute to coronary artery disease (Arvind et al., 2002).

In diabetic rats there was a significant increase in total cholesterol and triglycerides (P<0.05). In MECH, MEBP and MEBA treated rats there was a reduction in total cholesterol and triglycerides which showed the hypolipidemic effect of this plant.

The hypolipidemic effect may be due to inhibition of fatty acid synthesis (Kumar et al., 2011; Chi et al., 1982). The repeated administration of test extracts for a period of 21 days resulted in a significant improvement in lipid parameter levels when compared to the diabetic control.

**Streptozotocin (STZ) induced diabetes in rats**

STZ is a nitrosourea compound produced by Streptomyces achromogenes, which specifically induces DNA strand breakage in β-cells causing diabetes mellitus. This leads to insulin deficiency which in turn increases the blood sugar level. In our study, MECH, MEBP and MEBA extract significantly reduced the hyperglycemia caused by STZ.

In the study, normal healthy animals were found to be stable in their body weight whereas diabetic animals showed reduction in body weight. Decrease in weight in diabetes was due to the increased muscle wasting and loss of tissue protein. (Rangachari et al., 2012; Kumar et al., 2011; Swanston-Flat et al., 1990). Decrease in the weight in diabetes is also due to continuous excretion of glucose and decrease in peripheral uptake of glucose and glycogen synthesis. The reduction of body weight was diminished by extracts treatment and proved them significant in diabetes (Defronzo et al., 1992).

**Screening of anti-inflammatory and analgesic activity**

**Hot plate method**

The plant extracts showed significant analgesic activity in the Hot Plate test. With reference to previous Studies analgesic effect produced by the extract may be via
peripheral mechanisms involved in the inhibition of prostaglandins, leukotrienes and other endogenous substances that are key players in pain and inflammation (Pasero et al., 1999).

**Carageenan induced Anti inflammatory activity**

Carrageenan has been widely used as an inflammmagen capable to induce experimental inflammation used for the screening of compounds possessing anti-inflammatory activity. It induces an inflammatory reaction in two different phases. The initial phase has been attributed to the release of histamine, serotonin and bradykinin on vascular permeability (Vinegar et al., 1969) and the later phase has been due to over production of prostaglandin in tissues (Di Rosa, 1974).

In anti-inflammatory activity carageenan shows biphasic increase in paw volume due to release of diff mediators. Maximum anti-inflammatory activity had shown in dose 200 mg/kg of MECH at 6 hours which was comparable with standard diclofenac sodium and also inhibition was dose dependent manner. Anti-inflammatory activity of MECH, MEBP and MEBA may be due to high Steroids content of the plants. Telang et al. had also demonstrated the anti-inflammatory activity of *Vitex negundo in mice.* (Telang et al., 1999).

Several flavonoids isolated from medicinal plants have been discovered to possess significant anti-inflammatory and analgesic effects (Gulnur et al., 2004; Bujbal et al., 2008). The anti-inflammatory and analgesic activities of kigelia may be due to the presence of flavonoidal compounds present in Kigelia pinnata flower (Scogin, 1980). The mechanism and the bioactive principles responsible for these actions remain to be explained. Flavonoids are present in MECH, MEBP and MEBA therefore it may posses anti-inflammatory activity.

**Acetic acid induced writhing test**

Acetic acid acts indirectly by inducing the release of endogenous mediators which stimulate the nociceptive neurons sensitive to non-steroidal anti-inflammatory drugs and opiods. In this studies acetic acid writhing test was used because of its sensitivity that could provide different grades of noxious stimuli in chemically induced
tissue damage and the response is thought to be mediated by peritoneal mast cells, acid sensing ion channels and the prostaglandin pathways (Ranjit et al., 2006).

The acetic acid induced writhing allows the acid to act via central mechanisms and motor performance of the animal (Hossein et al., 2003; Chakraborty et al., 2004).

Diclofenac sodium (10mg/kg IP), a standard NSAID used as positive control, also produced significant inhibition of acetic acid-induced writhing response. Moreover, oral administration of MECH, MEBP and MEBA, 1 hour before the acid injection produced a significant ($P < 0.05$) inhibition of acetic acid induced abdominal constrictions in mice (Hunskaar and Hole, 1987).

**Screening of anti-hypertensive activity**

**DOCA salt induced Antihypertensive activity:**

Mineralocorticoid causes retention of salt and water as it has an aldosterone mimetic property. The uninephrectomized rat, treated with DOCA and salt water, develops severe hypertension, which is also known as salt sensitive high blood pressure (Rocha et al., 2001).

Increased concentrations of aldosterone lead to increased reabsorption of sodium ions and water from epithelial cells in the distal nephron of the kidney, thereby influencing blood pressure levels. In agreement with previous reports (Veeramani et al., 2011; Mink et al., 2007; Jalili et al., 2006) saponins, flavonoids and triterpene were shown to reduce hypertension in experimental animal models. Several studies have suggested that high intake of flavonoids decrease the risk of coronary heart diseases (Mink et al., 2007).

In the present study, SBP and DBP were increased persistently in DOCA salt treated nephrectomised rats as compared to normal rats in tail-cuff method; Due to above mechanism of DOCA salt, angiotensin converting enzyme (ACE) inhibitor- enalapril was used as standard (Vogel GH and Vogel WH, 1997). Results data were suggested that blood pressure (i.e. SBP and DBP) were decreased in standard group and in plant extract as compared to disease control group. Systolic and diastolic blood pressures were considerably increased in DOCA-salt hypertensive
rats might be due to increased oxidative stress and decreasing the bioavailability of nitric oxide (Jalili et al., 2006).

**Pentylenetetrazol induced convulsion test in rats**

PTZ, an agent widely reported to induce convulsion by inhibition and attenuation of GABAergic neurotransmission (Katzung et al., 2004). Hence, this accentuates the excitatory neurons to release neurotransmitters such as α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPA) and N-Methyl-D-aspartic acid (NMDA). This leads to neuronal excitotoxicity leading to convulsions (Yoo et al., 2007).

Experimental evidences clearly demonstrated that flavonoids exerts antiepileptic activity by modulating the GABA - Cl⁻ channel complex, as they are structurally similar to benzodiazepines (Avallone et al., 2000; Fernandez et al., 2006; Kavvadias et al., 2004; Nassiri-Asl et al., 2007; Park et al., 2007).

In this study MECH, MEBP showed significantly reduction in the duration of convulsion on PTZ induced rats and delayed the onset of convulsion. MEBA showed no recovery on PTZ induced rats. The mortality was also prevented in the test group of animals.

**Antianxiety (Light and Dark model)**

The light/dark box is widely used for rodents as a model for screening anxiolytic drugs, based on the innate aversion of rodents to brightly illuminated areas and on the spontaneous exploratory behavior of rodents in response to mild stressors, that is, a novel environment and light (Imaizumi et al., 1994). It has been reported that simply the measurement of the time spent in the light area, is the most consistent and useful parameter for assessing an anxiolytic action (Young et al., 1991).

The light and dark box test is based on the natural aversion of mice to brightly lit places (Bourin et al., 2003). The extracts reduce the natural aversion to light and increase the time spent in the lit compartment, dose dependently. The observed activity may be due to the agonistic effect on GABA/benzodiazepine receptor complex.
An increase in the number of entries into light arena was significant. Animals treated with diazepam showed more pronounced anxiolytic effect. The mean time spent by the mice in open arms was higher than mean time spent in closed arms. MECH and MEBP showed significant anxiolytic activity.