7.1 SUMMARY AND CONCLUSIONS

SUMMARY

In recent times, the reappearance of interest towards herbal drugs has been found globally. Even though the herbal medicines were used for treating many diseases, these medicines were mostly used inappropriately or exploited unscientifically. Thus detailed study of the herbal drugs is required with regards to modern medicine. Because of many reasons, most of the India’s populations are the victims of inflammatory and hepatic diseases. Even though huge developments were found in the latest therapeutic compounds but still the latest medicine system has no potentiality to give appropriate medicines for various diseases conditions. The current research significantly focuses on the advancements of useful anti-inflammatory and hepato-protective drugs.

Inflammation is a significant physiological reaction that happens in response to several stimulations primarily by bacterial infection, trauma, and chemicals (Nathan, 2002). Depending on the intensity of the inflammation may cause numerous infections and non-infectious diseases (Alzheimer’s, cancer, cardiovascular disease, RA, diabetes, and arteriosclerosis). Several classes of anti-inflammatory agents are prescribed nowadays. Among that indomethacin create its therapeutic as well as toxic effect by inhibiting PG/s synthesis in different tissues. A common adverse effect of NSAIDs is the hepatotoxicity upon long term usage.

Now a day the liver problems become too common in the age groups of the population. Even several drugs are available for the management of hepatotoxicity by allopathic medicine. The priority to the ethnomedicine and herbal drug formulations become very popular among the existed medications to the treatment of liver problems. All the activities of plant-based medicine due to the rich content of phytochemicals and are responsible for the possible therapeutic activity.

Indian Ayurvedic medical organization is a time-tested system of medicine. It has an edge over than another system of health care, management system (Rajasree
Herbal treatment also is known phytomedicine, in which organs of plants like barriers, roots, seeds, leaves, barks, flowers, etc., are used for medicinal purpose, by the people who live in rural areas as first-line treatment (Kamarapuet.al, 2015). This interest stems primarily from the belief that the green medicine as safe and dependable and it is essential to intensify the studies on medicinal plants to place them in folklore (Rajasree, et. al., 2012). More often the plant medicines are used in combinations rather than in a single to maximize the benefit from their combined strength (Sunil Mistry, et.al. 2012). It is even clear in allopathic medicines also that use of some drugs in combination gives rise to increase the desired therapeutic activity or to decrease the adverse effects of the drugs. Usually, the potentiating effect produced by one drug on other drug is called synergetic effect (Barik, et. al., 2015).

Epidemiological studies on the association between dietary habits and disease risk have exposed that food has a direct effect on health. It is usually established that plant-derived foods like fruits, nuts, vegetables, grains, legumes; spices etc. possess certain useful effects on human health, predominantly on age linked illnesses. The capacity of certain plant-derived food found to lessen the risk of chronic diseases has been reported (Philip J Tuso et al., 2013). Phytochemicals that are present in the diet, associated with health benefits include alkaloids, glycosides, flavonoids, phytosterols, anthocyanins, tannins, and terpenoids. Their bioactivity has been, to some extent related to their antioxidant properties which are elaborate in the onset of development of the chronic degenerative diseases (Espin et al., 2007).

The major cause of many chronic conditions of infectious and noninfectious conditions like cancer, asthma, diabetes, neurological problems, and arthritic problems is inflammation. Several drugs are existed in the treatment of both acute and chronic inflammatory conditions and based on perception. Most of the drugs are belongs to the class of nonsteroidal anti-inflammatory agents with potent action. But the long-term usage of these drugs produces adverse events like hepatic and gastrointestinal problems. Hence, research is focused on herbal based medicines with lesser adverse drug reactions with beneficial activities.
The functional homeostasis was majorly regulated by the liver. A number of metabolic functions are originated and controlled by the liver. The metabolic disorders finally result in the hepatic damage if left untreated. Herbs are known to use in the treatment and management of hepatic problems and others (Patel RK., 2008). More often the plant medicines are used in combinations rather than in a single to maximize the benefit from their combined strength (Sunil Mistry, et.al. 2012). It is even clear in allopathic medicines also that use of some drugs in combination gives rise to increase the desired therapeutic activity or to decrease the adverse effects of the drugs. Usually, the potentiating effect produced by one drug on other drug is called synergetic effect (Barik, et al., 2015).

**Review of literature**

Plant extraction is a major step in the process of the research activity and evaluation of any plant-derived products. There are a number advantages over the individual isolates to the whole plant extracts only for the reason that of the existence of a number of chemical components and its synergistic activity. The cumulative activities were not observed in individual isolates and are not much safety in a higher concentration (Umar , 2014). Since several plants were reported to had anti-inflammatory activity against CIPE like *Portulaca oleracea* (Agyare, C., 2015), *Salvia fruticosa* (Qnais, E.Y., 2010), *Corchorus olitorius* (Zakaria, Z.A., 2006) *Carica papaya* (Owoyele, B.V., 2008), *Desmodium gangeticum* (Yasmeen, N., 2013), *Morinda citrifolia* (Komalavalli, K., 2013) and *Sonerila tinneveliensis* (Benso, B., 2015) etc. The reported plants with HPA against PIHT such as Coriandrum sativum (Lewu, M.N., 2009), Colocasia esculenta (Chandra subhash, 2012), Brassica oleracea (Kanathur, N, 2001), Amorphophallus paeoniifolius (Pramod J.,2012), Lagenaria siceraria (Lakshmi, B.V.S., 2011) and Macrotyloma uniflorum (Parmar, H.B.,2012) etc.

**Statement of the problem**

ROS interact with tissue proteins, carbohydrates, lipid and nucleic acids due to highly reactive in nature (unpaired electron).

These ROS trigger the manufacture of pro-inflammatory cytokinin (TNF-a and IL 6) and chemokin mediators (CRP). Thus free radicals are significant mediators
stimulate inflammatory responses and these ROS are neutralized by antioxidants and radical scavengers to the control inflammation. NSAIDs are the first line drugs among all anti-inflammatory agents. Now a day, the usage of NSAID is common to treat mild and moderate types of pain. Because of the use of NSAIDs drastically reduced the threat of gastrointestinal ulceration; however, amplified rates of MI, HF, HTN, and acute renal insufficiency remained the same as that of earlier.

Aside from the pharmacological response extensive use of anti-inflammatory agents leads to toxicity and toward effects on the liver, kidney, and GIT upon long term usage. To over-come the above problem researchers looking on plant-based medicine with potent anti-inflammatory activity with lesser side effects.

**METHODOLOGY**

**In Vitro AO activity**

*Endogenous free radicals:*
- Superoxide radical scavenging activity (Martinez A C *et al.*, 2001)
- Hydroxyl radical scavenging activity (Nagai Myoda ., 2005)
- H2O2 scavenging activity (Gülçin H 2005)
- Nitric Oxide scavenging activity (Sreejayan N *et al.*, 1997)

*Exogenous free radicals:*
- DPPH (BloisMS *et al.*, 1958)

**In-vitro AI activity:**
- HRBC membrane stabilization assay(Gandhisanet al. 1991)
- Inhibition of albumin denaturation Assay(R. Perumal., 2008)

**Characterization**
- FTIR (Shimadzu, IR Affinity 1, Japan)
- DSC (Gortzi et al., 2006)

**Acute toxicity studies** (Itchfield and Wilcoxon, 1949)

**In vivo AI activity:**
Carrageenan induced edema of paw (Winter et al., 1962)

**Grouping of animals:**
After the induction of carrageenan (1.5% w/v, 0.1 ml/paw) was introduced into hind paw of right side at the plantar side. The rats were divided into eleven various groups of each containing 6 animals. All the samples controlled orally 60 min prior to carrageenan management. The paw edema was gauged (cm) before and then at 1, 2, 3, 4, 5 hours, after carrageenan injection. Indomethacin at 10 mg/kg was considered as the reference medication. The weight of the both the two paw of each rat was measured at every time interval after carrageenan injection

**Group 1:** Served as control rats received dist.-H2o and fed on a ordinary diet.

**Group 2:** Serve up as disease-control rats given carrageenan

**Group 3:** Received Indomethacin (10 mg/kg)

**Group 4:** Treated with (ALAG) (150mg/kg)

**Group 5:** Treated with (ALAG-300 mg/kg)

**Group 6:** Treated with (ALAA) (150mg/kg)

**Group 7:** Treated with (ALAA-300mg/kg)

**Group 8:** Treated with (ALLS) (150mg/kg)

**Group 9:** Treated with (ALLS) (150mg/kg)

**Group 10:** Treated with polyherbal extract (PHE 1)

**Group 11:** Treated with polyherbal extract (PHE 2)

**Estimation of Antioxidant enzymes (paw):**

- SOD
- CAT
- GSH
- MDA

**Estimation of inflammatory mediators (paw):**

- TNF α, IL 6
- CRP (C-reactive protein)

**Histopathology of paw**

*In vivo HPA:*

**Induction of HT:**
The rats were fasted for eighteen hours before to the experiment with H2O *ad libitum*. Paracetamol suspension was prepared with 5% gum acacia at a dose of 2g/kg (b.wt. p.o.) (Ramachandra setty, 2007). Treatment periods were once daily for 14 days according description in the chapter-6.

**Estimation of liver enzymes:**

SGOT or AST

Serum glutamate pyruvate transaminase (SGPT) or ALT Serum Alkaline Phosphatase (ALP)

Estimation of Antioxidant enzymes (liver):
- Total protein
- SOD
- CAT
- GSH
- MDA

Estimation of inflammatory mediators (liver):

TNF α, IL 6 and CRP

**Histopathology of liver**

**RESULTS AND DISCUSSION**

Scientists are really interested in researching more about the natural products to know their characteristics and properties and mainly they focus much on plants. From the starting time of human civilisation, plants which have medicinal values are collected and used by the people to cure the diseases of mankind. For thousands and thousands of years, nature acted as a source of providing medical values and large numbers of drugs were extracted from the natural sources. Most of the extractions are taken from the agents of traditional medicine. Plants (medicinal herbs, vegetables and fruits) gives a proper source of compounds like nitrogen compounds, Terpenoids, vitamins and phenolic compounds and other secondary metabolic are rich in valuable bioactivities are anti-inflammatory, antibacterial, antioxidant, antitumor, anti- carcinogenic, or antiviral, anti-mutagenic activities (Maridass and Britto. 2008). In many Asian countries like China, India etc. the traditional herbal medicines were used for
more than thousands of years. Herbal plants became as the main objects of pharmaceutics, biochemist, and chemist. This research plays a significant role in discovering and developing new drugs, effectiveness and there are no side actions like the effects of modern synthetic drugs. Other than focusing on components of plants, it focuses on folkloric reputation and traditional usage. Additionally, the extraction and identification on these plants are because of the activities like fraction and extracts. Therefore it is clear that studies on herbal plants are in demand for scientists of natural products. From all these natural products, a large number of herbal drugs are created through various forms like nutraceuticals, complementary alternative medicine and food supplement.

From the time of Vedic glory, medicinal plants are used as natural medicine in India (Gupta et al., 2008). Additionally, study on herbal plants are profounded to not discover the active compounds and also finds effective mechanism by developing them to treat all type of skin disease. Additionally, the studies supplies the constituents and plans to encourage the usage of herbal plants through food could improve health and prevent all types of disease.

Currently many developments are continuously occurs in chemistry field especially with the help of medicinal research. Regardless of this, developments occurs in fast-rate and drugs which are extracted from the plants are still cannot be produced through these advanced technologies. There are two strong reasons behind the above-mentioned statements. Compounds like reserpine and atropine are more expensive to synthesize and other useful drugs like cocaine, digitalis, ergotamine and morphine cannot be synthesized still now (Ernavithe, 2008). Therefore, the extractions of plant derived drugs are still having the same level of importance in discovering the drug. After plant derived drugs are isolated, it starts to act as a lead compound for a good start while creating new types of drugs. It allows the rational and design planning of new drugs along with the mimetic synthesis development and discovering of new biological activities which are completely not related to the known compounds (Hamburger and Hostertmann, 1991). For example, salicylic acid was properly synthesized to
replace phenol as antiseptic. The results have stated the activities of anti-rheumatic and antipyretic (Sneader, 2005).

Plants’ medicinal properties are used for antipyretic, antioxidant, antimicrobial effects of the phytochemicals (Adesokan et al., 2008).

In the current revise the chief objective was to identify a medicinal plant which can be exploit as an AO as well as a HP-agent with fewer/without side effects. *Apium graveolens, Allium amperifolium* and *Lactuca sativa* were selected for evaluation of AI and HP property.

Several researchers reported that natural herbs or plant-based medicine could suppress the production of oxidative stress by increasing the antioxidants systems (C. Tohda., 2006). In the present investigation was carried out on SPEs of *Apium graveolens, Allium amperifolium* and *Lactuca sativa* for the screening of *in vitro* antioxidant activity. All the three SPEs were preliminarily screened for FTIR and DSC analysis. Further, the extracts were evaluated for *in vitro* antioxidant activity by using several methods. The consequences of FTIR analysis confirmed the presence of alcohol, ethers, alkanes, aromatic amines and nitro groups from SPEs (Figure 4.01, 1.02 and 4.03) (Litwinienko, 1997). All the extracts were shown to have thermo stable in nature at a range between 110 °C to130°C, during DSC study. It indicates that the selected extracts were stable for auto oxidation (figure 4.04, 4.05 and 4.06).

Superoxide is the principle decrease result of atomic oxygen, an extremely dangerous radical, and the preeminent plentifully made in every single high-impact cell by a few enzymatic and non-enzymatic pathways, assaults various natural particles and prompts negative changes of biomolecules including DNA (Waris, 2004). The present study reveals that ALAG is a potent scavenger of deleterious free radicals formed during metabolic reactions as well as endogenous free radicals like O⁻ and OH⁻, at very low concentrations (Blois, 1958). The ALAA displayed significant dose-dependent inhibition of DPPH-RSA. Nitric oxide free radicals generated from the reactive oxygen free radicals are included in the pathogenesis of numerous chronic conditions like diabetes,
cancer, cardiac problems and inflammation (Moncada, 1991). The ALAA is shown to scavenging the NO free radical among all the SPEs (table 4.5). Several lysosomal enzymes are concerned in the course of inflammation and causing damage to the surrounding tissues (Amujoyegbe, 2012). The ALAG showed to have better membrane stabilization effect than that of all the SPEs, in a dose-dependent manner (table 4.6). The reason might be due to the production of autoantigens involved in the denaturation of protein and membrane lysis action. The ALAG had better activity against albumin denaturation among all the SPEs (table 4.7).

The free radicals are included in the procedures of inflammation and other complications. Since several plant derivatives with antioxidant potential reported to have anti-inflammatory and HPA; hence the SPEs were also screened for their AI activity and HPA in rats and was discussed in the following chapters. According to the acute toxicity studies, all the SPEs were found to be safe at 200mg/kg bd.wt. The present study 150mg/kg and 300mg/kg doses were selected for further investigation. The polyherbal mixture was prepared according to the different ratios of SPEs.

Acute inflammation is a process that involved the overproduction of free radicals, activation of complex enzyme, and discharge of several inflammatory and pro-inflammatory mediators. In anti-inflammatory studies, the SPEs exert the inhibitory activity of edema in both early-late phase of inflammation. The early phase is by released by the histamine and later phase is by the PG/s and lysosomal enzymes inhibition (Halici, 2007). The treatment with SPEs in CIPE showed significant reduction of paw thickness. Among all the SPEs ALAG showed better anti-inflammatory activity against CIPE Among all the treatment groups the PHE 2 presented substantial anti-inflammatory activity compared with the individual plant extracts (table 5.1 and 5.2). And finally, it stimulates the release of inflammatory mediators. In the present investigation total protein and AO-enzymes 'SOD, CAT, GSH, & MDA' were estimated in paw homogenates all the treatment groups. During carraggenan induction leads to the reduction of protein content in paw homogenates.
Treatment with plant extracts and PHE showed the elevation of protein content in a dose-dependent manner (table 5.3). Carrageenan induced rats showed a reduction of paw levels of ‘SOD, CAT & GSH’ and increase of MDA. But the management with SPEs and PHE showed the elevation of ‘SOD, CAT and GSH’ and lessening in MDA levels in carrageenan induced edema in paw homogenates. Among all the plant extracts the ALAG showed better antioxidant activity and PHE showed significant (p<0.05) elevation of antioxidant enzymes compared with carrageenan-induced inflammation (table 5.4, 5.5, 5.6 and table 5.7). But, it displayed that different functions of enzymatic and nonenzymatic antioxidants help to inhibit microbes from extreme production of ROS in the inflammatory states.

The tissue damage is characterized by the stimulation of pain perception and pain sensitivity during the inflammation (Cunha, T.M., 2005). In our research findings, induction of carrageenan leads to the triggering of TNF α, IL6, and CRP and was shown to be elevated. Treatment with SPEs and PHE showed a reduction of elevated inflammatory mediators in a concentration based manner. Among all the treatment groups the PHE showed significant anti-inflammatory activity (table 5.8, 5.9 and table 5.10).

Histopathology study of paw indicates that normal rats showed the general presence of epidermis and dermis short of any lesion. Carrageenan-induced rat showed heavy Polymorphonuclear infiltration (PMN) and a sponge-like appearance and bulla in the epidermis. Treatment with Indomethacin standard drug illustrated a considerable drop in the migration of PMN and oedematasis in the dermis without any spongy-like feature and bulla. The SPEs ALAG, ALAA, and ALLS showed a moderate reduction in PMN and near to normal appearance of the dermis. The PHF treated rats showed marked amelioration of infiltration and without any damage to the dermis.

The results obtained from the present chapter indicate the SPEs and polyherbal mixture showed potent antioxidant and anti-inflammatory agent by acting on several mechanisms. But the commercially available anti-inflammatory orthodox drugs have hepatotoxicity as a major drawback. Hence the research findings...
were further planned to elucidate the HPA of SPEs and polyherbal mixture in PIHT.

The liver is an organ of metabolism and the paracetamol was metabolized by glucuronide and as sulfate conjugations and resulted in the development of toxic metabolite NAPQI (Jollow, 1974). The NAPQI binds and interacts covalently to the sulfhydryl group of the liver proteins and resulted in liver necrosis due to the lipid peroxidation of liver tissue (Kapur et al., 1994). Liver function tests (SGOT, SGPT, and ALP) are useful in predicting the toxic effects of hepatotoxins. The Serum SGOT, SGPT, and ALP were estimated at last of 14 days of the test period and were found to be increased in be increased in PIHT. All the SPE’s and PHE are shown to decrease the elevated liver enzymes as shown as standard silymarin. And ALAG was the better hepatoprotective among the SPEs. Among all the treatment groups PHE was found to be more potent than standard Silymarin (Table 5.1, 5.2 and 5.3).

In general, the albumin levels are found to be lowered in the liver cirrhosis condition and due to the fall and disturbances in the protein synthesis. Serum bilirubin is one of the main sensitive tests used in the diagnosis of hepatic diseases (Wolf A., 1997). The Serum albumin, bilirubin, and total protein were estimated at the end of 14 days study period. The paracetamol-induced toxicity lead to the alteration of serum albumin and bilirubin and diminution of total protein levels in the serum.

All the SPEs and PHE is shown to decrease the elevated albumin, bilirubin and augment the total protein limits in paracetamol-induced rats in a dose-dependent manner (table 5.4, 5.5 and table 5.6). And ALAG was the better hepatoprotective among the SPEs. Among all the treatment groups PHE was found to be more potent than standard Silymarin. The activities might be due to the conditioning the hepatocytes to guard the veracity of the membrane from leakage induced by paracetamol of serum markers into transmission with SPEs and PHE treatment. Free radicals lead to protein oxidation, enzymatic inactivation and lipid peroxidation (Radosavljevic T., 2010). Paracetamol is a widely used analgesic but with an adverse effect like hepatotoxicity. The liver toxicity was caused by the
stimulation of oxidative stress and inhibition of antioxidant defense enzymes like SOD, CAT, and GST and by decreased byproducts like GSH, which are the major cause of hepatic damage (Medina, J., 2005; Dey, A., 2013).

The ‘total protein, SOD, CAT, GSH, & MDA’ in liver homogenate was calculated at the end of 14 days study period. The tissue ‘protein, SOD, CAT, & GSH’ ranges were declined and MDA levels were boosted in PIHT. This is measured as an important mechanism for centrilobular hepatic necrosis, leading to acute liver failure (Larson et al. 2005; Hanawa et al. 2008). All the SPEs and PHE is shown to revert back the protein, SOD, CAT, and GSH to the normal values and the elevated MDA were normalized in paracetamol-induced rats in a dose-dependent manner in liver homogenates (table 5.7, 5.8, 5.9 and table 5.10).

Among all the treatment groups The PHE was showed to be highly effective than standard Silymarin. And ALAG was the better antioxidant among the SPEs. The activity might be due to the inhibition and scavenging the oxidative radicals like superoxide, hydroxyl and H2O2 and nitrogen stress of SPEs as discussed in chapter. Due to the stimulation of Paracetamol induce toxicity lead to the stimulation of release of these inflammatory cytokines from the Kupffer cells of liver tissue which might increase inflammation by releasing of proinflammatory mediators like TNF α and IL 6 (Takahashi K., 2003). In the present investigation, the induction of paracetamol hepatotoxicity increased the release of these proinflammatory cytokines and CRP.

All the SPEs and PHE is shown to control the stimulation of proinflammatory cytokines TNF-alpha and IL-6, and biomarker CRP to the normal values in paracetamol-induced inflammation in a dose-dependent manner in liver homogenates (table 5.11, 5.12 and table 5.13). And ALAG was the better antiinflammatory among the SPEs. Among all the treatment groups The PHE was found to be more potent than standard Silymarin. The SPEs and PHE with suppressive effects on inflammatory cytokines and/or chemokines may be able to perk-up paracetamol-induced hepatic harm.

The histopathology study of the liver was conducted at the end of 14 days study period. In normal control rats the liver with normal structure and with lobular
architecture. In paracetamol treated rats, severe hepato-cellular degeneration and necrosis together with periportal mononuclear cell infiltration rats. Treatment with SPEs and PHE showed ameliorating the necrotic liver to normal architecture. The antioxidant capacity and inhibition of production and release of proinflammatory cytokines are the major contribution of its HPA.

The results achieved from the present research work designate the SPEs and poly herbal mixture showed potent antioxidant, anti-inflammatory, and hepatoprotective agent by acting on several mechanisms. The activities are due to the phytochemicals and phytonutrients present in the selected herbal extracts and with lesser adverse events.

CONCLUSION

Overall it can be concluded that SPEs *Apium graveolens*, *Allium amperfolium*, and *Lactuca sativa* flowers had free radical scavenging activity and activity against the inflammation and hepatotoxicity. All the activities might be due to the presence of volatile oils, sesquiterpene alcohols, limonene, pinene, camphene, terpinene, myristic, linoleic, palmitoleic, palmitic, oleic and stearic acid in *Apium graveolens*, amount of methiin and propiin, methyl and propyl propenyl disulfide, methyl and dimethyl propyl trisulfide from *Allium amperfolium* carotene, C-Vitamin and Vitamin E, triterpenoids, saponins and simple phenols present in *Lactuca sativa*. Hence these plants are used to discover new bioactive herbal based medicines for the treatment and prevention of multiple disorders and develop a neutraceutical based therapy. It is concluded that the poly herbal mixture was found to be more potent than that of the individual plant extracts. According to the ayudeva, the polyherbal mixture showed a greater therapeutic effect than the individual plants and is not sufficient to produce the desired effect at lower doses. The mixture of plants showed a synergistic effect with lesser toxicity. The polyherbal mixture of SPEs showed significant AI activity and the mixture also diminish the hepatotoxicity as produced by the conventional anti-inflammatory agent.
7.2 RECOMMENDATIONS

The present examination was carried-out to revise the effect of polyherbal extract (PHE) for its potency as an AI agent and also as a HP agent. The herbs used in the present study were

1. *Apium graveolens* (Apiaceae)
2. *Allium ampeloprasum* (Alliaceae)
3. *Lactuca sativa* (Asteraceae)

Various herbal products were already in the market for treating inflammatory disorders, but the present study with these endogenous herbs to provide a better formulation to treat the inflammatory disorders and to give hepatoprotective activity. The herbs used in the present study were proved to have various activities.

The results produced in the present study proved that the individual and the polyherbal extracts of the herbs used in the study have anti-inflammatory and hepatoprotective activity. The results showed that the activity produced by these herbs were significantly near or equal or slightly more than the activity produced by the activity of the standard drugs used in the study. Hence, these herbs used in the study can be used as an AI and HP-agent either by an individual or as a polyherbal formulation. The polyherbal extract (PHE) used in the study proved to have a better activity significantly equal to the activity of the standard drug used.

Further studies were needed to carry out to improve the polyherbal extract by isolating the chemical entities present in the crude drug responsible for the anti-inflammatory and hepatoprotective activities. Isolating the chemical entities present in the crude drug or herbs responsible for the activities can provide us to synthesize them in bulk using chemical synthesis in large quantities to formulate them to treat a large number of patients.

Further studies were also required to formulate the polyherbal extract (PHE) into a formulation to get good bio-availability and for also to increase the potency of the extract. Formulation of the drug is most important to get the activity of the drug biologically because the bio-availability and the extent of the drug release were depending on the formulation. More the bio-availability- more will be the
activity. The various methods used in this study to evaluate the AI and HP activities were well established and frequently used methods. Hence, change in the methods may also change the results. Inflammatory disorders are classified into various types, hence, it need to study the polyherbal extract for its specific use to treat an individual type of disorder e.g. gout. Further studies were needed to carry out the study for its specific use. Hence, according to the results produced by the polyherbal extract for anti-inflammatory and hepatoprotective activities with the evaluating methods used in the study the PHE was recommended as an anti-inflammatory and hepatoprotective agent. Further studies were recommended to carry out the energetic chemical article at hand in the herbal extract accountable for the above said activities and formulation studies were recommended to formulate the extract into the better formulation to provide good bio-available to increase the potency of the polyherbal extract.

7.3 FUTURE SCOPE

The current study was weighed up for the effectiveness of polyherbal extracts (PHE) as anti-inflammatory and hepatoprotective effects. The herbs used in the current study, namely

1. *Apium graveolens* (Apiaceae)
2. *Allium ampeloprasum* (Alliaceae)
3. *Lactuca sativa* (Asteraceae)

According to the outcomes produced in the study the endogenous herbs used in the test have high-quality potency to treat inflammatory disarray’s and also have a fine hepato-protective effect. The polyherbal extract set with the above herbs was also established to have first-class anti-inflammatory and hepatoprotective effects.

Synthetic anti-inflammatory managers on hand in the market is said to have lofty intense of severe adverse effects and toxicity, hence the patients were looking for an improved substitute medicine with stumpy side effects and toxicity. So, nowadays, the uses of herbal medicines were augmented. Hence, the polyherbal
extract considered in the current study, a good alternative to treat inflammatory disarrays and can also use as an anti-oxidant agent.

The endogenous herbs utilized in the tests were available in good quantity and not much of investigating work was carried out in the aspect of anti-inflammatory activity; hence, it has excellent scope for the canvassers to carry examine in this field to perk up. The individual effect of the herbs with various other animal models can be carried out in upcoming to make out the variability of the results with vary in the animal model or vary with change in the evaluation models for the said activities.

This study has grand potential for the reason that the incidence or occurrence of inflammatory disorders and heap-to toxic disorders are escalating day by day, and it is fetching a frightening circumstance to look on to care for these conditions with best patient compatibility i.e. with not as much of serious adverse events(SAEs) or side effects and toxicity. Because at present, the treatment of these conditions is more with synthetic agents which were said cause very serious adverse effects. Hence the present study makes available the best platform to work for the researchers to originate a polyherbal extract to care for the inflammatory disorders with less or no SAEs and toxicity. This study can be served up as a reference for researchers to carry research to classify the active chemical molecules to treat the anti-inflammatory disorders and hepatoprotective activity. The chief benefit of this study is providing a therapy to treat or cure inflammatory diseases and protect the liver from toxicity with less or no side effects. As the extract worn in the study is from herbs it is also a cheap, cost-effective and non-toxic.

7.4 LIMITATIONS OF RESEARCH WORK

FTIR analysis:
- The dipole moment is required to analyse a compound, therefore a compound with no dipole moment cannot be analysed because they cannot absorb the infrared rays.
- Analysing a mixture compounds causes masking effect because molecules which can be analysed at same or nearbywavelength absorbs infrared rays.
and cannot be differentiated. Peaks are masked because of this masking effect.

- Molecules with positive charging like CO₂ cannot be analysed because the electronegativity of carbon and electropositivity of oxygen cancel.

**Spectrophotometric analysis:**
The speed of analysis, limit of detection and sensitivity are less in Spectrophotometric analysis.

**Herbal extract:**
The availability of the herbs in nature is limited; hence the production of the polyherbal extract for treating the numerous patients suffering from the disorder is also not possible. Cultivating the herbs required is one of the alternative ways to meet the need.

These are obtained from the extraction of some of the parts of herbs or whole herbs are used for extraction. Hence, a herbal extract contains maximum compounds or molecules present in the herbs which are miscible in the solvent used for the extraction process. So, molecules extracted into the extract may react each other and/or may interfere with results. So, isolation of the molecules responsible for the required activity may need to be done for getting the best potency. The shelf-life of the extract is most important and it is very hard to determine for the herbal extracts.

**Method of analysis:**
It is exceptionally significant to consider the choice of method to analyze, as a mis-choice of the method leads to errors in the results and also causes a huge waste of valuable time, money and hard work. The method chosen must be familiar with the practical data of other techniques & of the theoretical principles upon what they are based. The techniques of analysis have a differing degree of sensitivity, selectivity, and cost & also of the time required. Before starting the analysis, the researcher should consider the following;
- What type of sample to collect and to be analyzed?
- Identify any other molecules likely to be interfering with the results?
- Reduce the chance of interference of the impurities in the herbs while extraction and prevent the chance of getting impure while storing the extracted crude drug.

**Animals used:**
Selection of animals are most important in analysis. The various other factors influence the results are as follows:

- Animal's sex
- Age group
- Weight
- Pathological conditions
- Feeding

**Sampling:**
Various factors limits are as follows:

- Selection of sampling method
- Type of sample to be collected
- Method of processing the sample,
- Storage of sample
- An analytical method to be used for analyzing the sample
- The quantity of the sample to be collected
- The frequency of sampling also interferes with the results.