Determination of Analgesic & CNS Activity of Chloroform Extract of *Ixora coccinea*

This dissertation is submitted for the partial fulfilment of the requirements for the degree of Bachelor of Pharmacy

East West University

Submitted By

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December, 2016

Endorsement by Head of the Department

This is to certify that the dissertation entitled **"Determination of Analgesic and CNS Depressant Activity of Chloroform Extract of *Ixora coccinea*"** is a genuine research work carried out by **Md. Didarul Islam**, under the supervision of **Meena Afroze Shanta** (Senior Lecturer, Department of Pharmacy, East West University). I further certify that no part of the thesis has been submitted for any other degree and all the resources of the information in thus connection are duly acknowledged.

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Certificate by the Supervisor

This is to certify that the dissertation entitled **"Determination of Analgesic and CNS Depressant Activity of Chloroform Extract of *Ixora coccinea*"**, submitted to the Department of Pharmacy, East West University, Dhaka, in partial fulfilment of the requirements for the Degree of Bachelor of Pharmacy, was carried out by **Md. Didarul Islam**, ID: 2013-1-70-027 under my supervision and no part of this dissertation has been or is being submitted elsewhere for the award of any Degree/ Diploma.

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Declaration by the Candidate

I, **Md. Didarul Islam**, hereby declare that the dissertation entitled **"Determination of Analgesic and CNS Depressant Activity of Chloroform Extract of *Ixora coccinea*"** submitted by me to the Department of Pharmacy, East West University, in the partial fulfillment of the requirement for the award of the degree Bachelor of Pharmacy, under the supervision and guidance of **Meena Afroze Shanta**, Senior Lecturer, Department of Pharmacy, East West University. The thesis paper has not formed the basis for the award of any other degree/diploma/fellowship or other similar title to any candidate of any university.

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This Thesis Paper

is dedicated to

MY FAMILY

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First, all praise and glory to **ALLAH** for all the bounties granted to me and only with his guidance and help this achievement has become possible.

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Abstract

Recently worldwide different researches on medicinal plants have grabbed attention. A number of experiments were found where medicinal plants have been used for different complementary and traditional systems to promote newer and safer drugs. Keep this in mind the current study was designed to find out CNS depressant and analgesic principles from chloroform solvent extracts of *Ixora coccinea.* The traditional use of this plant is in the treatment of dysentery, leucorrhoea, dysmenorrhoea, haemoptysis and catarrhal bronchitis. It is also claimed that the plant is a good reservoir of such compounds which have the possibility to be a better source of CNS depressant and analgesic therapeutic agents in living organism. In this study, above mentioned pharmacological activities of the experimental plant extract was checked in swiss albino mice. By the open field and hole cross method, CNS depressant activity was inspected with the decline of locomotor activity on mice. Analgesic activity was evaluated by acetic acid induced pain method. Here chloroform extract of *Ixora coccinea* were administered to mice at a dose of 200 mg/kg and 400 mg/kg. All the results of the experiments were statistically significant ($p<0.001$). In CNS depressant activity tests, the movement of mice decreased in a dose depending manner comparing to the standard diazepam. The experimental extracts also gave good analgesic effect comparing to control group and represented by decreased amount of writhes in acetic acid induced pain method. Percent inhibition is 30.43% for 200 mg/kg body weight and 37.46% ($p < 0.05$) for 400 mg/kg body weight, whereas for Standard drug (Indomethacin) the percent inhibition is 86.96% ($p < 0.001$) for 10 mg/kg body weight. In conclusion it can be said that chloroform extract of *Ixora coccinea* possesses good CNS depressant as well as analgesic activity.

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LIST OF ABBREVIATIONS

CMC	Carboxy Methyl Cellulose
CNS	Central nervous system
COX	Cyclooxygenase
EC	Effective-dose Concentration
GABA	Gamma aminobutyric acid
GABAA	Gamma aminobutyric acid Type A
gm	Gram
ICC	Chloroformic Extract of *Ixora coccinnea*
i.p.	Intraperitoneal
IC_{50}	Inhibitory Concentration with 50% scavenging
LC_{50}	Lethal Concentration with 50% mortality
mg	Milligram
min	Minute
ml	Millilitre
PG	Prostaglandin
p.o.	Per oral
WHO	World Health Organisation

Chapter 01

Introduction

1.1 Introduction

Plants can be subjected to successive extraction and purification procedures to isolate the compounds of interest, which can themselves be active and used directly as a drug, examples being quinine, digoxin and ergotamine, or they can be used as precursors (e.g. diosgenin) in hemi-synthetic processes or as models for total synthesis, with well-defined pharmacological activity or structure–activity relationship studies determining a prototype drug (e.g. morphine).

According to the OPS (Arias,1999) a medicinal plant is:

- ✓ any plant used in order to relieve, prevent or cure a disease or to alter physiological and pathological process, or
- ✓ any plant employed as a source of drugs or their precursors.

A phytopharmaceutical preparation or herbal medicine is any manufactured medicine obtained exclusively from plants (aerial and non-aerial parts, juices, resins and oil), either in the crude state or as a pharmaceutical formulation. A medicine is a product prepared according to legal and technical procedures that is used for the diagnosis, prevention and treatment of disease and has been scientifically characterised in terms of its efficacy, safety and quality (WHO, 1992). A drug is a pharmacologically active compound, which is a component of a medicine, irrespective of its natural, biotechnological or synthetic origin.

1.2 History and the earliest known medicines to mankind

For thousands of years natural products have played a very important role in healthcare and prevention of diseases. The ancient civilizations of the Chinese, Indians and North Africans provide written evidence for the use of natural sources for curing various diseases (Phillipson, 2001). The earliest known written document is a 4000 year old Sumerian clay tablet that records remedies for various illnesses (Kong, 2003). For instance, mandrake was prescribed for pain relief, turmeric possesses blood clotting properties, roots of the endive plant were used for treatment of gall bladder disorders, and raw garlic was prescribed for circulatory disorders. These are still being used in

several countries as alternative medicines. However, it was not until the nineteenth century that scientists isolated active components from various medicinal plants. Friedrich Sertürner isolated morphine from *Papaver somniferum* in 1806, and since then natural products have been extensively screened for their medicinal purposes. Atropine obtained from *Atropa belladonna*, strychnine, a CNS stimulant, ziconotide, identified from a cone snail, *Conus magus*, and Taxol® obtained from the bark of the Pacific yew tree are a few examples of active components isolated from natural sources (Newman, 2003).

The use of plants as medicines has a long history in the treatment of various diseases. The earliest known records for the use of plants as drugs are from Mesopotamia in 2600 B.C., and these still are a significant part of traditional medicine and herbal remedies. To date, 35,000-70,000 plant species have been screened for their medicinal use. Their contribution to the world market for herbal remedies is as shown in Figure 1.1 (WHO, 2002).

1: Europe - 33%
2: Asia - 26%
3: North America - 20%
4: Japan - 11%
5: Others - 10%

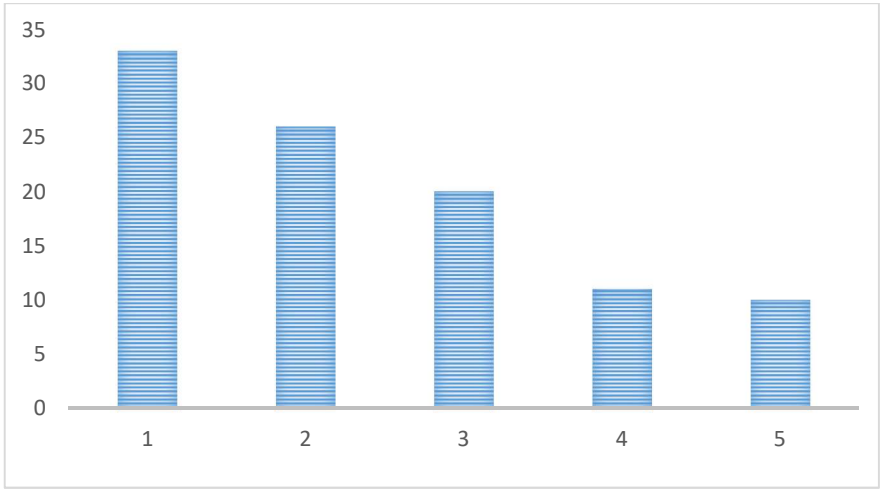

Figure 1.1: World market for drugs from plant sources (*Environmental Health Perspectives* 1999)

1.3 Natural products as medicines

Collectively, plants produce a remarkably diverse array of over 100,000 low molecular mass natural products, also known as secondary metabolites. Secondary metabolites are

distinct from the components of intermediary (primary) metabolism in that they are generally non-essential for the basic metabolic processes of the plant. Most are derived from the isoprenoid, phenyl propanoid, alkaloid or fatty acid/ polyketide pathways. This rich diversity results in part from an evolutionary process driven by selection for acquisition of improved defence against microbial attack or insect/ animal predation. (Pichersky, 2000)

Natural compounds can be lead compounds, allowing the design and rational planning of new drugs, biomimetic synthesis development and the discovery of new therapeutic properties not yet attributed to known compounds (Hamburger and Hostettmann., 1991). In addition, compounds such as muscarine, physostigmine, cannabinoids, yohimbine, forskolin, colchicine and phorbol esters, all obtained from plants, are important tools used in pharmacological, physiological and biochemical studies (Williamson *et al.*, 1996).

1.3.1 Benefits of plants

The use of natural products with therapeutic properties is as ancient as human civilisation and, for a long time, mineral, plant and animal products were the main sources of drugs (De Pasquale, 1984). The Industrial Revolution and the development of organic chemistry resulted in a preference for synthetic products for pharmacological treatment. The reasons for this were that pure compounds were easily obtained, structural modifications to produce potentially more active and safer drugs could be easily performed and the economic power of the pharmaceutical companies was increasing. Furthermore, throughout the development of human culture, the use of natural products has had magical-religious significance and different points of view regarding the concepts of health and disease existed within each culture.

Obviously, this approach was against the new modus vivendi of the industrialised western societies, in which drugs from natural resources were considered either an option for poorly educated or low income people or simply as religious superstition of no pharmacological value. However, even if we only consider the impact of the discovery of the penicillin, obtained from micro-organisms, on the development of anti-

infection therapy, the importance of natural products is clearly enormous. About 25% of the drugs prescribed worldwide come from plants, 121 such active compounds being in current use. Of the 252 drugs considered as basic and essential by the World Health Organisation (WHO), 11% are exclusively of plant origin and a significant number are synthetic drugs obtained from natural precursors. Examples of important drugs obtained from plants are digoxin from *Digitalis* spp., quinine and quinidine from *Cinchona* spp., vincristine and vinblastine from *Catharanthus roseus*, atropine from *Atropa belladona* and morphine and codeine from *Papaver somniferum*. It is estimated that 60% of anti-tumour and anti-infectious drugs already on the market or under clinical trial are of natural origin (Yue-Zhong Shu, 1998). The vast majority of these cannot yet be synthesised economically and are still obtained from wild or cultivated plants.

1.3.2 Disadvantage of synthetic compound

In recent years, there has been growing interest in alternative therapies and the therapeutic use of natural products, especially those derived from plants (Goldfrank *et al.*, 1982; Vulto and Smet, 1988; Mentz and Schenkel, 1989). This interest in drugs of plant origin is due to several reasons, namely, conventional medicine can be inefficient (e.g. side effects and ineffective therapy), abusive and/ or incorrect use of synthetic drugs results in side effects and other problems, a large percentage of the world's population does not have access to conventional pharmacological treatment, and folk medicine and ecological awareness suggest that "natural" products are harmless. However, the use of these substances is not always authorised by legal authorities dealing with efficacy and safety procedures, and many published papers point to the lack of quality in the production, trade and prescription of phytomedicinal products.

1.3.3 Natural products as medicine in global market

It is estimated that, in 1997, the world market for over-the-counter phytomedicinal products was US$10 billion, with an annual growth of 6.5% (Soldati, 1997). The WHO considers phytotherapy in its health programs and suggests basic procedures for the validation of drugs from plant origin in developing countries (Vulto and Smet, 1988; OMS, 1991). Eastern countries, such as China and India have a well-established herbal

medicines industry and Latin American countries have been investing in research programs in medicinal plants and the standardisation and regulation of phytomedicinal products, following the example of European countries, such as France and Germany. In Germany, 50% of phytomedicinal products are sold on medical prescription, the cost being refunded by health insurance (Gruenwald, 1997). In North America, where phytomedicinal products are sold as "health foods" (Brevoort, 1997; Calixto, 2000), consumers and professionals have struggled to change this by gathering information about the efficacy and safety of these products, and new guidelines for their registration are now part of FDA policy (Israelsen, 1997). In 1997, the North American market for products of plant origin reached US$ 2 billion (Brevoort, 1997).

According to recent studies conducted by the World Health Organization (WHO), about 80% of the world's population relies on traditional medicine. About 121 drugs prescribed in USA today come from natural sources, 90 of which come either directly or indirectly from plant sources. Forty seven percent of the anticancer drugs in the market is representation of the contribution of natural products to drug discovery.

V = Vaccine
B = Biological
NP = Natural Product
NPD = Natural Product Derivative
SNP = Synthetic Derived from NP
S = Synthetic

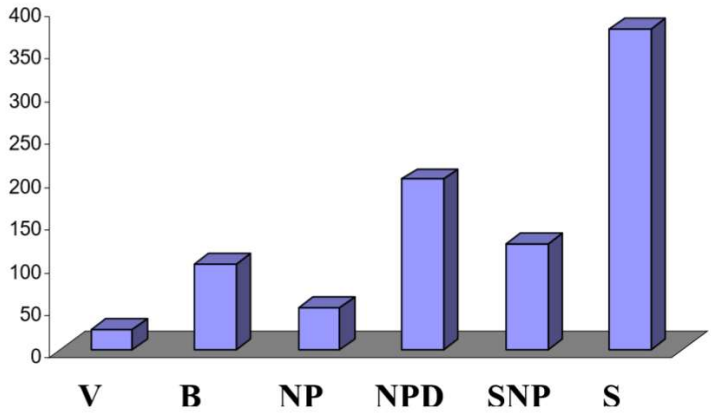

Figure 1.2: Distribution of natural products as drugs (J. Nat. Prod. 2003)

Thus, the modern social context and economic view of health services, the needs of the pharmaceutical market and the recognition that research on medicinal plants used in folk medicine represents a suitable approach for the development of new drugs (Elisabetsky, 1986; Calixto, 1996) have led to an increase in the number of publications

in this field, and private and governmental institutions are now financially supporting research programmes worldwide.

The NCI (National Cancer Institute, USA) has tested more than 50,000 plant samples for anti-HIV activity and 33,000 samples for anti-tumour activity. In 1993, the International Program of Co-operation for Biodiversity (IPCB) was launched in order to promote natural products in Latin America and Africa, linking universities, industries and governments in a multidisciplinary programme for the sustained development and preservation of the environment (Rouhi, 1997). Large pharmaceutical companies, such as Merck, CIBA, Glaxo, Boehringer and Syntex, now have specific departments dedicated to the study of new drugs from natural sources (Reid *et al.*, 1993). However, the potential use of higher plants as a source of new drugs is still poorly explored. Of the estimated 250,000–500,000 plant species, only a small percentage has been investigated phytochemically and even a smaller percentage has been properly studied in terms of their pharmacological properties. In most cases, only pharmacological screening or preliminary studies have been carried out. It is estimated that 5000 species have been studied for medical use (Payne *et al.*, 1991). Between the years 1957 and 1981, the NCI screened around 20,000 plant species from Latin America and Asia for anti-tumour activity, but even these were not screened for other pharmacological activities (Hamburger and Hostettman, 1991).

1.4 Challenges of isolating lead compounds from medicinal plants

Research into, and development of therapeutic materials from plant origin is a hard and expensive task (Borris, 1996; Turner, 1996; Williamson *et al.*, 1996). Each new drug requires an investment of around US$100–360 million and a minimum of 10 years of work, with only 1 in 10,000 tested compounds being considered promising and only 1 in 4 of these being approved as a new drug. Up to 1992, the NCI had only found 3 plant extracts active against HIV out of 50,000 tested, and only 3 out of 33,000 plant extracts tested were found to have anti-tumour activity (Williamson *et al.*, 1996). Quantitative considerations regarding the average yield of active compounds and the amount of starting crude plant material required for the discovery, development and launch of a

new drug on the market were presented by McChesney (1995): 50 kg of raw material are necessary to provide 500 mg of pure compound for bioassays, toxicology, and "in vivo" evaluation; full pre-clinical and clinical studies can require 2 kg of pure compounds obtained from 200 ton of raw material. The process is multi-disciplinary (De Pasquale, 1984; Verpoorte, 1989). The basic sciences involved are botany, chemistry and pharmacology, including toxicology. Any research into pharmacological active natural compounds depends on the integration of these sciences. The way they are integrated and the extent of integration depend on the objectives of the study. In any case, a particular discipline should not be seen as secondary to another; quite the opposite, as each step must be carried out considering the theoretical and technical background of each of the sciences involved, otherwise the results may not be robust enough and may lead to break down of the process.

Other fields of challenges may also be involved if the long path from plant to medicine is taken into account. Anthropology, agronomy, biotechnology and organic chemistry can play very important roles. In addition, pharmaceutical technology is fundamental to the development of any drug, including drugs of plant origin (Petrovick *et al.*, 1997; Sharapin, 1997).

Concerning drugs of plant origin, it is important to bear in mind certain conceptual distinctions. Plants can be used as therapeutic resources in several ways. They can be used as herbal teas or other homemade remedies, when they are considered as medicinal plants. They can be used as crude extracts or "standard enriched fractions" in pharmaceutical preparations, such as tinctures, fluid extracts, powder, pills and capsules, when they are considered as phyto pharmaceutical preparations or herbal medicines.

1.5 Selecting a plant

The approach for drug development from plant resources depends on the aim. Different strategies will result in a herbal medicine or in an isolated active compound. However, apart from this consideration, the selection of a suitable plant for a pharmacological study is a very important and decisive step. There are several ways in which this can be

done, including traditional use, chemical content, toxicity, randomised selection or a combination of several criteria (Ferry and Baltassat-Millet, 1977; Soejarto, 1996; Williamson *et al.*, 1996).

The most common strategy is careful observation of the use of natural resources in folk medicine in different cultures; this is known as ethnobotany or ethnopharmacology. Information on how the plant is used by an ethnic group is extremely important. The preparation procedure may give an indication of the best extraction method. The formulation used will provide information about pharmacological activity, oral versus non-oral intake and the doses to be tested. However, certain considerations must be taken into account when the ethnopharmacological approach of plant selection is chosen. For instance, each ethnic group has its own concepts of health or illness, as well as different health-care systems (Elisabetsky and Posey, 1986). The signs and symptoms should be translated, interpreted and related to western biomedical concepts, thus allowing a focused study of a particular therapeutic property.

Selection based on chemical composition uses phylogenetic or chemotaxonomic information in the search, mainly in certain genera and families, for compounds from a defined chemical class with known pharmacological activity (Gottlieb and Kaplan, 1993; Souza Brito, 1996).

The search for highly specific potent drugs for therapeutic use and, more precisely, as an investigation tool in biological research has been quite productive in toxic plants. A number of important compounds now used in research came from toxic plants and several examples have been mentioned earlier (Williamson *et al.*, 1996). Observation of the plant's environment has led to the isolation of active compounds, mainly anti-bacteria and anti-insect drugs (Harmburger and Hostettman, 1991). Another method of selecting a plant is that the investigator decides on a well-defined pharmacological activity and performs a randomised search, resulting in active species to be considered for further study. The search for anti-tumour drugs is a good example of the use of this strategy.

The search for drugs active against tumours, viruses and cardiovascular and tropical diseases is a priority. The largest research fields, as defined by the number of publications describing bioactive plant-derived compounds in the last few years, are anti-tumour drugs, antibiotics, drugs active against tropical diseases, contraceptive drugs, anti-inflammatory drugs, immune modulators, kidney protectors and drugs for psychiatric use (Hamburger and Hostettman, 1991). Taxol is both an example of the importance of natural products and of the complexity and necessity of finding alternative routes by which it can be obtained. It is the most important natural product-derived diterpene with anti-tumour activity found in recent years. Taxol is isolated from Taxus (*T. brevifolia* and *T. bacata*). However, the biggest obstacle to its clinical use is obtaining the material.

In order to produce 2.5 kg of taxol, 27,000 tons of *T. brevifolia* bark are required and 12,000 trees must be cut down. Due to the high demand, this species of Taxus will soon be extinct if no alternative source of taxol can be developed. An economically possible and technically realistic alternative is its partial synthesis, in considerable yield, from an analogue found in other species of Taxus, as well as the production of other hemi-synthetic analogues (Hamburger and Hostettman, 1991; Wall and Wani, 1996).

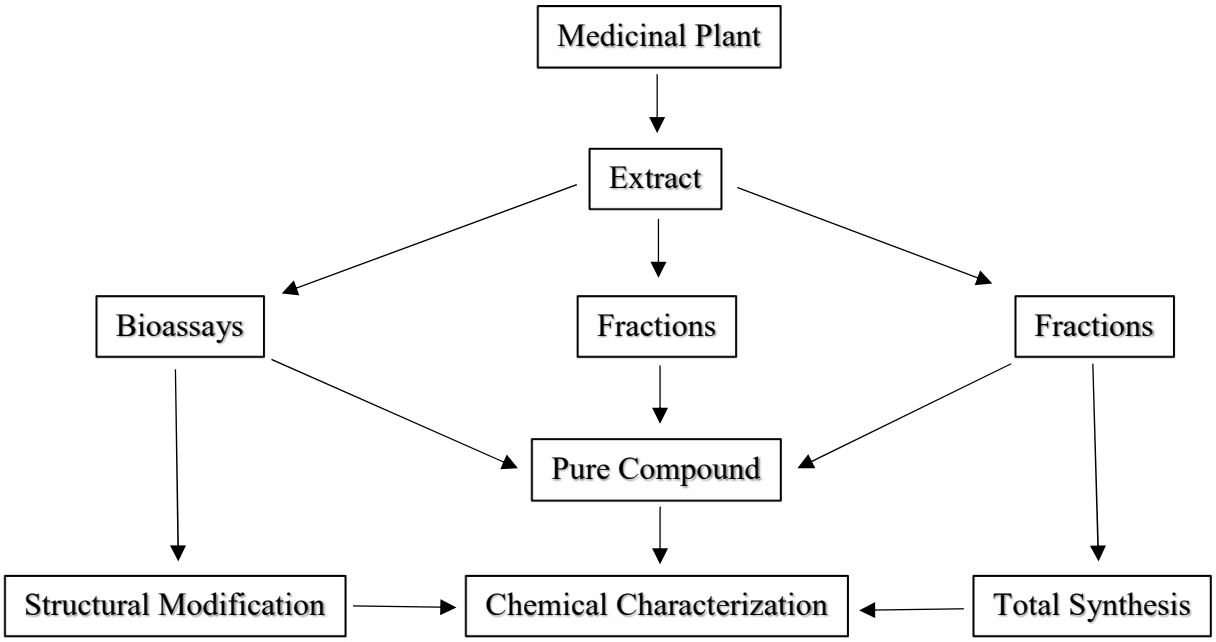

Figure 1.3: Methods for obtaining active substances from plants

1.6 Steps of drug development

Once the plant is chosen, the next step is its collection and botanical identification. Then it should be submitted to a stabilisation process. It is important that plant recollection involves a professional botanist who is able to correctly identify the species and prepare part of the material for herbarium preservation in order to have a reference material ("voucher specimen"). Preferably, the place and date of recollection should be recorded and the information retained for further collection, if necessary. Stabilisation is usually by drying the material at ambient temperature in a shady place, but can also be carried out in an oven with controlled airflow and temperature. When the stability of the compounds is unknown or if they are known to be unstable, the fresh plant should undergo a stabilisation process consisting of freezing, lyophilisation, use of alcohol vapour etc. (Williamson *et al.*, 1996).

The dried or stabilised plant material should then be powdered and subjected to a suitable extraction process. When the chemical nature of the compounds involved is known (once again, chemotaxonomic information and data - bank consultation are crucial), extraction methods should be directed at obtaining these compounds in as high a yield and purity as possible. When the chemical composition is unknown, the extraction procedure can be based on how the plant is used in folk medicine, or several extractions with solvents of increasing polarity can be performed (Williamson *et al.*, 1996). To obtain isolated active compounds, the plant extracts are first qualitatively analysed by thin layer chromatography (TLC) and/or other chromatographic methods and screened to determine the biological activity or to obtain a general evaluation of biological activities. For purification and isolation, the active plant extracts are sequentially fractionated (Verpoorte, 1989), each fraction and/or pure compound being subjected to bioassay and toxicity evaluation in animals. This strategy is called bioactivity-guided fractionation. Bioassays can be performed using micro-organisms, molluscs, insects, cellular systems (enzymes, receptors etc.), cell culture (animal and human), and isolated organs or in vivo (mammals, amphibians, birds etc.) (Hamburger and Hostettman, 1991; Souza Brito, 1996).

All these methods have advantages and disadvantages and the appropriate method must be carefully selected at each step of any biological study aimed at the development of a drug or the understanding of the biological basis of a particular pathology or even the discovery of the mechanism of action of already known drugs.

Furthermore, new techniques that can fulfil different needs and be adjusted to the classical pharmacological study of natural compounds should be sought. There is also a need for the improvement and establishment of experimental models not yet extensively used in the evaluation of natural products.

After verifying the purity of an isolated active compound, the structure is determined by spectroscopic methods (UV, IR, mass spectrum or NMR) (Verpoorte, 1989). Once the chemical structure is defined, total or partial synthesis and preparation of derivatives and/or analogues can be considered, and modulation of the biological activity and definition of the structure–activity relationship can be carried out. After completing all these steps, large-scale isolation (it may necessary to collect the plant again) or partial or total synthesis is required for pharmacological evaluation in pre-clinical, clinical and toxicological trials aimed at future therapeutic use (Hamburger and Hostettman, 1991; Borris, 1996). As mentioned above, the final result of this strategy, the drug, is expensive. However, the study of medicinal plants also allows their use “in natura” and/or in pharmaceutical formulations obtained from them, called phytomedicines or herbal remedies. This approach also requires efficacy and toxicity studies, but these are less time-consuming, as the steps of fractionation, purification and bioassay are basically not required or are far less complex (Elisabetsky, 1987).

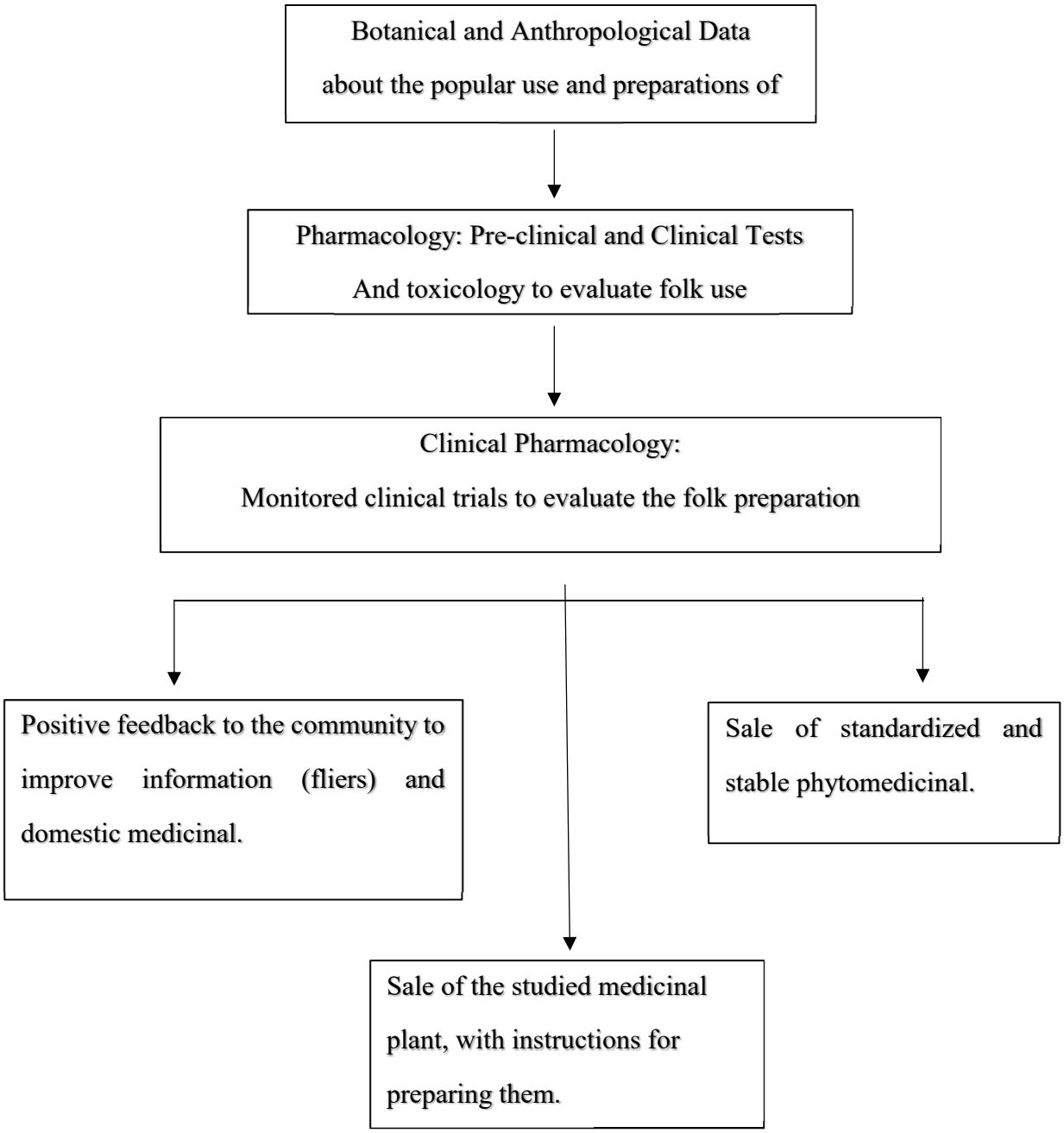

Figure 1.4: Methods for pharmacological validation of the popular use of medicinal plants

1.7 Status of plant research in various countries

Research into medicinal plants and the search or plant-derived drugs require a multidisciplinary approach with integrated projects, financial and technical support, and a very carefully planned strategy. The aims should consider demands in terms of

public health, preservation of biodiversity and the technical qualification of each laboratory or research group involved. Advances in technology and knowledge of natural products must be viewed not merely from the perspective of drug development, but also as a special tool for the understanding of biological phenomenon in order to contribute to the well-being of humanity.

Various countries around the world have taken steps to do in detail study of medicinal plants. The activities carried out in this field by some of the countries are given below:

Table 1.1: Activities of various countries concerning medicinal plants

Country	Activities	Remark
Armenia	Rich history of use and export of medicinal plants.	Over 3200 species described and conserved for use and export.
Australia	Asian-Australian Centre for the Study of Bioactive Medicinal Plant Constituents was set up in 1992 at La Trobe University for conduction of research in collaboration with Chulalongkorn and Chieng-Mai Universities in Thailand.	Inter-university research work focuses on: - The bioactive constituents of turmeric e.g. curcumin - Islamic medicinal plants - Antifungal proteins and secondary metabolites in crop plants, e.g. in cotton and yellow mustard Sinapis alba - Marine toxins - Collaboration on medicinal plants with scientists from Indonesia, Thailand, Bangladesh, Singapore, Kuwait and New Zealand.

Country	Activities	Remark
Bangladesh	Research on cultivation and biochemical aspects of medicina plants.	Bangladesh Council for Scientific and Industrial Laboratory at Chittagong oversees development of appropriate chemical technologies, and pharmacopoeia of plants.
Bhutan	Sustainable protection and use of forest resources and development of plant tissue culture.	Capacity building in plant biotechnology for rural markets and development of forest seedlings Indigenous Hospital in Thimphu has recorded 180 species of medicinal and aromatic plants.
Canada	Southern Crop and Food Production Centre of Agriculture and Agrifood Canada mandated to develop novel technologies in production and protection of new crops inclusive of medicinal plants.	Active research on crop production, genetics, germplasm improvement, micropropagation, and protection of medicinal herbs. Attention is given to research in developing base-line agronomic information, elucidating the chemistry of bioactive principles, etc.
China	Chinese root extracts from Astragalus membranaceus have been developed for us as a general tonic food and for boosting immunity. Institute of Medicina Plants established in 1983 with branches in provinces of Yunnan, Hainan and Guangxi.	Used in Chinese herbal medicine to strengthen the vital energy Qi in general health and well being. Product widely used in China, and South Asia. Work deals with the development, conservation and utilisation of medicinal plant resources, and the discovery of new potent drugs. The Institute is also recognised as a WHO Collaborating Centre on Traditional Medicine.

Country	Activities	Remark
Domician Republic	Launched in 1982 as a traditional medicine for the islands (TRAMIL) network with support from IRDC, activities focus on developing scientific proven medicinal plant remedies as alternatives to patent drugs that are expensive and difficult to obtain in rural populations.	Regional node was established in Panama in 1994 to cover area from Belize to Panama. Over 150 medicinal plants evaluated and results disseminated in Caribbean Pharmacopoeia.
Estonia	Network of plant genetic resources inclusive of medicinal plants.	Development of computerised genebank system at Jogeva Plant Breeding Institute linking inputs from the Polli Horticultural Institute and the Estonian Agricultural University and Botanical Garden.
Guatemala	Farmaya Laboratory, following screening of 700 different plants, has developed 15 pharmaceutical products using traditional knowledge of indigenous and rural groups. Farmaya engaged in organic cultivation of medicinal plants, pharmacological research, production of plant-derived pharmaceuticals, and engaged in developing protocols for the safe use of medicinal plants.	Created a National Commission for the Use of Medicinal Plants which serves as a model for other Latin American countries in developing guidelines and standardised protocols for production of plant-based pharmaceuticals. Co-operates in the IDRC project on the Application, Research and Dissemination of the Use of Medicinal Plants in the Caribbean. Collaborates with the Central America Centre of Studies on Appropriate Technologies (CEMAT)

Country	Activities	Remark
India	Herbal Gene Bank at the Tropical Botanic Garden Research Institute at Thiruvananthapurnam. Germplasm Bank, Point Calimere Wildlife Sanctuary Tamil Nadu.	All-India ethnobiological project for the development of drugs from medicinal plants and herbs. Promotion of ethnopharmacological research. More than 40 species of medicinal plants are maintained and protected. Examples are Manilkara hexandra to treat jaundice, Salvadora persicum to treat ulcers; Mucuma purata used for preparation of a health tonic.
Japan	Research Centre of Medicinal Resources Medicinal Plant Gardens, Chiba University.	Chemical, biochemical and pharmacological studies on plant secondary metabolites. Pharmacological studies of neurotoxic proteins in Lathyrus sativus. Screening for biologically active products in Asian medicinal plants.
Malaysia	Network of plant genetic resources.	Screening of marine and terrestrial biochemical diversity for medicinal principles, phytomedicinals and nutraceuticals.
Malta	Medicinal plants are widely used as part of folk medicinal.	Well-known Maltese examples are: *fejgel, faqqus il-hmir* and *hobbeja*.
Myanmar	Conservation of plant genetic resources and medicinal plants.	Research programmes at Yangon (formerly Rangoon) University focus on folk medicinal herbs; pharmacognostic studies; and bioassay of plants credited with anti-tumour, antipyretic and anti-diabetic properties.

Country	Activities	Remark
Nepal	Plant biotechnology, mushroom cultivation, bioenergy production, environmental microbiology and medicinal plants.	University programmes in plant tissue culture and environmental microbial-based technologies; medicinal plants widely cultivated in Shivpuri, Doti, Tistung, Urindavan and Tarakava Herbal farms. Herbal products widely marketed as Ayurvedic therapeutics.
New Zealand	Conservation of medicinal plants used in Maori medicine.	Coprosma robusta – a sacred Maori medicinal plant and Aristotelia serrata used by early settlers maintained in nurseries.
Nigeria	Preservation of Nigerian genetic patrimony comprised of 5000 acquisitions of edible, fodder, forest, industrial and medicinal plants.	Research supported by National Centre for Genetic Resources and Biotechnology which functions also as affiliate of International Centre for Genetic Engineering and Biotechnology.
Norway	Collaborative project between UNESCO and Governmental agency.	Conduction of research work in the origins, uses, trades and constraints in the cultivation of medicinal plants in Mozambique and Madagascar by students at M.Sc. level.
Pacific Islands	Noni, a Tahitian herbal tonic derived from *Morinda citrifolia* is used as a general tonic food and energiser	Product widely used in China and South Asia and widely marketed throughout the pacific islands.
Russia	Medicinal Plants reared and protected as economic bioresource in Karadag Reserve.	Karadag Reserve serves as a base for studying the bioecological properties for Crimean medicinal plants such as Rosa canina.

Country	Activities	Remark
Sri Lanka	General biotechnologies, medicinal plants.	Possesses rich history of medicinal plants intricately linked with religious and cultural practices. Ayurvedic system of medicine is widespread.
Thailand	Laboratory of Natural Products – research on medicinal plants.	Species been investigated are: Tai Bai (Phyllanthus amarus), Chai aim Thai (Derris escalata and Carophyllum inophyllum). Based at the Chulabhorn Research Institute, research activities deal with the preparation of dietary supplements and therapeutics from traditional medicinal plants.
USA	National Germplasm Resources Laboratory of the US Department of Agriculture hosts Phytochemeco, a phytochemical/geographic database.	Contains unique blend of phytochemicals taxonomic, ecological, geographic and climatic aspects - phytochemical database contains data on over 16,000 chemical compounds present in some 16,000 plants of economic importance, and of some 1500 specific activities of some 4,000 plant-derived chemicals. - taxonomic database contains plant names of over 8,000 taxa - ecological database contains growing locations of some 6,000 taxa - yield database contains crop yields of some 239 taxa

(Hoareau and Da Silva, 1999)

1.8 Medicinal plants in Bangladesh

About 500 medicinal plants grow in Bangladesh, where 80 per cent of the rural population depends on traditional remedies for ailments such as cough, cold, fever, headache and dysentery. Neem, for example, is used to treat skin disease and in beauty care products.

Turmeric is used as an anti-inflammatory, to treat digestive disorders and skin diseases, and in wound healing (Dold & Cocks, 2001). Despite this, and the presence of more than 400 companies producing herbal medicines, more than 90 per cent of the plants and products needed to meet demand are imported by Bangladesh from other countries such as India, Nepal and Pakistan. According to a December 2003 study by the World Bank's South Asia Enterprise Development Facility and a Swiss development organization 'Inter-cooperation', Bangladesh's medicinal plant market is worth US $14 million each year at wholesale for 17,000 tonnes of final product. The report predicted that the demand for imported raw materials would increase by US $4.9 million within five years. But at present medicinal plants are not commercially farmed in Bangladesh and are only used when gathered from the wild. Plants such as garlic, mint, turmeric and neem could boost Bangladesh's economy if planted on a larger scale, even if it is just in villagers' backyards. Although Bangladesh has no government policy or regulations about growing, conserving and marketing medicinal plants, some universities and non-governmental organizations are collaborating to boost the country's production of the plants (Schippmann *et al.*, 2002).

1.9 Plant Profile

1.9.1 Botanical Name

Ixora Coccinea L.

The species name *coccinea* is a latin derivative which means scarlet coloured.

1.9.2 Synonym

Ixora grandiflora Bot, *Ixora bandhuca* Roxbg.

1.9.3 Taxonomic Hierarchy of the Plant

Kingdom: Plantae

Order: Gentianales

Family: Rubiaceae

Subfamily: Ixoroideae

Tribe: Ixoreae

Genus: *Ixora*

Species: *Ixora coccinea*

1.9.4 Vernacular Names

Table 1.2: List of vernacular names of *Ixora coccinea*

Bengali	Rangan, Ranjan
Tribal	Kaya Machaoi (Marma)
English	Jungle-flame lxora, Flame of the Woods, Jungle Geranium

1.9.5 Habitat and Distribution

Ixora coccinea is Cultivated in gardens throughout Bangladesh.

It is also a common flowering shrub native to Southern India and Sri Lanka and widely cultivated in Indonesia, Malaysia, the Philippines, Vietnam, Cambodia, Laos and Thailand. It has become one of the most popular flowering shrubs in South Florida – USA gardens and landscapes.

It grows in tropical areas with in medium annual rainfall in well drained soils. (Plants Rescue, N.D.)

1.9.6 Description

The plant is a dense, multi – branched evergreen shrub, commonly 4-6 ft (1-2-2m) in height, but capable of reaching up to 12ft (3.6 m). Leaves are oblong are about 10 cm long, with entire margins and are carried in opposite pairs or whorled on the stem. They are sessile to short – petiolate, blades elliptic, oblong or obovate, usually leathery, base cordate to rounded, apex rounded, mueronate or shortly tapering; stipules basally

sheathing, lobes Triangular and strongly acon – tipped. Flowers sessile; calyx lobes short, triangular, persistent, corolla tube usually 1-1.5 inches long, lobes lanceolate to ovate, less than 0.25 inches long, acute or sometimes obtuse fruit thinly fleshy and reddish black. (Vadivu R *et al.*, 2009)

Figure 1.5: *Ixora coccinea*

1.9.6.1 Leaves

Leaf arrangement: whorled

Leaf type: simple

Leaf margin: entire

Leaf shape: ovate

Leaf venation: pinnate

Leaf type and persistence: evergreen

Leaf blade length: 2 to 4 inches

Leaf color: green

Figure 1.6: Leaves of *Ixora coccinea*

1.9.6.2 Flower

Flower color: Red, Yellow, Pink, White, Orange

Flower characteristic: Year-round flowering

Figure 1.7: Flowers of *Ixora coccinea*

1.9.6.3 Fruits

Fruit shape: round

Fruit length: less than .5 inch

Fruit cover: fleshy

Fruit color: purple

Figure 1.8: Fruits of *Ixora Coccinea*

1.9.7 Traditional Uses of *Ixora Coccinea*

Roots are sedative and stomachic; used in hiccup, fever, gonorrhoea, diarrhoea and dysentery. The flowers are used in the treatment of dysentery, leucorrhoea, dysmenorrhoea, haemoptysis and catarrhal bronchitis. The leaves are used in diarrhoea (Yusuf *et al.* 2009). EtOH(50%) extract of aerial parts is spermicidal and CNS depressant (Asolkar *et al*., 1992).

1.10 Study Design

Modern medicines are intimately related to chemistry and detailed examinations of active principles of plants and other products from an essential part of it. The healing properties of the plants are due to the presence of physiologically active chemical compounds inside the plant materials. The phytochemical investigation or screening is an evaluatory process for the detection of plant constituents through chemical analysis; phytochemical screening is co-related with phytochemical study. The compounds isolated through phytochemical study are applied on treated animal to find out the pharmacological effect either beneficial or toxic and thus toxic plants are separated.

In this research work Chloroformic extract of *Ixora coccinea* were evaluated for central nervous system depressant activity and analgesic activity. It is found that chemical constituent such as epicatechin, ixoratannin A-2, procyanidin A2, cinnamtannin B-1 and the flavon-3-ol rhamnosides is present in the leaves of Ixora coccineaa which can be extracted out by Chloroform. (Lim, 2014)

1.11 Study Protocol

Present study was designed so that the medicinal effect of Chloroform extract of *Ixora coccinea* plant can be observed. The study protocol consists of following steps:

1. Preparation of Chloroform extract of *Ixora coccinea* plant.
2. Investigation of CNS depression activity of Chloroform extract on Swiss albino mice.
3. Investigation of analgesic activity of Chloroform extract on Swiss albino mice.

1.12 Study Objective

The objective of this study was to biologically evaluate the effect of Chloroform extract of *Ixora coccinea* on Central Nervous System, as well as to evaluate its analgesic property. There is medical flow about this plant and that is possesses various medicinal properties due to which it was extensively used as traditional medicine. Therefore, the objective of this work is to explore the possibility of developing new drug candidates from this plant for the treatment of various diseases.

Chapter 02

Literature Review

2.1 Pharmacological Studies

2.1.1 Anti-oxidant activity

The anti-oxidant activity of the methanol extract of *Ixora coccinea* L by DPPH free radical scavenging assay, reducing power and total antioxidant capacity using phosphor molybdenum method. Preliminary phytochemical screening revealed that the extract of the flower of *Ixora coccinea* possesses flavonoids, steroids and tannin materials. The methanolic extract showed significant activities in all antioxidant assays compared to the standard antioxidant in a dose dependent manner and remarkable activities to scavenge reactive oxygen species (ROS) may be attributed to the high amount of hydrophilic phenolics. In DPPH radical scavenging assay the IC_{50} value of the extract was found to be 100.53 µg/mL while ascorbic acid had the IC_{50} value 58.92 µg/ml. Thus *Ixora coccinea* extract showed strong reducing power and total antioxidant capacity. (Moni Rani *et al.*, 2008)

2.1.2 Anti-inflammatory activity

The anti-inflammatory potential of an aqueous leaf extract (ALE) of *Ixora coccinea* (Rubiaceae) in rats by carrageenan-induced paw edema (acute inflammatory model) and cotton pellet granuloma tests (chronic inflammatory model) at oral. In the former test, ALE significantly impaired both early and late phases of the inflammatory response and also the edema maintained between the two phases. In the latter test, it significantly suppressed granuloma formation (only highest dose tested). Collectively, these data show promising anti-inflammatory activity against both acute and chronic inflammation. (Ratnasooriya *et al.*, 2005)

2.1.3 Anthelmintic activity

The anthelmintic activity of *Ixora coccinea* roots in different extracts against Indian earthworm Pherituma posthuma. Chloroform soluble fraction showed good anthelmintic activity than Ethyl acetate soluble, Methanolic and petroleum ether extract. (Surana *et al.*, 2011)

2.1.4 Anti-asthmatic activity

Study of an hydroalcoholic leaf extract in ovalalbumin-induced asthmatic rat model showed anti-asthmatic activity suppressing airway inflammation and airway hyperactivity. It also showed inhibitory effect on immediate allergic reactions probably mediated by reducing the release of mediators such as histamine from mast cells. (Missebukpo *et al.*, 2011)

2.1.5 Anti-diarrhoeal Activity

The anti-diarrhoeal activity of aqueous extract of the leaves of *Ixora coccinea* against a castor oil induced diarrhoea model in rats. The gastrointestinal transit rate was expressed as the percentage of the longest distance which was traversed by the charcoal, divided by the total length of the small intestine. The weight and the volume of the intestinal content induced by castor oil were studied by the enteropooling method. Loperamide was used as a positive control. The plant-extract showed significant ($P < 0.001$) inhibitor activity against castor oil induced diarrhoea and castor oil induced enter pooling in rats at the dose of 400 mg/kg. There was significant reduction in gastrointestinal motility by the charcoal meal test in rats. (Prabu *et al.*, 2010)

2.1.6 Hypoglycaemic and Hypolipidaemic activity

The hypoglycaemic and the hypolipidaemic activity of the aqueous extract of the leaves of *Ixora Coccinea* Linn. in alloxan induced diabetic albino rats. The aqueous extract of leaves of *Ixora Coccinea* showed significant reduction ($p < 0.01$) in the blood glucose levels and the serum lipid profile levels, with 400 mg/kg of body weight in the alloxan induced diabetic rats as compared to the controls. (Yasmeen and Prabu, 2011)

2.1.7 Hepatoprotective activity

Extract of IC flowers showed significant hepatoprotective effect against paracetamol overdose-induced hepatotoxicity in rats. (Latha *et al.*, 2010)

2.1.8 Wound healing activity

The wound healing activity of alcoholic extract of the flowers of *Ixora coccinea* by using a dead space wound model in rats. Significant increases in granuloma tissue

weight, tensile strength, hydroxyproline and glycosaminoglycan content were observed in extract treated rats. The prohealing actions seem to be due to increased collagen deposition as well as better alignment and maturation. The drug induced a hypertropic effect on the thymus gland but had no effect on the adrenals. (Nayak *et al.*, 1999)

2.1.9 Antinociceptive activity

Study showed the aqueous leaf extract of IC possesses considerable antinociceptive activity mediated centrally via a dopaminergic mechanism. In addition, the antioxidant activity may play a role in inducing antinociception. The dopaminergic and antioxidative activities may arise from alkaloid and flavonoid constituents, respectively. (Nayak *et al.*, 1999)

2.1.10 Cytotoxic and Antitumour activity

Study of the active fraction of Ixora coccinea flowers showed greater activity on ascitic tumors than solid tumors. It had no toxicity to normal lymphocytes but was toxic to lymphocytes from leukemic patients. (Latha and panikar, 1998)

2.1.11 Anti-ulcer activity

Study of the fresh leaf extract of Ixora coccinea was found to possess potent anti-ulcerogenic property and could be a potential therapeutic agent against ulcer disease. (Arunachalam *et al.*, 2009)

2.1.12 Antimicrobial activity

The antimicrobial activity was performed on 50% ethanolic extract of *Ixora coccinea*. The effective inhibitory concentration of extract for both bacteria and fungus was found to be 125 µg/ml beyond which the inhibitory activity declined and organism started reviving from antimicrobial principle. (Latha PG *et al.*, 1995)

2.2 Chemical Constituents

The essential oil of *Ixora coccinea* flower was obtained by hydrodistillation and analyzed by Gas Chromatograph (GC). Fifty-four components have been identified in the essential oil of *Ixora coccinea* flower, representing 99.97% of the total components detected. The oil is composed mainly of triterpenes 62.60%, monoterpenes 31.73%, sesquiterpenes 3.35% and an ester 2.29%. The major constituents of triterpenes were ursolic acid (27.34%), oleanolic (20.16%) and lupeol (15.10%). *Ixora coccinea* flower is of ursolic acid chemotype. Geranyl Acetate (8.74%) is the major monoterpenes, followed by Linalyl acetate (6.79%), α–Terpineol acetate (4.91%), and Borneol acetate (4.77%); Ethyl cinnamate (2.29%) an ester while the sesquiterpenes are Cyperene (2.72%) and α–Copaene (0.63%). (Gloria and Gibson, 2011).

Chapter 03

Materials & Methods

3.1 Collection and Preparation of *Ixora coccinea*

3.1.1 Collection and Identification of the Plant material

The leaves of Ixora coccinea were collected from Narayanganj, in March, 2016. The sample was collected and identified by experts in Bangladesh National Herbarium, Mirpur, Dhaka, where a Voucher specimen has been deposited for future reference. In the local area this plant is known as 'Rangan'.

The accession number is: *Ixora coccinea* L. - 43416

The specimen samples are kept in the Bangladesh National Herbarium.

3.1.2 Preparation of *Ixora coccinea* extract

3.1.2.1 Drying of the fresh leaves of *I. coccinea*

The plant was thoroughly washed with water. All the unwanted materials were discarded and spread in thin layers on poly bag and placed for shadow drying for 1 week.

3.1.2.2 Grinding and storage of the dried samples

The dried leaves were ground to coarse powder with a mechanical grinder (Grinding Mill). This process breaks the plant parts to smaller pieces thus exposing internal tissues and cells to solvents thus facilitating their easy penetration into the cells to extract the constituents. Then the powdered sample was kept in clean closed glass containers till extraction. During grinding of sample, the grinder was thoroughly cleaned to avoid contamination with any remnant of previously ground material or other extraneous matters deposited on the grinder. The weight of the total dry powder was 945 gm.

3.1.2.3 Extraction of dried powdered sample

Total amount of dried powder of sample was 945 gm. From the total amount, 315 gm of powder was soaked in Chloroform for extraction.

The powder is soaked in 1000 ml of Chloroform in amber color glass container for 7 days. When the powder became exhausted of its chemical constituents and to facilitate the process regular shaking was continued for those 7 days. After the completion of extraction process, the liquid was filtered in three steps. At first, it was done by using

sterilized cotton cloth, then by using sterilized cotton filter and finally by No. 1 Whatman filter paper. Then solvent was completely evaporated by rotary evaporator at 60 degree celsius. The yield is preserved in a petridish in refrigerator.

3.2 Drugs

Diazepam and Indomethacin were used for current study which was supplied from Square Pharmaceuticals Ltd, Bangladesh. SANDOZ respectively.

3.3 Experimental Animal

For the pharmacological investigation 40 mice were collected from ICDDRB, Bangladesh. The average weights of the mice were 20 to 25 gm. Standard environmental situation was maintained to keep the mice. The condition was 55-65% relative humidity, 12 hours light/dark cycle and 24.0 ± 2°C temperature. Also sufficient amount of food and water was supplied all the time.

3.4 Ethnical Approval

Institutional animal ethical committee accepted the guidelines which were followed for animal test.

3.5 Pharmacological Investigation of Plant Extract

The following pharmacological investigations were done to determine the medicinal effect of the experimented extracts:

- ✓ CNS depressant activity and
- ✓ Analgesic activity

3.5.1 Study of CNS Depressant Effect of Chloroform Extract

CNS Depressant drugs are the agents which slow down the activity of brain. These types of drugs are prescribed by doctor for the treatment of panic attack, anxiety, insomnia etc. Mostly CNS Depressants activate GABA neurotransmitter. This helps in decreasing brain activity.

The CNS depressant action of *Ixora coccinea* plant extracts were observed by comparing with the standard diazepam in the experimented rodents. CNS depressant activity was determined by using two techniques. They are:

- Open field test
- Hole cross test

3.5.1.1 The Design of the CNS depressant Experiments:

In both methods 24 mice were chosen randomly and then divided into 4 groups. They were group 1 to group 4 where 6 mice were in each group. A particular treatment was given to each group. Before this specific treatment, weight of every mouse was measured accurately as well as marked. Also the dosage of the sample and standard were also settled according to body weight.

Group 1 - Chloroform 200 mg/kg
Group 2 - Chloroform 400 mg/kg
Group 3 - Standard (Diazepam)
Group 4 - Control (Distilled Water)

3.5.1.2 Preparation of drug and chemical solution:

In order to administer the crude extract of chloroform at dose 200 & 400 mg/kg body weight of mice. The extract was collected by calculating of mice weight & was sonicated in unidirectional way by the addition of 3 ml of distilled water. For proper mixing, small amount of suspending agent CMC was slowly added. The final volume of the suspension was made up to 4.5 ml. To stabilize the suspension it was stirred well. For the preparation of positive control group (1 mg/kg) Diazepam is taken & a suspension of 4.5 ml is made.

Table 3.1: Test samples used in the estimation of CNS Depressant activity of *Ixora coccinea* plant

Group	Treatment	Dose	Route of Administration
Group 1 (Extract)	ICC	200 mg/kg	Orally
Group 2 (Extract)	ICC	400 mg/kg	Orally
Group 3 (Standard)	Diazepam	1 mg/kg	Orally
Group 1 (Control)	Distilled Water	10 ml/kg	Orally

3.5.1.3 Open Field Test

Gupta's open field method (Gupta *et al.*, 1971) was followed to carry out open field test. The box was half square meter as well as divided into squares each. On the other hand the box was black and white colour like a chess board. The apparatus had a wall which was 40cm in height. For 3 minutes, each square was counted which was visited by mice. Also, during the study period, several results were taken on 0, 30, 60, 90 and 120 minutes.

Figure 3.1: Open field test

The flow chart of procedure for evaluation of CNS depressant effect of *Ixora coccinea* plant by open field test is shown below:

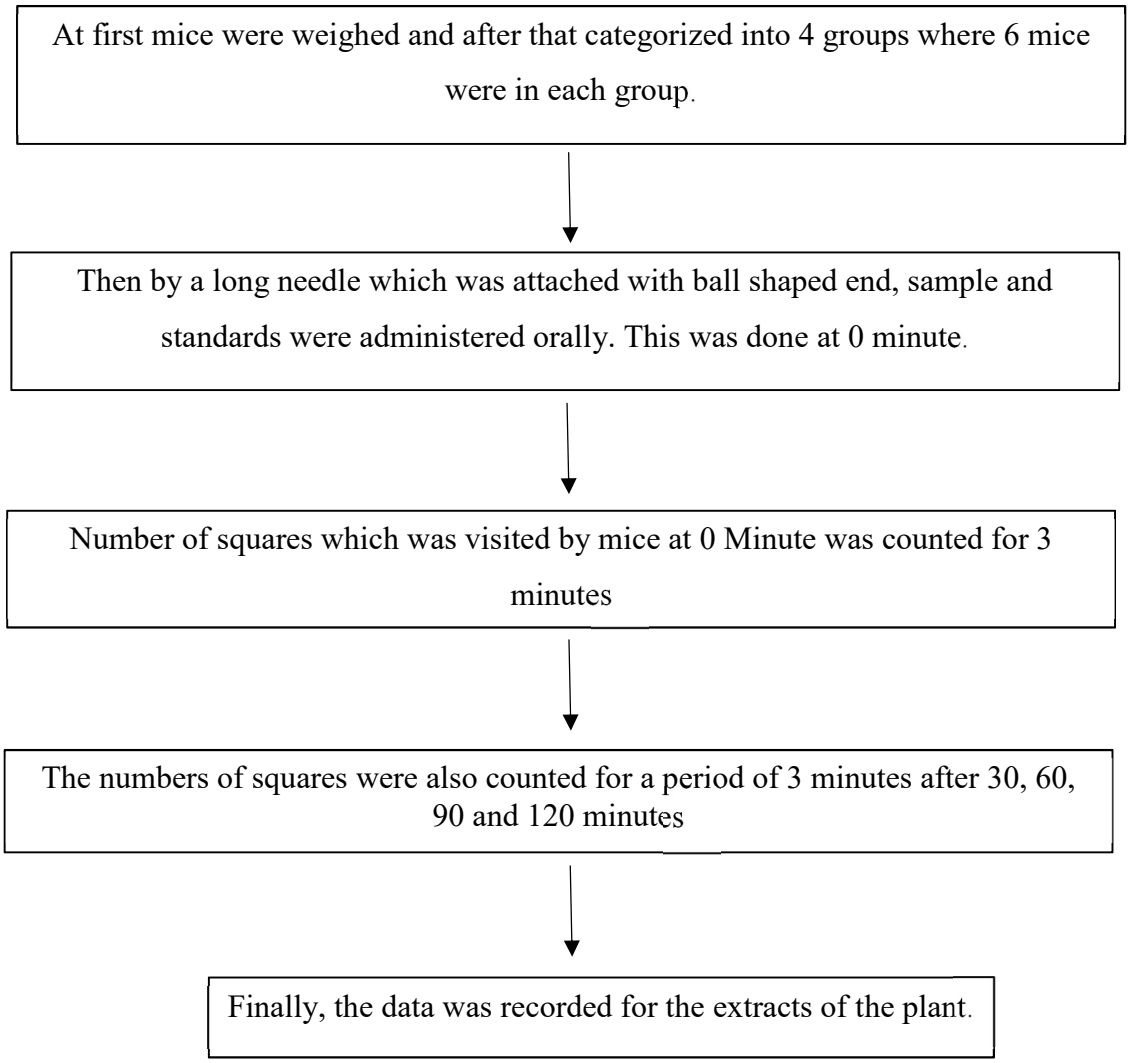

Figure 3.2: Flow chart of process for CNS depressant activity on mice by open field test

3.5.1.5 Hole Cross Test

The main purpose of Hole Cross test to analyze the locomotor and exploratory effects of the extract by using the hole-board on mice. Takagi's method (Takagi *et al.*, 1971) was followed to examine the test. The box where the hole-board test was tested, a size of 30 x 20 x 14 cm was measured.

The flow chart of procedure for evaluation of CNS depressant effect of *Ixora coccinea* plant by Hole Cross test is shown below:

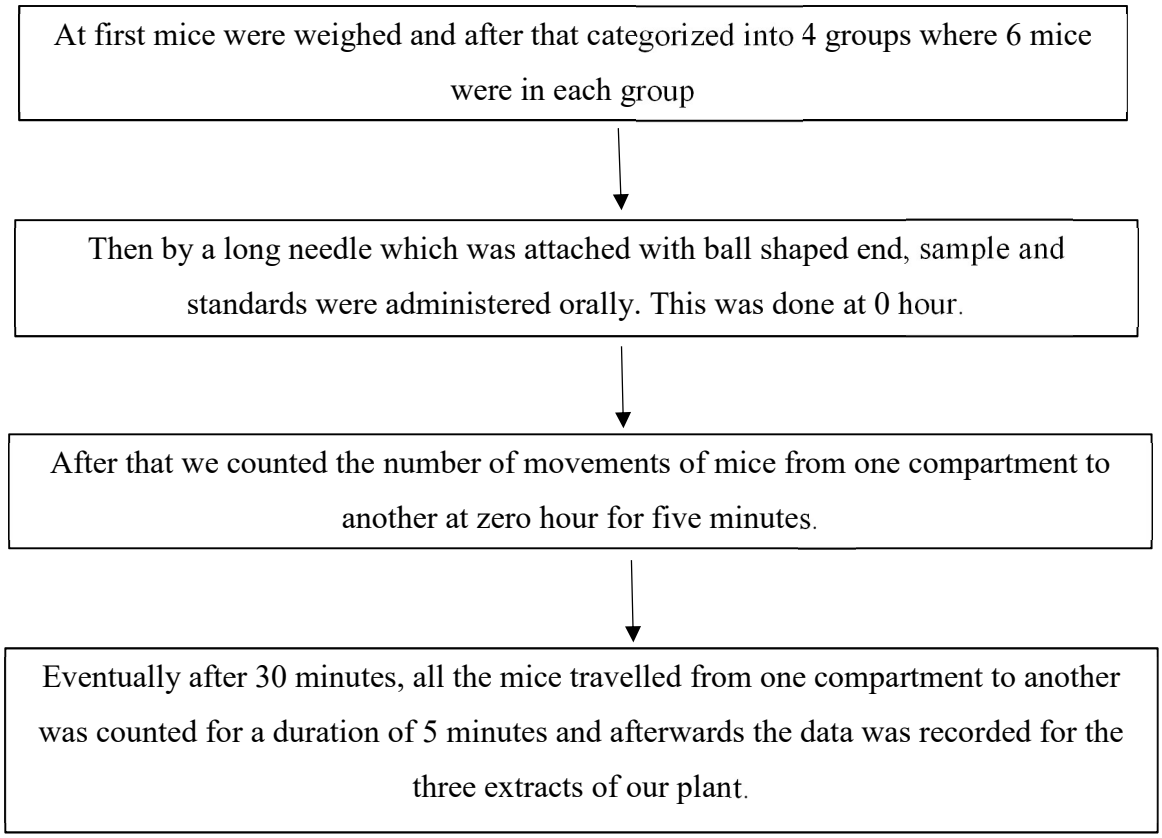

Figure 3.3: Flow chart of process for CNS depressant activity on mice by Hole Cross method

Figure 3.4: Hole cross test

3.5.2 Analgesic activity of *Ixora coccinea* plant extracts:

Drug which is used to relieve pain is called analgesic drug. These drugs are also known as painkiller. The analgesic test was done by acetic acid induced writhing technique.

3.5.2.1 Design of the analgesic experiments:

24 mice were chosen anyway and divided into 4 groups where the groups were from group 1 to group 4 as well as 6 mice were in each group. Each group got a specific treatment. Before the treatment, each mouse were weighed properly as well as marked. Then the dosage of the test sample and control materials was also settled according to body weight.

Group 1 – Chloroform 200 mg/kg

Group 2- Chloroform 400 mg/kg

Group 3- Standard (Indomethacin)

Group 4 – Control (Distilled Water)

3.5.2.2 Acetic acid-induced writhing technique:

Acetic acid induced writhing test is a technique where analgesic behaviour is observed. In this method (Ahmed *et al.*, 2001) intra-peritoneally acetic acid was administered to the mice so that pain sensation generates. Here, indomethacin was considered as standard. At first the distilled water, extracts at a dose of 200 mg/kg and 400 mg/kg as well as standard drug were administered orally. After 30 minutes, the solution of 0.7% v/v acetic acid was administered intraperitoneally. After administration of solution of acetic acid, no writhing was counted for 5 minutes. After 5 minutes, writhing was counted for 15 minutes. For that each mouse was placed on observation table and noticed the number of writhing of mice. The mice did not give full writhing all the time. They gave half writhing also. So, two incomplete writhing were counted as one complete writhing.

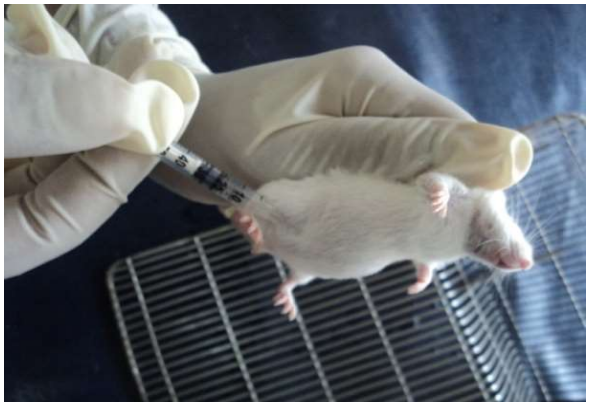

Figure 3.5: Peritoneal acetic acid administration

3.5.2.2.1 Preparation of standard drug and crude drug

Standard drug, Indomethacin at dose of 10 mg/kg body weight was prepared by adding 10 mg indomethacin in 10 mL distilled water and the solution was triturated in a unidirectional way.

Crude extract solution was prepared at a dose of 200 mg/kg and 400 mg/kg body weight. For that, extracts were mixed with distilled water by sonication. CMC was added as suspending agent for proper mixing.

Table 3.2: Test samples used in the estimation of analgesic activity of *Ixora Coccinea* by acetic acid induced writhing technique.

Group	Treatment	Dose	Route of Administration
Group 1 (Extract)	ICC	200 mg/kg	Orally
Group 2 (Extract)	ICC	400 mg/kg	Orally
Group 3 (Standard)	Indomethacin	1 mg/kg	Orally
Group 1 (Control)	Distilled Water	10 ml/kg	Orally

3.5.2.2.2 Procedure of analgesic activity of *Ixora coccinea* extract by acetic acid induced writhing technique

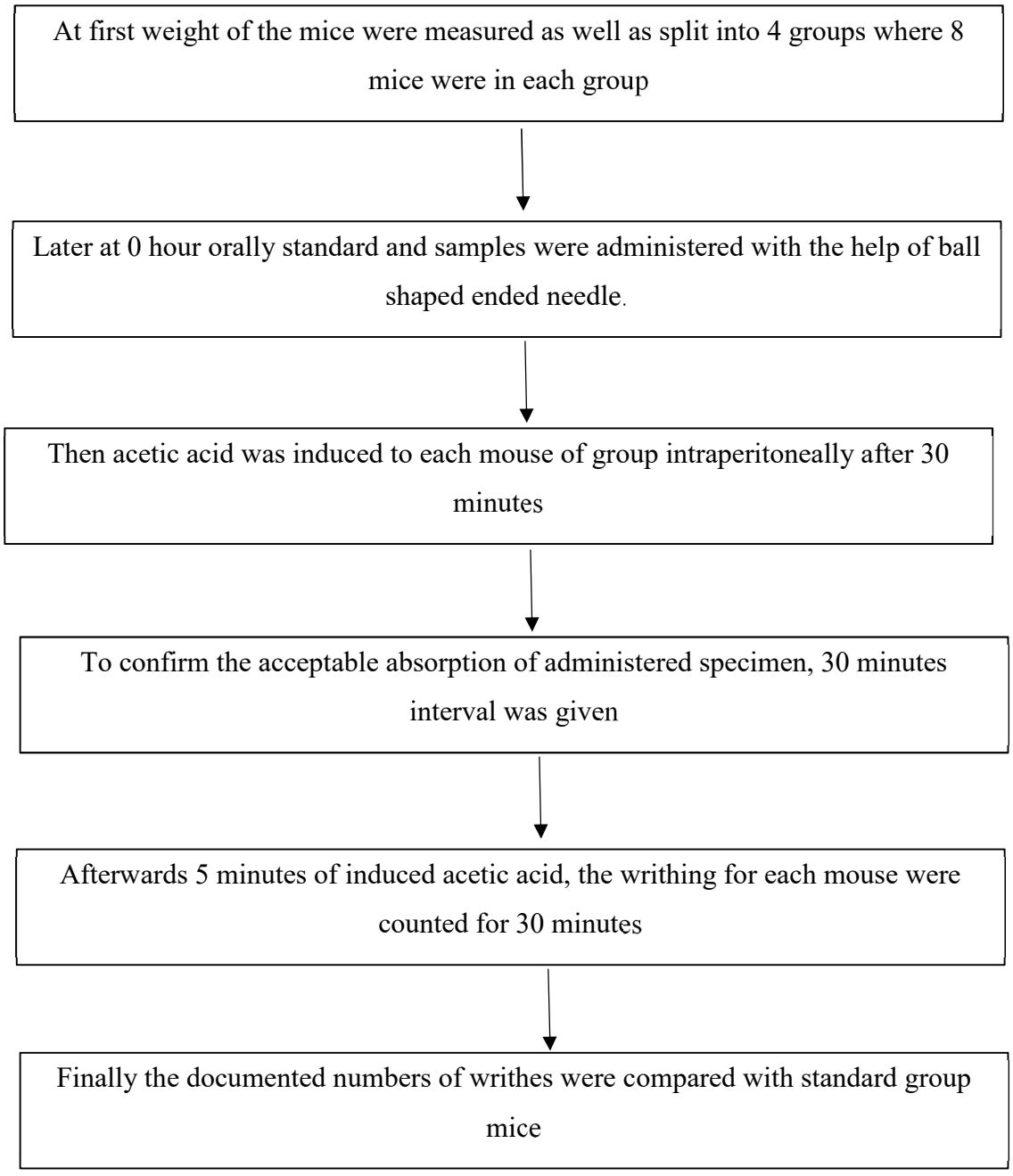

Figure 3.6: Flow chart of process for analgesic activity on mice by acetic acid induced technique

Chapter 04

Result & Discussion

4.1 Open Field Test

4.1.1 Result

From the Table 4.1 it is found that, central and peripheral locomotion count for chloroformic extract of *I. coccinea* for both 200 & 400 mg/kg body weight was increased as the time proceeded it was due to the sedative effect and reduction in spontaneous locomotion. Significant ($P < 0.001$) result was found at 60 min, 90 min and 120 min for both the strengths. In contrast, for standard (Diazepam) at 1 mg/kg body weight the result was significant ($P < 0.001$) at 30 min, 60 min, 90 min and 120 min.

Table 4.1: Effect of chloroformic extracts of *Ixora coccinea* in mice by Open-field method

Group	Treatment	Dose	Number of Movement				
			0 Minute	30 Minute	60 Minute	90 Minute	120 Minute
Group - 1 (Extract)	ICC	200 mg/ kg	113.33 ± 18.24	98.5 ± 11.78	85.17 ± 26.42***	61 ± 20.03***	57.83 ± 27.78***
Group - 2 (Extract)	ICC	400 mg/ kg	148.5 ± 26.30	74.33 ± 28.11**	38.33 ± 30.76***	38.67 ± 29.53***	36.17 ± 27.52***
Group - 3 (Standard)	Diazepam	1 mg/ kg	137.5 ± 44.64	36.17 ± 26.09***	68.33 ± 39.40***	35.67 ± 26.64***	30.33 ± 39.07***
Group - 4 (Control)	Water	10 ml/ kg	137.5 ± 44.64	126 ± 32.79	159.67 ± 15.47	162.5 ± 25.33	153 ± 23.04

Here, ICC means *Ixora coccinea* in Chloroform. Number of writhing values are mean ± S.E.M., (n=6). *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ significantly different from control; done by Dunnet test using Excel 2007 and SPSS 17.00 software.

4.1.2 Discussion:

The most important step in evaluating drug action on CNS is to observe its effect on locomotor activity of the animal. The locomotor activity is a measure of the level of excitability of the CNS, and decreased activity results from CNS depression (Uma *et al.,* 2011). The result showed that the 200 mg and 400 mg of ICM causes sedative effect,

reduction in spontaneous motor activity, exploratory behavior and motor coordination. Decreasing central locomotion count and peripheral locomotion count, supports the evidence of reduction of motor activity in mice. The standard drug (Diazepam) exerts CNS depressant action by stimulating GABAA, ligand-gated chloride-selective ion channels that are activated by GABA to inhibit the release of neurotransmitter. (Camposoria *et al.*, 2006) *Ixora coccinea* leaves are rich in flavonoids and tannin contents such as epicatechin, ixoratannin A-2, procyanidin A2, cinnamtannin B-1 and the flavon-3-ol rhamnosides (Donthan *et al*., 2015). Flavonoids can produce sedation followed by depression effect by stimulating GABA. (Hernandez et al., 2016). Therefore, the result of the experiment and reports of having high flavonoids strongly suggest that the mechanism of action of ICC may be linked to GABA stimulation and neurotransmitter release inhibition.

4.2 Hole Cross Test

4.2.1 Result

Chloroformic extract of *I. coccinea* at both 200 mg/kg body weight dose, produced insignificant and significant (p<0.001) at 0 min and 30 min, respectively, and at 400 mg/kg body weight dose the result is significant (p<0.001). This indicates decrease of locomotion from its initial value during the period of experiment by the Hole-cross method. Maximum suppression of locomotor activity was for reference drug diazepam at 1 mg/kg body weight which was significant (p<0.001).

Table 4.2: Effect of chloroformic extracts of *Ixora coccinea* in mice by Whole-cross method

Group	Treatment	Dose	Number of Movement	
			0 Minute	30 Minute
Group - 1 (Extract)	ICC	200 mg/ kg	48.17±10.15	30.83 ±10.07***
Group - 2 (Extract)	ICC	400 mg/ kg	28.17 ±6.55***	19.67 ± 8.48***
Group - 3 (Standard)	Diazepam	1 mg/ kg	19 ± 5.22***	9.83 ±6.31***
Group - 4 (Control)	Water	10 ml/ kg	58.5 ± 4.93	56.33 ±4.50

Here, ICC means *Ixora coccinea* in Chloroform. Number of writhing values are mean ± S.E.M., (n=6). *$P <$ 0.05, **$P < 0.01$, ***$P < 0.001$ significantly different from control; done by Dunnet test using Excel 2007 and SPSS 17.00 software.

4.2.2 Discussion

The study shows decrease in locomotion of mice from its initial value during the period of experiment by the hole cross method. Since locomotor activity is a measure of the level of excitability of the CNS, this decrease in spontaneous motor activity could be attributed to the CNS depressant effect of the plant extracts. The activity may be due to the flavonoids present in the extracts (Hasan, 2009) that may interact with the gamma aminobutyric acid (GABA) type A receptors in the brain.

4.3 Acetic Acid Induced Writhing Test

4.3.1 Result

Table 4.3 represents the effect of chloroformic extracts of *Ixora coccinea* in Acetic Acid Induced Writhing test. The chloroform extract inhibited writhes in a dose dependent manner. Percent inhibition is 30.43% for 200 mg/kg body weight and 37.46% ($p < 0.05$) for 400 mg/kg body weight, whereas for Standard drug (Indomethacin) the percent inhibition is 86.96% ($p < 0.001$) for 10 mg/kg body weight. So, it can be conclude that the acetic acid induced pain inhibitory activity of 400 mg/kg body weight of chloroformic extract of *I. coccinea* is significant ($p < 0.05$).

Table 4.3: Effect of chloroformic extracts of *Ixora coccinea* in Acetic Acid Induced Writhing test

Group	Treatment	Dose	No. of Writhing (Average ± S.E.M)	Percent of Inhibition
Group - 1 (Extract)	ICC	200 mg/ kg	34.67±10.61	30.43
Group - 2 (Extract)	ICC	400 mg/ kg	31.17±4.96*	37.46
Group - 3 (Standard)	Indomethacin	10 mg/ kg	6.50 ± 4.59***	86.96
Group - 4 (Control)	Water	10 ml/ kg	49.83 ± 12.48	---

Here, ICC means *Ixora coccinea* in Chloroform. Number of writhing values are mean ± S.E.M., (n=6).

*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ significantly different from control; done by Dunnet test using Excel 2007 and SPSS 17.00 software.

4.3.2 Discussion

Acetic acid induced writhing model represents pain sensation by triggering localized inflammatory response. Such pain stimulus leads to the release of free arachidonic acid from tissue phospholipids (Ahmed *et al*., 2006). The acetic acid induced writhing response is a sensitive procedure to evaluate peripherally acting analgesics. The response is thought to be mediated by peritoneal mast cells (Ronaldo *et al*., 2000), acid sensing ion channels (Voilley, 2004) and the prostaglandin pathways (Hossain *et al.*, 2006).

Chapter 05

Conclusion

5. Conclusion

From the thousands of years, nature is giving us medicinal gift which act as natural source of modern drugs. One of those gifts is *Ixora coccinea* which contains pharmacological activity. To examine the pharmacological activity, a number of experiments were done. Among them one was analgesic test (acetic acid induced writhing method) and the other was CNS depressant test (open field method and hole cross method) which was compared with the standard indomethacin and diazepam respectively. After observing the results of recent study, it can be said that chloroform extract of the experimental plant at dose of 200 mg/kg and 400 mg/kg showed significant ($p<0.001$) CNS depressant activity compare to control group. The extract inhibited writhes in a dose dependent manner in analgesic test. At dose of 400 mg/kg showed significant ($p < 0.05$) analgesic activity compare to control group.

So it is clear that the experimental plant is helpful plant and the work was only preliminary effort which will require further comprehensive exploration as well as depiction of active compounds and necessitates preformulation studies for expansion of a potential dosage form.

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