INTRODUCTION

Success in drug discovery and development depends greatly on the therapeutic value of the bioassay performed in the first screening and on the effort required to identify the first promising lead compounds which can act on the basis for starting powerful structure/activity relationship studies. Traditionally performed screening attempts of crude extracts and subsequent isolation of the bioactive principle dominate natural products screening up to the present. The traditional natural product screening is done by testing crude extracts, followed by the crucial work of back tracking the active compounds from the 'hit' extracts. The drugs used by ancient civilizations were extracts of plants or animal products with a few inorganic salts. Issues of interest not only comprise bioactivity at the target of interest but also applicability, bioavailability, biostability, metabolism, toxicity, specificity of bioactivity, distribution, tissue selectivity, cell penetration properties and as a key prerequisite for development access to large quantities of the compound for clinical studies and commercialization has to be guaranteed (Grabley and Thiericke, 1999).

The ethnopharmacological approach provides the way for developing new plant-derived drugs, particularly in the areas of cancer, immunosuppression, hypocholesterolemic agents, inflammation, rheumatism, pain, diarrhea, and diabetes. In the last few years efforts have been directed to identify new potent analgesics, anti-inflammatory, antipyretic, anti-diarrheal and anti-ulcer drugs from natural sources. Plants are known for sources such as carbenoxolone from Glycyrrhiza glabra, solon from sophoradin, clerodane diterpenes and gefarnate from cabbage (Souza-Brito et al. 1998; Lewis & Hanson 1991) for various activities. Some terpenes or terpene derivatives display gastroprotective activity in different models of induced gastric lesions in animals.
(Giordano et al, 1990; Lewis & Hanson, 1991; Matsuda et al, 1998). A powerful antiulcerogenic effect has been demonstrated for some.

Indigenous remedies may also indicate pharmacological activity for maladies such as schizophrenia, for which the biochemical mechanisms have yet to be discovered. Ethnopharmacological information can be used to provide three levels of resolution in the search for new drugs: (1) as a general indicator of non-specific bioactivity suitable for a panel of broad screens; (2) as an indicator of specific bioactivity suitable for particular high-resolution bioassays; (3) as an indicator of pharmacological activity for which mechanism-based bioassays have yet to be developed (Cox, 1994).

A number of scientific studies have been done on a large number of medicinal plants in India. Inspite of this, we have not developed a considerable number of marketable drugs as plant derived chemical entities or traditional herbal preparations with modern standards of safety and efficacy. This is due to the fact that in India to a large extent integrated research focusing the development of commercially viable and useful phytomedicine is not carried out. Although opportunities for developing Ayurvedic and other traditional herbal drugs with international acceptance are vast, at present there is hardly any concerted effort in this direction in our country. Many research publications on medicinal plants show marginal or insufficient pharmacological activities, which are not satisfactory to develop into a herbal drug or phytomedicine. Further in some cases, a medicinal plant is credited with innumerable pharmacological activities without focusing practical therapeutic benefits.

Nonsteroidal antiinflammatory drugs (NSAID) induced gastropathy is now well-recognised (Roth and Bennett, 1987). The incidence of ulcers in chronic NSAID users is estimated to be 20% (Gabriel and Bonbardier, 1990). Approximately 2000-4000 deaths/year may occur in the USA due to gastrointestinal complications in rheumatoid
patients who are chronic NSAID uses (Fries, 1988). The characteristics of NSAID gastropathy contrast with their classic peptic ulcer disease (Roth and Bennett, 1987). The development of less GI-toxic NSAIDs is therefore, an important objective.

*Calotropis gigantea* R.Br. Asclepiadaceae, commonly known as milkweed or swallow-wort, is a common wasteland weed (Singh and Chaudhuri, 1996). *Calotropis* belongs to Asclepiadaceae or Milkweed or Ak family which includes 280 genera and 2,000 species of world-wide distribution but most abundant in the sub-tropics and tropics, and rare in cold countries. Other familiar plants of *Calotropis* are Milk weed or Silk weed (*Asclepias syriaca* L.), Butterfly weed (*Asclepias tuberosa* L.) and *Calotropis procera* (Ait.) Ait.f. Native to India (Lindley, 1985), *Calotropis* grows wild up to 900 meters throughout the country (Sastry and Kavathekar, 1990) on a variety of soils in different climates, sometimes where nothing else grows.

*Calotropis* is used as a traditional medicinal plant (Rastogi and Mehrotra 1991; Oudhia and Dixit 1994; Oudhia 1999a,b,c,d) with unique properties (Oudhia and Tripathi 1998, 1999a). Traditionally calotropis is used alone or with other medicinals (Caius, 1986) to treat common disease such as fevers, rheumatism, indigestion, cough, cold, eczema, asthma, elephantiasis, nausea, vomiting, diarrhea (Das, 1996). According to Ayurveda, dried whole plant is a good tonic, expectorant, depurative, and anthelmintic. The dried root bark is a substitute for ipecacuanha. The root bark is febrifuge, anthelmintic, depurative, expectorant, and laxative. The powdered root is used in asthama, bronchitis, and dyspepsia. The leaves are useful in the treatment of paralysis, arthralegia, swellings, and intermittent fevers. The flowers are bitter, digestive, astringent, stomachic, anthelmintic and tonic (Agharkar 1991; Warrier et al. 1995). *Calotropis* is also a reputed Homoeopathic drug (Ghos, 1988; Ferrington, 1990).
Although its blooms can be seen all through the year, its flowers are particularly abundant during *Ganesh puja* season; in earlier days it was the white flowered species that was used for this puja and other auspicious occasions; but it is no longer common. It has been used with marked success in the treatment of *syphilis* following Mercury, elephantiasis, leprosy, acute dysentery, pneumonic phthisis and tuberculosis. It increases the circulation in the skin; has powerful effects as a sudorific. In the secondary symptoms of syphilis, where Mercury has been used but cannot be pushed safely any farther, it rapidly recruits the constitution, heals the ulcers and blotches from the skin, and perfects the cure. *Primary anaemia of syphilis. Heat in stomach* is a good guiding symptom. *Obesity,* while flesh decreases, muscles become harder and firmer (William Boericke, 1999).

The plant has been studied extensively for its important chemical constituents, proteinases calotropain $F_1$ and $F_2$ of latex (Abraham and Joshi, 1979). The milky sap was studied in blood group serology as a proteolytic enzyme (Patil, et al., 1993). Isorhamnetin-3-O-rutinoside, Isorhamnetin-3-O-glucopyronoside, taraxasterol acetate and flavonol trisaccharide were isolated and characterized from the aerial part and their structures were established by combination of fast atom bombardment mass spectroscopy, $^1$H and $^{13}$C NMR spectra and some chemical degradations (Sen, et al., 1992). Pal and Sinha in 1980 isolated, crystallized and studied the properties of cysteine proteinases Calotropins $D_1$ and $D_2$ (Sen Gupta, et al., 1984). Two new oxiopregnane-oligoglycosides named calotropis A and B have been isolated from the root and their chemical structures have been elucidated by chemical spectroscopy methods (Kitagawa et al., 1992). The cytotoxic principles cardenolide glycosides, calotropin frugoside and 4-O-Beta-D-Glucopyranosyl frugoside were also obtained as the cytotoxic principles (Kiuchi, et al., 1998).
However, only few considerable pharmacological properties of this plant have been studied including uterus stimulation (Dhawan and Saxena, 1968), anticancer (Bhakuni, et al., 1969; Dhar, et al., 1968), cardiac stimulant (Kulkarni, et al., 1976), antibacterial (Sasidharan, 1997), antimalarial (Neraliya and Srivastava, 1996) activities. Recently Calotropis gigantea has been studied for its fungicidal and insecticidal properties (Oudhia, 2001). Development-inhibiting activity against Sitophilus zeamais Motschulsky (Haque and Nakakita, 2000). The other plant belonging to the genus calotropis i.e. Calotropis procera was reported to possess a number of biological activities, viz., proteolytic (Atal and Seth, 1961), antimicrobial (Malik and Chaughati, 1979), antifertility (Prakash et al., 1978), larvicidal (Girdhar, et al., 1984), nematocidal (Masood et al., 1980), anticancer activity (Dhar et al., 1968; Ayoub and Kingstron, 1981), anti-inflammatory (Kumar and Basu, 1994; Basu and Nagchandhuri, 1991), anti-diarrheal (Kumar, et al., 2001), etc. However, Calotropis gigantea of the same genus has not been pharmacologically studied. There is a need to explore the pharmacological properties of Calotropis gigantea with safety and efficacy of the plant preparation by studying its toxicological aspects.

The rationale behind this work was to scientifically test the therapeutic folk claims of the plant for its anti-inflammatory, analgesic, antipyretic, antiulcer and antidiarrheal activities along with its detailed acute and chronic toxicological actions. The outcome of the present study will assist to bring out cheap, safe and reliable herbal formulation(s) for its clinical use.

1.2 Objectives

The present study has been designed with following objectives;
1. Evaluate anti-inflammatory, analgesic, antipyretic, antiulcer and antidiarrheal activity of hydroalcoholic extract of *Calotropis gigantea* using experimental animals.

2. Evaluate acute and chronic toxicological effects of *Calotropis gigantea* in mice and rats.

1.3 **Present Investigation**

In regard to the pharmacological actions of *Calotropis gigantea* very little information is available. Accordingly, the objective of the present study have been designed to assess the possible mechanisms involved in the properties of *Calotropis gigantea* by investigating the effects of hydroalcoholic (50:50) extract in various animal models to understand the pharmacological basis for the use of *Calotropis gigantea* in folk medicine for the treatment of various types of illnesses. To our knowledge, this is the first attempt to demonstrate the detailed pharmacological and toxicological activities of *Calotropis gigantea* extract. The extraction procedure adopted was intended to obtain an extraction similar in chemical composition to the one used in the traditional medicine, this may not hold true as far the proportion of individual components are concerned. Traditionally, the administration of the plant extract may also last from weeks to months. The extract was administered intraperitoneally, inspite of the fact this is not the route employed in folk medicine.

The anti-inflammatory properties of hydroalcoholic (50:50) extract of the aerial part of *Calotropis gigantea* was evaluated on the basis of its inhibitory effect on topical, acute and chronic methods by using xylene-induced ear oedema in mice, carrageenan-induced hind paw oedema and cotton-pellet induced granuloma in the rats. The analgesic activity of the plant was evaluated by using the tail-flick latency in rats to study the inhibitory effect on peripheral reflexes, hot-plate model to study the analgesic effect
against thermal stimuli involving central reflexes, and acetic acid-induced abdominal constriction for evaluating peripheral effects using mice. It was evaluated for its antipyretic activity by using yeast-induced and TAB vaccine-induced pyrexia in rats and rabbits respectively. The anti-diarrheal effect of hydroalcoholic (50:50) extract of aerial part of *Calotropis gigantea* was studied against castor oil-induced-diarrhea model in rats. The gastrointestinal transit rate was expressed as the percent of the longest distance traversed by the charcoal divided by the total length of the small intestine. The weight and volume of intestinal content induced by castor oil were studied by enteropooling method. The anti-ulcer activity of extract of *Calotropis gigantea* was studied in pyloric ligation and stress models of ulcer in rats. To determine the safety and viability to use the drug for therapeutic purpose, the acute toxicity was evaluated in mice and chronic toxicity was evaluated in rats.

The thesis reports the results of our investigations carried on these lines. It comprises of four chapters. The first chapter evidently provides a brief introduction to the medicinal plants, their contribution to betterment of health, role of these plants in pharmaceutical preparations, scope of these plants in new drug discovery and development of new drug delivery system. This chapter also gives a brief introduction of the plant selected for the present study.

The second chapter consists of the detailed survey of literature available in this line. The present national and international status of the present investigation is covered in review of literature. It also comprises of the physiological consideration and various screening models can be selected for the evaluation of anti-inflammatory, analgesic, antipyretic, anti-diarrheal and anti-ulcer properties of the medicinal plants. The detailed survey of the studies done on *Calotropis gigantea* is also given in this chapter.
The third chapter deals with the detailed descriptions of materials and methods used for the study. The authentication, collection, preparation of plant extract, methodology of pharmacological and toxicological investigation, statistical analysis to calculate the significance values of the treated groups compared with the control groups.

The fourth chapter deals with the results of the present investigation highlighting the significant differences in the results of treated groups and the control groups. Each table has a brief heading, explanatory matter in footnotes, short or abbreviated column heads has been used and the results are expressed as mean ± S.E. (standard error of the mean). It is followed by the discussion of the results obtained in the present investigation and correlating these results with previous studies to elaborate the possible mechanism of drug action. This section relate to the significance of the work to existing knowledge in the field and indicating the importance and contribution of this study, with a supported hypotheses and speculations.

The last and fifth chapter consists of the summary of present work, explains the methodology used, significant results obtained and the conclusion drawn by present results.