ANTIPYRETIC AND CNS ACTIVITY OF SEEDS FROM RED AND WHITE TYPES OF LOTUS (*Nelumbo nucifera*) IN ALBINO RATS

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Thesis submitted in partial fulfillment of the requirement for the degree of

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DECLARATION

I hereby declare that the thesis entitled **"ANTIPYRETIC AND CNS ACTIVITY OF SEEDS FROM RED AND WHITE TYPES OF LOTUS (*Nelumbo nucifera*) IN ALBINO RATS"** is a bonafide record of research work done by me during the course of research and that this thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

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CERTIFICATE

Certified that this thesis, entitled **"ANTIPYRETIC AND CNS ACTIVITY OF SEEDS FROM RED AND WHITE TYPES OF LOTUS (*Nelumbo nucifera*) IN ALBINO RATS"** is a record of research work done independently by **Dr. Deepa, P.K.** under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, associateship or fellowship to her.

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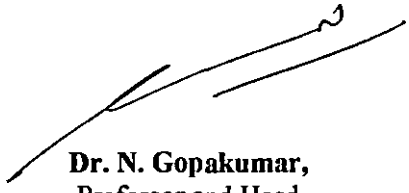

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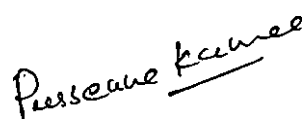

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Introduction

1. INTRODUCTION

Plants and plant derived products form part of health care systems since ancient human civilizations. In India, the history of health care goes back to 5000BC, where use of plants and polyherbal formulations were mainly used for health care practices. Texts like Charaka Samhitha give a detailed description on the use of herbal medicine for a variety of ailments. Throughout the history of drug development, plants are safer and important source for the discovery of novel therapeutically active compounds. WHO estimates that about 80 per cent of the population living in developed countries relies on traditional medicine for their primary health care needs. About 35,000 to 70,000 plant species are being used world wide in health care systems. About 425 of the best selling pharmaceutical products are biologicals or natural products (Mukherjee and Wahile, 2006). Many of the herbal medicines are alternatives for use in various ailments, to which modern medicine has no answer.

Nelumbo nucifera Gaertn. (nymphaeaceae), the Sacred Indian Lotus, commonly known as Kamala, Padma, or Pundarika is the National Flower of India. It is an aquatic herb with stout creeping yellowish white coloured rhizomes and white or red coloured flowers, found everywhere in India (Chopra *et al.*, 1958). It is often cultivated in India for its elegant, sweet scented flowers and in China and Japan in terraced fields for its edible rhizomes and seeds (Anon., 1966).

Almost all parts of this plant are used in traditional medical practice to treat various diseases. The rhizomes, flowers, stalks and leaves are used in the form of infusion in fever as refrigerant and diuretic (Nadkarni, 1992). The rhizomes were also found to be an aromatic and good tonic, increasing the mental facilities and quietening the spirits. Antipyretic property of ethanolic extracts of *Nelumbo nucifera* rhizomes were reported in rats (Mukherjee *et al.*, 1996a). The rhizomes also possessed significant tranquillizing property (Mukherjee *et al.*, 1996b).

Lotus seeds are sold in Indian market in the name of kamal gatta, as a vegetable. Nelumbo seeds are commonly used in folk medicine in the treatment of tissue inflammation, cancer, emesis and given to children as diuretic and refrigerant in skin diseases. The seeds have proven free radical scavenging and antifertility activity along with the ability to suppress cell cycle progression, cytokine gene expression and cell proliferation in human peripheral mononuclear cells (Rai *et al.*, 2006).

Regulation of body temperature in homeotherms requires a delicate balance between the internal production and external loss of heat. The Organum Vasculosum Lamina Terminalis (OVLT) region of hypothalamus is the regulatory centre for the control of body temperature. Pyrexia (fever) indicates a rise in body temperature above the normal range specified for a particular species of animal.

Fever may be the result of infection or sequalae of tissue damage, inflammation, graft rejection, malignancy or other disease states. In these conditions there is increased formation of cytokines like Interleukin-1 β, Interleukin-6, Interferons α and β and Tissue Necrosis Factor α. These are called endogenous pyrogens. The cytokines increase the synthesis of prostaglandin E_2 in circumventricular organs in and near the preoptic hypothalamic area and prostaglandin E_2 trigger the hypothalamus to elevate the body temperature by promoting an increase in heat generation and decrease in heat loss (Simmons *et al.*, 2004).

Fever can be reduced or eliminated when prostaglandin synthesis is blocked by drugs. Such drugs which reduce the body temperature are called antipyretics. Different groups of compounds called Non Steroidal Antiinflammatory Drugs (NSAIDS) are commonly employed as antipyretics. One such compound is aspirin, a salicylic acid derivative, which irreversibly inhibits cyclooxygenase enzyme which converts arachidonic acid to prostaglandinE_2 (Roberts and Morrow, 2001).

Tranquillizers are agents which have a calming and quietening effect on

animals. In veterinary medicine, they are valuable in chemical restraint of animals for various diagnostic and clinical procedures.

Tranquillizers depress the motor activity and cause prolongation of sleeping time induced by hypnotics. They exert their sedative action by depressing the brain stem and connections to the cerebral cortex. They also decrease the spontaneous motor activity in animals. It does not affect the coordinated motor responses of the animal and so arousal from tranquillization is easy.

Various classes of tranquillizers commonly employed are phenothiazine derivatives like chlorpromazine, trifluprómazine and its congeners, butyrophenone derivatives, thioxanthine derivatives and rauwolfia alkaloids. The principal central activity of these classes of drugs is the blockade of dopamine in the central nervous system (Booth, 2004).

The present study was undertaken to evaluate the antipyretic potential and tranquillizing property of alcoholic extract of *Nelumbo nucifera* seeds and to compare the effect between the seeds of white and red lotus.

2. REVIEW OF LITERATURE

2.1 NELUMBO NUCIFERA

Almost all parts of *Nelumbo nucifera* are used in traditional medicine for the treatment of various diseases. The rhizomes are used as nutritive, mucilaginous, demulcent, diuretic, cholagogue, and in treatment of dyspepsia and diarrhoea (Nadkarni, 1992).

Wu *et al.* (2004) isolated alkaloids liensinine, and its analogues, isoliensinine and neferine from embryo of the seed of *Nelumbo nucifera* employing preparative counter current chromatography. 1102 mg of crude alkaloid yielded 350 mg neferine, 100 mg isoliensinine and 95 mg liensinine with 95% purity.

Kashiwada *et al.* (2005) reported the isolation of alkaloids like coclaurine, norcoclaurine, quercetin 3-O-beta–D-glucuronide from the leaves of *Nelumbo nucifera.*

Ling *et al.* (2005) isolated procyanidins from *Nelumbo nucifera* seed pod which exhibited strong antioxidant activity, inhibited the lipooxygenase activity by more than 90% at a concentration of 62.5 μg/ml.

2.2 PLANTS HAVING ANTIPYRETIC PROPERTY

Poli *et al.* (1992) reported that the intraperitoneal administration of the aqueous and hydroalcoholic extracts of whole plant of *Elephantopus scaber* at the dose rates of 0.3-6 g/kg reduced brewer's yeast induced hyperthermia in rats. But oral administration of the same failed to produce the effect.

Antiinflammatory, analgesic and antipyretic properties of petroleum ether extract of *Litchi chinensis* in rats was reported by Besra *et al.* (1996). Since the extract did not inhibit arachidonic acid induced paw inflammation it is evident that it inhibited cyclooxygenase pathway of arachidonic acid metabolism.

The antipyretic activity of methanolic extract of rhizomes of *Nelumbo nucifera* at dose rates of 200, 300 and 400 mg/kg orally was studied in rats with normal body temperature and yeast induced pyrexia (Mukherjee *et al.*, 1996a). They observed a significant dose dependant lowering of normal body temperature and yeast provoked elevation of body temperature in rats. The effect was comparable with that of paracetamol at a dose rate of 150 mg/kg intraperitoneally.

Jain *et al.* (1997) demonstrated that the ethanolic extracts of the whole plant parts of *Cassia italica* could reduce pyrexia and carrageenan induced paw swelling at the dose rate of 100 mg/kg body weight in rats.

The alcoholic extract of *Clerodendron serratum* roots produced significant antipyretic, antinociceptive and antiinflammatory activities in experimental animal models at the dose rates of 50, 100 and 200 mg/kg orally (Narayanan *et al.*, 1999).

So *et al.* (1999) reported that the antipyretic effects of *Spiraea prunifolia* var.*simpliciflora* root extract resulted in direct suppression of nitric oxide and decreased super oxide generation.

Dewan *et al.* (2000) demonstrated the antipyretic effects of latex of *Calotropis procera* at dose rates of 250 mg/kg and 500 mg/kg in male albino rats. The latex at both dose rates produced a significant decline in rectal temperature in baker's yeast induced hyperthermia.

According to Hajare *et al.* (2000) the ethanolic extracts of *Dalbergia sissoo* leaves when administered orally at the dose rates of 100 and 300 mg/kg produced a significant antipyretic activity in brewer's yeast induced pyrexia in rats and mice. The extract at the dose rates of 1000 mg/kg and aspirin at the dose rate of 300 mg/kg showed antipyretic activity up to 6 hours.

Sinha *et al.* (2000) evaluated the antipyretic potential of the ethanolic extract of stalks of *Nelumbo nucifera* in normal body temperature and yeast induced hyperthermia in rats. The extract at dose rates of 200 and 400 mg/kg orally was found

to reduce the normal body temperature up to three hours and six hours respectively. The extracts could also produce a dose dependent lowering of body temperature up to four hours in yeast induced pyrexia.

Experiments by Ahmadiani *et al.* (2001) in rats showed that the ethanolic extract of *Trigonella foenum-graecum* leaves can reduce hyperthermia induced by brewer's yeast, when given at the dose rate of 1000 and 2000 mg/kg body weight intraperitoneally as well as orally.

Adzu *et al.* (2002) demonstrated that the methanolic extracts of *Diospyros mespiliformis* when administered at the dose rate of 100 mg/kg body weight intraperitoneally to mice possessed significant antipyretic effect.

Al-Yousuf *et al.* (2002) demonstrated that the methanolic extract of *Salvia aegyptiaca* at the dose rates of 0.5 and 1 g/kg orally did not affect the rectal temperature of hyperthermic mice, 0.5 and 1 hour post administration of the extract. But the acetone extract at dose rates of 0.25-2 g/kg body weight was effective in significantly reducing the rectal temperature 0.5 and 1 hour post administration of the extract. Both the extracts possessed significant antiinflammatory and CNS activity.

Methanolic extracts obtained from *Berberis cratagenia* root was screened for antipyretic activity in Freund's complete adjuvant induced pyrexia in mice and rats. The significant antipyretic effect of the plant could be attributed to the major active ingredient Berberine (Yeileda and Kupeli, 2002).

Evaluation of the antipyretic potential of the methanolic extract of *Cleome viscosa* was done by Devi *et al.* (2003) in normothermic and yeast induced hyperthermic albino rats. The extract at doses of 200, 300 and 400 mg/kg body weight showed significant reduction in body temperature in normothermic and hyperthermic rats in dose dependent manner. The effect extended up to five hours after drug administration and was comparable to that of paracetamol at dose rate of 150 mg/kg orally.

Gupta *et al.* (2003) reported that the methanolic extracts of *Caesalpinia bondeculla* leaves possessed significant antipyretic, antiinflammatory and analgesic activities at the dose rates of 50, 100 and 200 mg/kg body weight orally in yeast induced pyrexia in rats.

Studies conducted by Iwalewa *et al.* (2003) on chloroform, methanolic and ether extract of *Vernonia cinerea* leaf revealed the antipyretic property of the plant in mice when administered at the dose rates of 100, 200 and 400 mg/kg intraperitoneally in brewer's yeast induced pyrexia.

Makonnen *et al.* (2003) assessed the antipyretic effect of the aqueous and ethanolic extracts of the leaves of *Ocimum sauve* and *Ocimum lamiifolium* in mice. Both ethanolic and aqueous extracts of *Ocimum sauve* were more potent than *Ocimum lamiifolium* in the antipyretic activity. Aqueous extract of *Ocimum sauve* and ethanolic extract of *Ocimum lamiifolium* were more potent than their respective counterpart extracts.

Mutalik *et al.* (2003) observed that the dry residue of leaf juice of *Solanum melongena* produced significant antipyretic effect in a dose dependant manner and an appreciable antipyretic effect was noticed at 500 mg/kg body weight orally in mice and rats.

Pharmacological studies on *Clerodendrum petasites* revealed that the methanolic extract of the plant possessed antipyretic effect when tested in yeast induced hyperthermic rats (Panthong *et al.*, 2003). They suggested that the antipyretic action was caused by the inhibition of the prostaglandin synthesis.

According to Trongsakul *et al.* (2003) the hexane extract of the dry stem of *Diospyros variegate* elicited antipyretic action in yeast induced hyperthermic rats in addition to the antiinflammatory effect.

Aqueous extract of *Acanthus montanus* leaves was tested for antipyretic, antiinflammatory and analgesic properties in rats. It significantly reduced fever at

doses greater than 100 mg/kg within six hours, besides possessing antiinflammatory and analgesic properties (Asongalem *et al.*, 2004).

Panthong *et al.* (2004) showed that the methanolic extracts from heart wood, stem bark and stem wood of *Ventilago hermandiana* have excellent antipyretic property on yeast induced pyrexia in rats.

Perianayagam *et al.* (2004) reported that the ethanolic and aqueous extracts of *Emblica officinalis* fruits have antipyretic and analgesic properties. A single oral dose of both the extract at dose rates of 500 mg/kg showed significant reduction in brewer's yeast induced hyperthermia in rats.

The methanolic extract obtained from *Bauhinia racemosa* when administered orally at dose rates of 50, 100 and 200 mg/kg body weight significantly reduced fever in yeast induced hyperthermic rats (Gupta *et al.*, 2005).

2.3 PLANTS HAVING CENTRAL NERVOUS SYSTEM ACTIVITY

Shukia *et al.* (1987) reported that Brahmi rasayan, an ayurvedic preparation exhibited a sedative effect and significantly prolonged the hypnotic action of pentobarbitone. It produced a variable blockade of conditioned avoidance response. They also suggested the involvement of GABAergic system in the mediation of CNS effects of Brahmi rasayan.

Kulkarni *et al.* (1988) demonstrated that the alcoholic extract of *Clitoria ternatea* in dose rates of 230 and 460 mg/kg intraperitoneally, produced increased sedation, diminished alertness, inhibited conditioned avoidance response and induced hypothermia equivalent to chlorpromazine at dose rates of 10 mg/kg intraperitoneally in rats and mice. But the extracts could not produce any significant anticonvulsant activity.

The water soluble dried powder of alcoholic extract of roots and rhizomes of *Acorus calamus* when administered at doses of 10, 25, and 50 mg/kg intraperitoneally

antagonized spontaneous motor activity and also amphetamine induced hyperactivity in mice (Panchal *et al.*, 1989).

The lyophilized extract of *Euphorbia hirta* was evaluated for behavioural effects in mice by Lanhers *et al.* (1990). Sedative properties could be demonstrated with doses of 100 mg/kg body weight, by a decrease in the behavioural parameters measured by non familiar environment tests.

Hsieh *et al.* (1991) evaluated the anticonvulsant, sedative and hypothermic effects of ethanolic extract of *Periostracum cicadae* in rats. It enhanced the 5 hydroxy-tryptamine induced decrease in locomotor activity and reduced the increase in locomotor activity produced by levadopa plus benserazide.

Viola *et al.* (1994) demonstrated that the alcoholic extracts of inflorescence from *Tilia tomentosa* has sedative and anxiolytic properties in both elevated plus maze and hole board tests in rats.

Nalini *et al.* (1995) reported that celastrus oil extracted from *Celastrus panniculatus* decreased the dopamine level in rat brain along with reduction of norepinephrine and 5-hydroxy tryptamine.

Zia *et al.* (1995) observed that the methanolic extract and bioassay directed fraction of fresh undried uncrushed leaves of *Nerium oleander* reduced the locomotor activity, rotarod performance, potentiated the hexobarbital induced sleeping time in mice and possessed potent analgesic activity.

Mukherjee *et al.* (1996b) reported that the methanolic extract of rhizomes of *Nelumbo nucifera* reduced spontaneous motor activity in rats, decreased exploratory behaviour, reduced muscle relaxant activity by rotarod, 30° inclined screens and traction test and also potentiated the pentobarbitone induced sleeping time in mice at dose rates of 200, 300 and 400 mg/kg intraperitoneally.

According to a study conducted by Soulimani *et al.* (1997) lyophilized hydroalcoholic extracts of aerial parts of *Passiflora incarnata* produced psychotropic

and anxiolytic properties at 400 mg/kg body weight and aqueous extract at the same dose produced sedative effect and prolonged the pentobarbitone induced sleeping time in mice.

Hellion-Ibarrolla *et al.* (1999) reported that the oral administration of 100 mg/kg of crude hydroalcoholic rhizome extract of *Kyllingia brevifolia* induced a significant decrease in spontaneous locomotor activity, piloerection and palpebral ptosis in rats. The oral administration of the extract at dose rates of 1, 10 and 100 mg/kg produced a significant increase in the hypnotic effect induced by pentobarbital in a dose dependant manner.

Gilani *et al.* (2000) demonstrated the sedative, anticonvulsant and antispasmodic activities of the aqueous and methanolic extract of *Lavandula stoechas* flowers in mice at dose rates of 600 mg/kg .The extract could prolong the pentobarbital sleeping time similar to that of diazepam.

The ethanolic extract of *Ziziphus jujuba* was screened for CNS properties (Peng *et al.*, 2000). It was found that the extract at dosage of 0.5-1 g/kg body weight increased the percentage of time spent and the percentage of arm entries in the open arms of elevated plus maze test. At the dose rate of 1 g/kg, it prolonged the hexobarbital induced sleeping time in mice and decreased locomotor activity in rats.

Neuropharmacological effects of the aqueous extract of *Sphaeranthus senegalensis* was studied by Amos *et al.* (2001). The extract at dose rates 50 and 100 mg/kg orally produced reduction in spontaneous motor activity, exploratory behaviour and motor coordination and prolonged pentobarbitone induced sleeping time in mice.

A bioactive phytomoiety derived from the methanolic extract of *Passiflora incarnata* was observed to exhibit significant anxiolytic effect at a dose rate of 10 mg/kg in mice using elevated plus maze model of anxiety (Dhawan *et al.*, 2001). They also suggested the possibility of a phytoconstituent having benzoflavone nucleus as the basic moiety which is responsible for the potent anxiolytic activity of the plant.

The anticonvulsant activity of roots and rhizomes of *Glycyrrhiza glabra* was studied by Ambawade *et al.* (2002). They observed that the extract significantly delayed the onset of clonic convulsions induced by pentylene tetrazole at a dose rate of 100 mg/kg in rats.

Agarwal *et al.* (2003) showed that both alcoholic and aqueous extract of *Tinospora cordifolia* produced decrease in learning scores in Hebb-William maze and retention memory test, indicating learning and memory enhancement. It also decreased the neurodegenerative changes of hippocampus in cyclosporine treated rats.

Chindo *et al.* (2003) studied the central nervous system activity of methanolic extract of *Ficus platyphylla* bark and found that the extract significantly reduced the locomotor and exploratory activities in mice and prolonged pentobarbital sleeping time in rats in a dose dependant manner.

Achliya *et al.* (2004) reported that Unmadnashak ghrita, an ayurvedic formulation containing *Ferula narthex, Gardeniaa gummifera, Ellataria cardamom, Bacopa monneria* and cow's ghee, showed central nervous system depressant activity in gross behavioural test, potentiated pentobarbitone sleeping time and significantly deceased the spontaneous locomotor count in mice, when administered at the dose rate of 50,100, 200 and 300 mg/kg orally.

The experiments conducted by Misar *et al.* (2004) showed that the methanolic extracts of fruits of *Luffa acutangula* significantly reduced the exploratory activity of mice in dose dependant manner, enhanced pentobarbitone sleeping time when administered at dose rates of 10 and 50 mg/kg orally.

Aqueous extract of *Coriandrum sativum* seed was found to possess anxiolytic and sedative effect (Emamghoreishi *et al.*, 2005). Aqueous extracts at 50, 100 and 500 mg/kg significantly reduced the spontaneous locomotor activity and neuromuscular coordination in albino rats.

Mora *et al.* (2005) observed that the hydroalcoholic extract of leaves from *Casimiora edulis* administered intraperitoneally in male and female albino rats and mice caused considerable reduction of locomotor and exploratory activities and increased the exploration of the elevated plus maze open arms in a similar way as that of diazepam.

2.4 OTHER PHARMACOLOGICAL ACTIONS OF NELUMBO NUCIFERA

Petroleum ether extract of seeds of *Nelumbo nucifera*, when administered intraperitoneally to sexually mature female albino Swiss mice at a dose rate of 3 mg/kg body weight on alternate days for 15 days produced significant contraceptive, antioestrogenic, antiprogestational activities and reduced the ovarian and uterine weights. (Mazumder *et al.*, 1992).

Yu and Hu (1997) reported that neferine, a dibenzyl isoquinoline alkaloid isolated from *Nelumbo nucifera,* significantly inhibited rabbit platelet aggregation induced by adenosine triphosphate, collagen, arachidonic acid and platelet activating factor.

The ethanolic extract of *Nelumbo nucifera* seeds exhibited antioxidant and hepatoprotective effect by inhibiting the production of serum enzymes and cytotoxicity induced by intraperitoneal injection of carbontetrachloride and oral administration of aflatoxin B_1 in rats (Sohn *et al.*, 2003).

Wu *et al.* (2003) reported that the methanolic extract of the leaf of *Nelumbo nucifera* possess free radical scavenging and metal binding ability. The extract also exhibited concentration dependent antioxidant activities against haemoglobin induced linoleic acid peroxidation.

The effect of isoliensinine, a bisbenzyl alkaloid extracted from the seed embryo of *Nelumbo nucifera,* on bleomycin induced pulmonary fibrosis in mice was studied by Xiao *et al.* (2005). They observed a significant inhibitory effect on bleomycin induced

pulmonary fibrosis which could be attributed to its antioxidant and anti-inflammatory activity.

Ono *et al.* (2006) reported that the extract of leaves of *Nelumbo nucifera* prevented increase in body weight, parametrial adipose tissue weight and liver triacyl glycerol levels in mice with obesity induced by high fat diet. The extract caused a concentration dependent inhibition of alpha amylase and lipase activity and upregulated lipid metabolism.

The antioxidant activity of hydroalcoholic extract of *Nelumbo nucifera* was found to have strong free radical scavenging activity and it also increased the level of superoxide dismutase and catalase, decreased the level of thiobarbituric acid reacting substances in liver and kidney on carbon tetra chloride induced oxidative stress (Rai *et al.*, 2006).

Materials and Methods

3. MATERIALS AND METHODS

3.1 EXPERIMENTAL ANIMALS

The study was conducted in adult albino rats weighing 150-200 g of either sex, procured from Small Animal Breeding Station, Mannuthy. Rats were maintained on identical feeding and management practices in the laboratory for one week before the commencement of studies.

3.2. PREPARATION OF HERBAL EXTRACTS

The dried seeds of *Nelumbo nucifera,* both red and white types were collected from Nagercoil of TamilNadu (plate 1). The seeds were pulverized to a coarse powder using an electric pulverizer. The powder was extracted in Soxhlet extraction apparatus using methanol. The extract was evaporated to dryness with the help of a rotary vacuum evaporator and kept in refrigerator in an airtight container. 100 g dried powder of the lotus seed gave 10.0 g of the extract.

The experiment was carried out in three stages.

3.3. PHYTOCHEMICAL SCREENING

The methanolic extract of *Nelumbo nucifera* seeds were tested for the presence of various active chemical constituents namely steroids, alkaloids, tannins, phenolic compounds, flavonoids, glycosides, diterpenes, triterpenes, and saponins as per the procedure quoted by Harborne (1991).

3.3.1 Tests for Detection of Steroids

3.3.1.1 Salkowski Test

About five mg of the extract was mixed with three ml of chloroform and then shaken with three ml of concentrated sulphuric acid.

A. Red Lotus

B. White Lotus

3.3.1.2 Leiberman Burchardt Test

About five mg of the extract was mixed with three ml chloroform. Then five drops of acetic anhydride and one ml of concentrated sulphuric acid was added to it through the sides.

3.3.2. Tests for Detection of Alkaloids

About 0.5 g of the extract was mixed with five ml ammonia and then extracted with equal volume of chloroform. To this equal quantity of 0.1 N hydrochloric acid was added.

3.3.2.1 Mayer's Test

To one ml of the acid layer obtained, a few drops of Mayer's reagent (potassium mercuric iodide) were added.

3.3.2.2 Hager's Test

To one ml of acid layer, a few drops of Hager's reagent were added and mixed.

3.3.2.3 Dragendroff's Test

Two drops of Dragendroff's reagent (solution of potassium bismuth iodide) was mixed with one ml of acid layer.

3.3.3 Test for Detection of Tannins

3.3.3.1 Ferric Chloride Test

Two mg of the extract was mixed with three ml of one per cent ferric chloride solution.

3.3.4 Tests for Detection of Flavonoids

3.3.4.1 Ferric Chloride Test

To two ml of alcoholic solution of the extract, (0.5 g extract in 10 ml methanol), a few drops of neutral ferric chloride solution was added and mixed.

3.3.4.2 Lead Acetate Test

To two ml of the alcoholic solution of the extract (0.5 g extract in 10 ml methanol), a few drops of 10% lead acetate was mixed.

3.3.5 Tests for Detection of Glycosides

3.3.5.1 Benedicts Test

To about one ml of the extract (0.5 g of extract in one ml water), five ml of Benedict's reagent was added. The mixture was boiled for ten minutes.

3.3.5.2 Sodium Hydroxide Test

Mixed a small amount of the extract (about five mg) in one ml water and added five to six drops of sodium hydroxide solution.

3.3.6 Test for the Presence of Phenolic Compounds

About five mg of the extract was mixed with one ml of water and five drops of ten per cent ferric chloride solution was added to it.

3.3.7 Test for the Detection of Diterpenes

About five mg of the extract was mixed with three ml of copper acetate solution.

3.3.8 Tests for the Presence of Triterpenes

3.3.8.1 Salkowski Test

About three mg of the extract was mixed with three ml of chloroform and then shaken with concentrated sulphuric acid.

3.3.8.2 Leiberman Burchardt Test

A few drops of acetic acid and one ml of concentrated sulphuric acid were added to three ml of chloroform solution of the extract (about three mg of extract in three ml chloroform).

3.3.9 Test for the Detection of Saponins

3.3.9.1 Foam Test

A small amount of the extract (about five mg) was shaken with three ml of water.

3.4 ASSESSMENT OF ANTIPYRETIC ACTIVITY

Forty eight rats were divided into six groups of eight animals each. Body temperature of the rats was recorded continuously for seven hours at hourly interval for three days from 8 A.M. to 3 P.M. Blood was collected from all animals prior to the experiment from the retro orbital plexus using heparinised capillary tubes, for haematological and biochemical studies.

Hyperthermia was induced in all the six groups by subcutaneous injection of 20 per cent Brewers yeast suspended in normal saline @ 1ml/100g body weight in the back below the neck region of the rat (Turner, 1965). Five per cent gum acacia was used as a vehicle for the administration of drugs and extracts in all the groups.

3.4.1 Experimental Design

Group number	Treatment
I	Yeast (20 per cent suspension) 1ml/100g body weight subcutaneously
II	Yeast (20 per cent suspension) 1ml/100g body weight subcutaneously + aspirin 100 mg/kg body weight orally
III	Yeast (20 per cent suspension) 1ml/100g body weight subcutaneously + alcoholic extracts of *Nelumbo nucifera* seeds (red type) @ 400 mg/kg body weight orally.

IV — Yeast (20 per cent suspension) 1ml/100g body weight subcutaneously + alcoholic extracts of *Nelumbo nucifera* seeds (red type) @ 600 mg/kg body weight orally.

V — Yeast (20 per cent suspension) 1ml/100g body weight subcutaneously + alcoholic extracts of *Nelumbo nucifera* seeds (white type) @ 400 mg/kg body weight orally.

VI — Yeast (20 per cent suspension) 1ml/100g body weight subcutaneously + alcoholic extracts of *Nelumbo nucifera* seeds (white type) @ 600 mg/kg body weight orally.

The rectal temperature was recorded at an interval of one hour after yeast administration. The peak of pyrexia was determined to be at 19 hours after yeast administration, by conducting pilot experiments. Animals showing a rise in body temperature less than 0.5°C were excluded from the study. The drugs were administered at the time of peak pyrexia (19 hours after yeast administration). Thereafter the rectal temperature was recorded at an interval of one hour continuously for five hours.

3.5 ASSESSMENT OF CENTRAL NERVOUS SYSTEM (CNS) ACTIVITY

Forty eight adult albino rats were divided into six groups comprising eight animals each. These rats were trained for one week in actaphotometer and rotarod. Food was withheld for 12 hours to ensure complete absorption of drugs. Water was given adlibitum.

Chlorpromazine tablets (Tranchlor 25 mg*) purchased locally was pulverized into fine powder using clean mortar and pestle and suspended in five per cent gum

* Medopharma, Chennai

acacia and was used as the standard drug.

3.5.1 Equipments

Two equipments, actaphotometer and rotarod were used to assess the reduction in spontaneous and forced motor activity respectively and thereby the degree of tranquillization (Turner, 1965).

3.5.1.1 Actaphotometer

The spontaneous motor activity (locomotor activity) was measured using actaphotometer. It consisted of a cubical box with photoelectric cells in perpendicular direction to the source of light (fig. 1). The movement of the animal cuts off a beam of light falling on the photocell and a count was recorded and displayed digitally. The count will be proportional to the spontaneous motor activity. Each rat was placed individually in the actaphotometer for ten minutes and readings were taken at 30, 60, 90, 120, 150, 180, 210, 240 minutes respectively after administration of drugs (Thakur and Mengi, 2005).

3.5.1.2 Rotarod

This instrument consists of a rough metallic rod rotating at fixed speeds which can be regulated with a counter (fig. 2). The animals were trained to maintain balance on the rod rotating at the speed of 25 rpm. Only those rats, which could balance themselves for more than three minutes were selected for the study. Rats were placed on the rotarod individually and their time permanence on the rod in seconds was taken as a measure of forced locomotor activity. Readings were taken at 30 minutes interval for four hours after administration of drugs (Bigoniya and Rana, 2005).

3.5.2 Experimental Design

Group number	Treatment
I	Control group. 5 per cent gum acacia in water

Fig. 1. ACTAPHOTOMETER

II	Chlorpromazine (standard drug) @ 7mg/kg body weight orally.
III	Alcoholic extracts of *Nelumbo nucifera* seeds (red type) @ 400 mg/kg body weight orally.
IV	Alcoholic extracts of *Nelumbo nucifera* seeds (red type) @ 600 mg/kg body weight orally.
V	Alcoholic extracts of *Nelumbo nucifera* seeds (white type) @ 400 mg/kg body weight orally.
VI	Alcoholic extracts of *Nelumbo nucifera* seeds (white type) @ 600 mg/kg body weight orally.

3.6 HAEMATOLOGICAL STUDIES

Blood was collected from retro orbital plexus into sterile vials containing disodium salt of Ethylene Diamine Tetra Acetic acid (EDTA Sodium) at the rate of 1mg/ml for the estimation of haematological parameters like Total Leukocyte Count, Differential Leucocyte Count, Total Erythrocyte Count and Haemoglobin Concentration as described by Schalm *et al.* (1975).

3.7 BIOCHEMICAL STUDIES

Blood was collected in centrifuge tubes without anticoagulant for collecting serum for the estimation of alanine amino transferase and aspartate amino transferase.

3.7.1 Estimation of Serum Alanine Amino Transferase (ALT)

Estimation of serum alanine amino transferase was done using kits manufactured by Agappe diagnostics (Reitman and Frankel, 1957).

3.7.2 Estimation of Serum Aspartate Amino Transferase (AST)

Estimation of serum aspartate amino transferase was done using kits manufactured by Agappe diagnostics (Reitman and Frankel, 1957)

In antipyretic study the blood samples were collected before drug administration (at the peak of pyrexia) and five hours after drug administration.

In central nervous system study, all the blood samples were collected at the peak of tranquillization, which was determined using pilot studies.

3.8 STATISTICAL ANALYSIS OF DATA

Results were analysed using Tukeys multiple comparison test for comparison between and within various groups in both the studies as described by Hogalin *et al.*(1991). Results were expressed as mean $\pm$ standard error.

Results

4. RESULTS

4.1 SCREENING OF ALCOHOLIC EXTRACTS FROM SEEDS OF *Nelumbo nucifera* (RED AND WHITE TYPES) FOR ACTIVE PRINCIPLES

4.1.1 Steroids

As per Salkowski test, red colour was obtained with the extract and Lieberman Burchardt test gave a reddish ring at the junction. Thus it could be concluded that steroids were present in the alcoholic extract of *Nelumbo nucifera* seeds.

4.1.2 Alkaloids

A creamy white precipitate as per Mayer's test and a reddish brown coloured precipitate as per Hager's test were obtained. Dragendroff's test gave a reddish brown precipitate. Thus the tests revealed the presence of detectable level of alkaloids in the seed extract of *Nelumbo nucifera.*

4.1.3 Tannins

Blue colour was not obtained in ferric chloride test and a white precipitate was not obtained in gelatin test. These results indicated the absence of tannins in the extracts.

4.1.4 Flavonoids

A green colour in the ferric chloride test and a yellow precipitate in lead acetate test indicated the presence of flavonoids in the extracts.

4.1.5 Glycosides

As per Benedict's test, brown colour was developed indicating the presence of glycosides. A yellow colour was obtained by mixing the extract with sodium hydroxide reagent that also indicated the presence of glycosides.

Table 1. Screening of Alcoholic Extracts From Seeds of *Nelumbo nucifera* (Red and White Type) for Active Principles

Sl.no	Active Principles	Alcoholic Extract
1.	Steroids	Present
2.	Alkaloids	Present
3.	Tannins	Not detected
4.	Flavonoids	Present
5.	Glycosides	Present
6.	Phenolic compounds	Present
7.	Diterpenes	Present
8.	Triterpenes	Present
9.	Saponins	Present

4.1.6 Phenolic Compounds

The extract mixed with 10 per cent ferric chloride produced dark brown colour indicating the presence of phenolic compounds.

4.1.7 Diterpenes

Diterpenes were detected in the extract as indicated by the green colour when it was mixed with copper acetate solution.

4.1.8 Triterpenes

As per Salkowski test, lower layer turned to yellow on standing and by Lieberman Burchardt test, a deep ring appeared at the junction of two layers. These results indicated the presence of triterpenes in the *Nelumbo nucifera* seed extract.

4.1.9 Saponins

In the foam test, foam persisted for 10 minutes indicating the presence of saponins.

The results obtained in the above phytochemical study are summarised in Table 1.

4.2 STUDY OF THE ANTIPYRETIC EFFECT OF ALCOHOLIC EXTRACT FROM SEEDS OF *Nelumbo Nucifera* (RED AND WHITE TYPES)

The results are presented in table 2 and fig 3. The mean body temperature of animals at zeroth hour of study (peak of pyrexia) were 39.23± 0.09 °C, 39.10± 0.14 °C, 39.32± 0.06 °C, 39.40± 0.06 °C, 39.42± 0.12 °C and 39.35± 0.05 °C for groups I to VI respectively.

During the first hour of study, the mean body temperature of animals of group I to VI were 39.23± 0.09 °C, 38.47± 0.11 °C, 39.20± 0.04 °C, 39.20± 0.05 °C, 39.15± 0.12 °C and 38.73 ± 0.16 °C respectively. Group II (aspirin 100 mg/kg body weight) and group VI (white lotus seed extract @ 600 mg/kg bodyweight) showed significant ($P<0.05$) antipyretic effect when compared with control (group I).

Table 2. Effect of Alcoholic Extracts from seeds of *Nelumbo nucifera* (Red and White Type) on Body Temperature in Yeast Induced Pyrexia, °C

Time	Group I	Group II	Group III	Group IV	Group V	Group VI
0th hour	39.23± 0.09	39.10± 0.14	39.32± 0.06	39.40± 0.06	39.42± 0.12	39.35± 0.05
1st hour	39.23± 0.09	38.47± 0.11	39.20± 0.04	39.20± 0.05	39.15± 0.12	38.73± 0.16
2nd hour	39.15± 0.08	37.46± 0.09	38.98± 0.18	38.70± 0.09	38.55± 0.15	38.44± 0.03
3rd hour	39.07± 0.05	37.28± 0.14	38.52± 0.08	38.10± 0.17	38.33± 0.27	37.80± 0.08
4th hour	38.93± 0.06	37.05± 0.11	38.31± 0.11	37.55± 0.11	37.68± 0.21	37.52± 0.07
5th hour	38.84± 0.07	36.93± 0.08	37.95± 0.22	37.31± 0.10	37.38± 0.07	37.33± 0.19

Table 3. Tukeys Multiple Comparison Test – P Values for Pyrexia Study

Comparison	0 hr	1 hr	2 hr	3hr	4hr	5hr
Group I vs Group II	P > 0.05	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001
Group I vs Group III	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P < 0.05	P < 0.001
Group I vs Group IV	P > 0.05	P > 0.05	P > 0.05	P < 0.001	P < 0.001	P < 0.001
Group I vs Group V	P > 0.05	P > 0.05	P < 0.05	P < 0.001	P < 0.001	P < 0.001
Group I vs Group VI	P > 0.05	P < 0.05	P < 0.001	P < 0.001	P < 0.001	P < 0.001
Group II vs Group III	P > 0.05	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001
Group II vs Group IV	P > 0.05	P < 0.001	P < 0.001	P < 0.01	P > 0.05	P > 0.05
Group II vs Group V	P > 0.05	P < 0.001	P < 0.001	P < 0.05	P < 0.05	P > 0.05
Group II vs Group VI	P > 0.05	P > 0.05	P < 0.001	P > 0.001	P > 0.05	P > 0.05
Group III vs Group IV	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P < 0.001	P < 0.01
Group III vs Group V	P > 0.05	P >0.05	P < 0.01	P < 0.05	P < 0.01	P < 0.05
Group III vs Group VI	P > 0.05	P < 0.05	P < 0.05	P < 0.05	P < 0.05	P < 0.05
Group IV vs Group V	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05
Group IV vs Group VI	P > 0.05	P < 0.05	P < 0.05	P > 0.05	P > 0.05	P > 0.05
Group V vs Group VI	P > 0.05	P < 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05

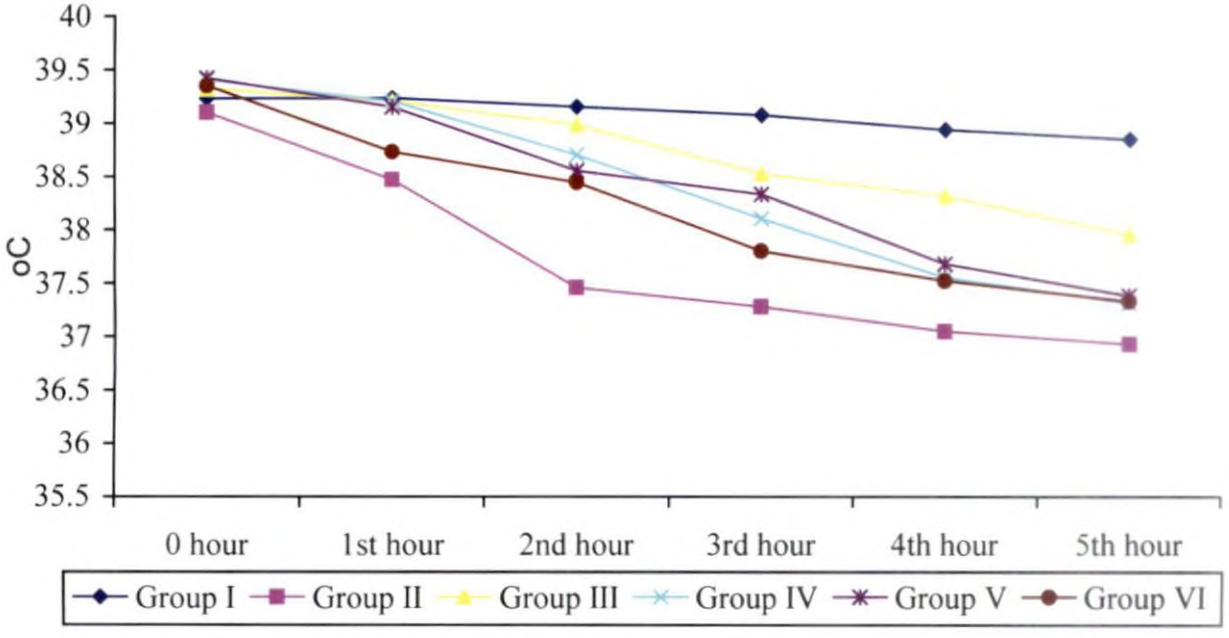

Fig. 3. Effect of Alcoholic Extracts from Seeds of *Nelumbo nucifera* (red and white type) on Body Temperature in Yeast Induced Pyrexia

The mean body temperature of animals at second hour of study were 39.15± 0.08 °C, 37.46± 0.09 °C, 38.98± 0.18 °C, 38.70± 0.09 °C, 38.55± 0.15 °C and 38.44± 0.03 °C for groups I to VI respectively. The aspirin treated group showed highest antipyretic activity and the animals of group VI showed significant ($P< 0.05$) reduction in body temperature when compared to control group (table 3).

The mean body temperature of animals from group I to VI, recorded at third hour of study were 39.07± 0.05 °C, 37.28± 0.14 °C, 38.52± 0.08 °C, 38.10± 0.17 °C, 38.33± 0.27 °C and 37.80± 0.08 °C respectively. All the extract treated groups except group III (red lotus seed extract @400 mg/kg) showed significant antipyretic activity when compared with the control (group I). Among the extract treated groups, white lotus seed @ 600 mg/kg (group VI) showed highest antipyretic activity followed by red lotus seed @ 600 mg/kg (group IV) and white lotus seed @ 400 mg/kg (group V).

During the fourth hour of the study the mean body temperature of animals recorded were 38.93± 0.06 °C, 37.05± 0.11 °C, 38.31± 0.11 °C, 37.55± 0.11 °C, 37.68± 0.21 °C, 37.52± 0.07 °C. The highest antipyretic activity was shown by animals of group II (Aspirin treated group) followed by those of group VI. The extract treated groups at higher doses (both red and white @ 600 mg/kg) showed similar antipyretic activity ($P>0.05$).

The mean body temperature of animals at fifth hour of study from I to VI were 38.84± 0.07, 36.93± 0.08 °C, 37.95± 0.22, 37.31± 0.10 °C, 37.38± 0.07 °C and 37.33± 0.19 °C respectively. All the extract treated groups exhibited a significant antipyretic activity. There was no significant difference ($P>0.05$) between the standard (group II) and the extract treated groups indicating a similar potency.

4.3 STUDY OF THE CNS ACTIVITY OF THE ALCOHOLIC EXTRACTS FROM SEEDS OF *Nelumbo nucifera* (RED AND WHITE TYPES)

4.3.1 Study of CNS Activity (Spontaneous Motor Activity) Using Actaphotometer

The results are presented in table 4 and fig 4.

After 30 minutes of drug administration, the mean actaphotometer counts per 10 minutes were 244.50± 8.72, 67.50± 3.21, 126.63± 2.26, 123.38± 3.02, 135.20± 3.34 and 115.00± 3.46 for groups I to VI respectively. A significant reduction ($P<0.001$) in spontaneous motor activity was observed in all treatment groups (table 6). But a comparison of the extract treated groups (group III, IV, V and VI) with that of group II (standard drug) indicated that standard drug was more potent in reducing spontaneous motor activity. Among the extract treated groups, group VI is showing more potent reduction in spontaneous motor activity.

The mean actaphotometer count at 60 minutes post drug administration for groups I to VI were 231.50± 10.53, 25.00± 1.64, 91.75± 3.50, 87.25± 3.42, 112.25± 2.45 and 93.50± 4.66 respectively. Among the extract treated groups, group IV (red lotus seed extract @ 600 mg/kg) significantly reduced ($P<0.05$) the spontaneous motor activity than group V (white lotus seed extract @ 400 mg/kg). But the standard group showed significantly reduced ($P<0.001$) count than the control and extract treated groups.

After 90 minutes of drug administration the mean actaphotometer counts recorded for groups I to VI were 212.88± 8.26, 25.13± 0.72, 98.75± 4.49, 89.38± 3.01, 113.50± 3.14 and 94.50± 4.12. The maximum inhibition of spontaneous motor activity was shown by animals of group II .The CNS activity exhibited by the red and white lotus seed @ 600mg/kg and the red lotus seed @ 400mg/kg were similar indicating same potency.

The mean actaphotometer counts at 120^{th} minute of study were 177.38± 0.87, 24.50± 15.77, 111.50± 1.47, 101.38± 3.22, 122.13± 5.03 and 101.25± 4.32 respectively for groups I to VI. The extract treated groups produced similar reduction in spontaneous motor activity, but the chlorpromazine continued to be more potent.

At 150^{th} minute study the mean actaphotometer counts for groups I to VI were167.00± 13.72, 23.88± 1.45, 119.88± 3.01, 101.13± 4.37, 126.75± 3.48 and 102.38± 3.81 respectively. The extract treated groups did not significantly ($P>0.05$)

Table 4. Effect of Alcoholic Extracts from Seeds of Red and White *Nelumbo nucifera* on Actaphotometer Counts at Various Time Intervals, counts/10 min

Time period	Group I	Group II	Group III	Group IV	Group V	Group VI
30 min	244.50± 8.72	67.50± 3.21	126.63± 2.26	123.38± 3.02	135.20± 3.34	115.00± 3.46
60 min	231.50± 10.53	25.00± 1.64	91.75± 3.50	87.25± 3.42	112.25± 2.45	93.50± 4.66
90 min	212.88± 8.26	25.13± 0.72	98.75± 4.49	89.38± 3.01	113.50± 3.14	94.50± 4.12
120 min	177.38± 0.87	24.50± 15.77	111.50± 1.47	101.38± 3.22	122.13± 5.03	101.25± 4.32
150 min	167.00± 13.72	23.88± 1.45	119.88± 3.01	101.13± 4.37	126.75± 3.48	102.38± 3.81
180 min	162.25± 8.12	30.88± 1.39	123.00± 3.28	107.75± 4.82	132.50± 3.70	107.50± 3.23
210 min	151.13± 11.58	40.38± 1.57	129.25± 1.68	112.75± 4.01	137.00± 2.67	117.63± 3.62
240 min	137.13± 11.34	42.38± 1.00	132.13± 1.25	126.00± 3.69	142.63± 2.63	120.88± 3.14

Table 5. Effect of Alcoholic Extracts from Seeds of Red and White *Nelumbo nucifera* on Rotarod Performance Time at Various Intervals, s

Time period	Group I	Group II	Group III	Group IV	Group V	Group VI
30 min	678.00± 10.05	160.13± 5.76	343.63± 5.76	240.00± 3.96	423.38± 3.78	329.13± 9.97
60 min	641.63± 13.56	160.13± 5.76	290.63± 9.96	211.50± 6.73	381.88± 7.33	296.50± 8.96
90 min	628.38± 12.52	131.75± 10.11	282.63± 15.70	216.13± 4.63	396.25± 7.17	302.63± 8.86
120 min	626.00± 14.37	118.75± 7.41	288.00± 12.67	221.75± 5.90	415.25± 12.34	306.63± 8.78
150 min	622.25± 15.85	128.13± 7.80	292.14± 11.98	232.88± 4.89	412.63± 5.57	306.25± 8.48
180 min	617.88± 12.56	133.75± 19.82	307.25± 16.01	238.50± 4.85	425.50± 11.22	316.88± 9.68
210 min	611.75± 17.73	137.50± 9.52	324.88± 14.18	241.00± 4.23	421.75± 4.13	325.00± 11.40
240 min	577.63± 20.60	144.50± 5.58	326.00± 16.07	250.38± 4.86	432.13± 5.71	331.88± 11.80

Table 6. Tukeys Multiple Comparison Test – P Values for Actaphotometer counts

Comparison	30 min	60min	90 min	120min	150 min	180 min	210 min	240 min
Group I vs Group II	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001
Group I vs Group III	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P > 0.05	P > 0.05
Group I vs Group IV	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P > 0.05
Group I vs Group V	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P > 0.05	P > 0.05
Group I vs Group VI	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.01	P > 0.05
Group II vs Group III	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001
Group II vs Group IV	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001
Group II vs Group V	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001
Group II vs Group VI	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001
Group III vs Group IV	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05
Group III vs Group V	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05
Group III vs Group VI	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05
Group IV vs Group V	P > 0.05	P < 0.05	P < 0.01	P > 0.05	P > 0.05	P < 0.01	P < 0.05	P > 0.05
Group IV vs Group VI	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05
Group V vs Group VI	P < 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P < 0.01	P > 0.05	P > 0.05

differ in their activity indicating similar reduction in spontaneous motor activity, but lesser when compared to the standard drug.

The mean actaphotometer counts at the end of 180th minute of study, were 162.25± 8.12, 30.88± 1.39, 123.00± 3.28, 107.75± 4.82, 132.50± 3.70 and107.50± 3.23 for animals of group I to VI respectively. The reduction in spontaneous motor activity exhibited by the treatment groups showed a similar pattern as that of 150th minute of study.

At the end of 210 minutes, the mean actaphotometer counts for groups I to VI were 151.13± 11.58, 40.38± 0.57, 129.25± 1.68, 112.75± 4.01, 137.00± 2.67 and 117.63± 3.62 respectively. There was no significant difference in the activity of the extract, indicating that they possess same CNS activity. The spontaneous motor activity was seen improving in all the groups.

After 240th minute of study, the mean actaphotometer counts for animals of group I to VI were 137.13± 11.34, 42.38± 1.00, 132.13± 1.25, 126.00± 3.69, 142.63± 2.63 and 120.88± 3.14 respectively. At the end of the experiment there was no significant difference between the control group and the extract treated groups, indicating that the animals of extract treated groups regained spontaneous motor activity to normal. Chlorpromazine (standard) continued to have significant difference ($P<0.001$) in activity when compared to control.

4.3.2 Study of CNS Activity (Forced Motor Activity) Using Rotarod

The results are presented in table 5 and fig 5.

The mean time spent on the rotarod by animals of group I to VI after 30 minutes of drug administration were 678 ± 10.05, 160.13± 5.76, 343.63 ± 5.76, 240.00 ± 3.96, 423.38± 3.78 and 329.13± 9.97 seconds for animals of group I to VI respectively. All the groups were showing statistically significant ($P<0.001$) reduction in forced motor activity when compared to control group (group I). But activity is less

when compared with the group treated with the standard drug, chlorpromazine (table 7).

After 60 minutes of drug administration, the mean time spent on rotarod by animals of group I to VI were 641.63± 13.56, 160.13± 5.76, 290.63± 9.96, 211.50± 6.73, 381.88± 7.33 and 296.50± 8.96 seconds respectively. A significant ($P<0.001$) reduction in forced motor activity was observed in groups III, IV,V,VI when compared with the control. Among the extract treated groups, group IV(red lotus seed extract 600 mg/kg) is showing maximum reduction in forced motor activity.

The mean values of rotarod performance time at 90 minutes of study were 628.38± 12.52, 131.75± 10.11, 282.63± 15.70, 216.13± 4.63 396.25± 7.17 and 302.63± 8.86 seconds respectively for groups I to VI respectively. All the groups showed significant reduction in forced motor activity when compared with the control (group I) but standard drug (group II) is showing more potent activity in comparison with the extract treated groups. The minimum time permanence was shown by animals of group IV (red lotus seed extract @ 600 mg/kg), among the extract treated groups. The red lotus seed extract @ 400 mg/kg (group III) exhibited activity similar ($P>0.05$) to that of white lotus seed extract @ 600 mg/kg (group VI).

At 120th minute of study, the mean time spent on rotarod by animals of group I to VI were 626.00± 14.37, 118.75± 7.41, 288.00± 12.67, 221.75± 5.90, 415.25± 12.34, 306.63± 8.78 seconds respectively. Forced motor activity was further reduced in chlorpromazine treated group (group II), but that of extract treated groups remained almost same as that of 90 minutes of drug administration. Group IV continued to show minimum time permanence on the rotarod among the extract treated groups.

The mean time spent on rotarod by animals of group I to VI at 150th minute of study were 622.25± 15.85, 128.13± 7.80, 292.14± 11.98, 232.88± 4.89, 412.63± 5.57 and 306.25± 8.48 seconds respectively. All the extract treated groups (group III, IV, V,

Table 7. Tukeys Multiple Comparison Test – P Values for Rotarod Performance Time

Comparison	30 min	60min	90 min	120min	150 min	180 min	210 min	240 min
Group I vs Group II	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001
Group I vs Group III	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001
Group I vs Group IV	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001
Group I vs Group V	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001
Group I vs Group VI	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001
Group II vs Group III	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001
Group II vs Group IV	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < .0.001	P < 0.001	P < 0.001	P < 0.001
Group II vs Group V	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001
Group II vs Group VI	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001
Group III vs Group IV	P < 0.001	P < 0.001	P < 0.001	P < 0.01	P < 0.01	P < 0.01	P < 0.001	P < 0.01
Group III vs Group V	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001
Group III vs Group VI	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05
Group IV vs Group V	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001
Group IV vs Group VI	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.01	P < 0.001	P < 0.001	P < 0.001
Group V vs Group VI	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001

Table 8. Total Leukocyte Count, number/mm^3 of blood

	Group I	Group II	Group III	Group IV	Group V	Group VI
Peak of pyrexia	6294±117.43	6875±188.04	7238±152.00	7713±174.68	7950±75.59	7588±164.14
5 hours after drug administration	6694±187.90	6950±139.19	7081±73.76	7031±77.88	7050±75.00	7225±91.12
At peak of tranquillization in CNS study	6894±148.64	7038±102.10	7100±73.80	7031±69.40	7025±71.34	7225±72.58

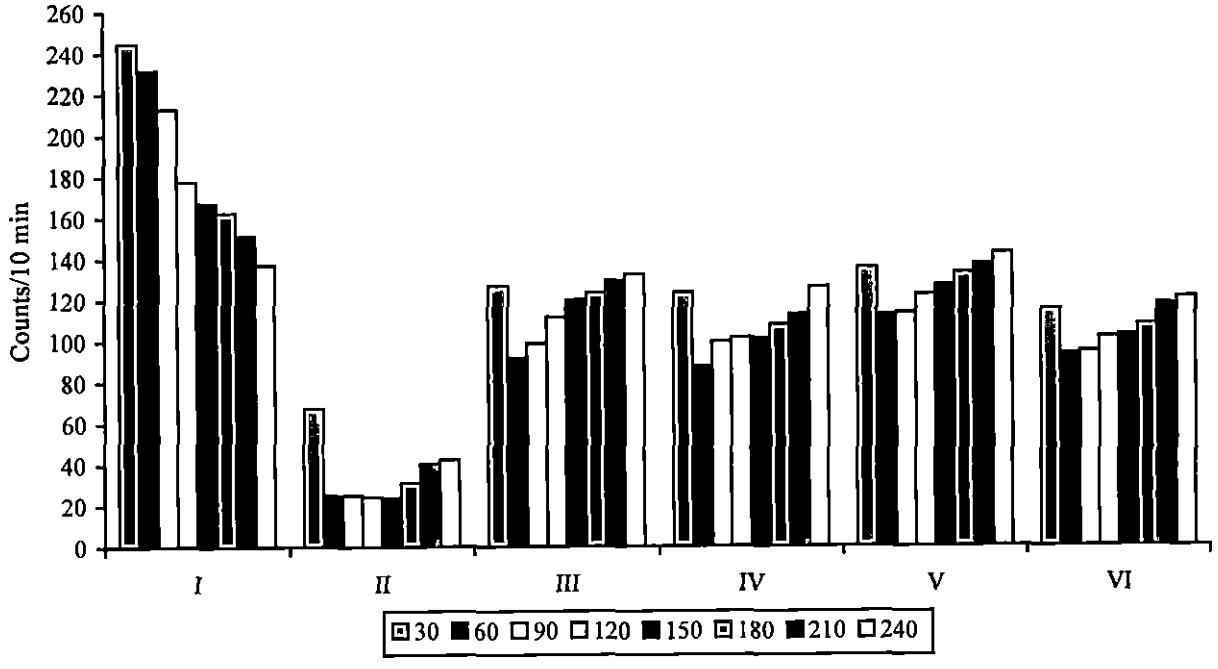

Fig. 4. Effect of Alcoholic Extracts from Seeds of Red and White Lotus on Actaphotometer Counts at Various Time Intervals

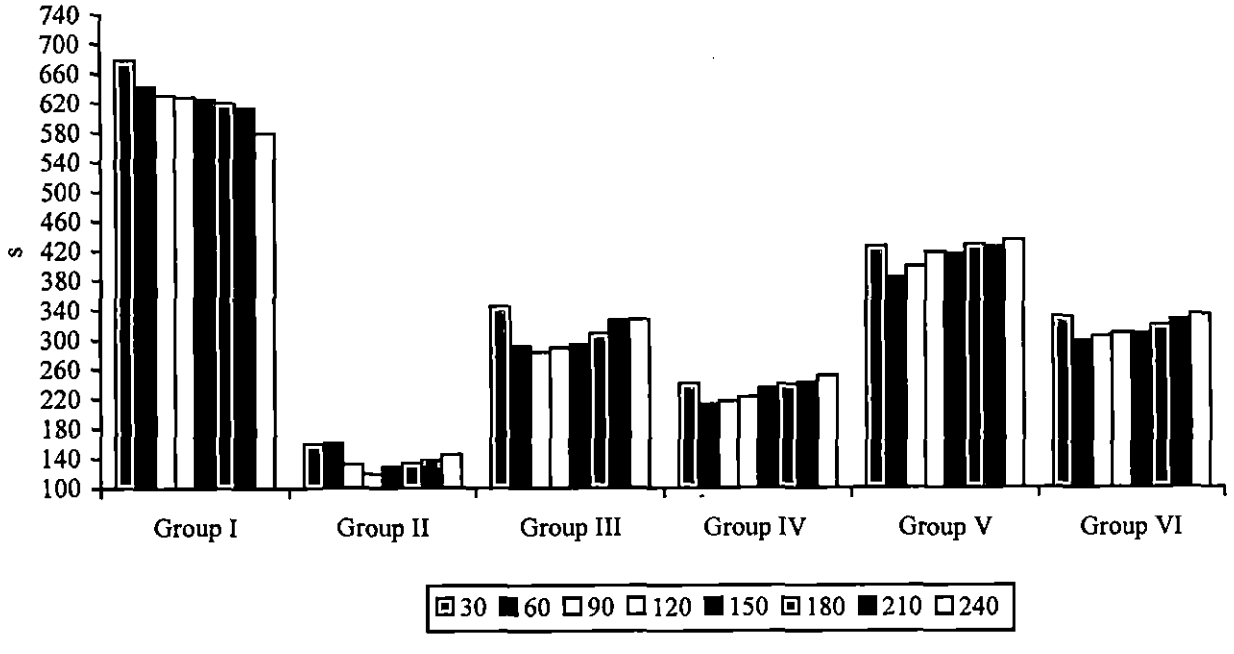

Fig. 5. Effect of Alcoholic Extracts from Seeds of Red and White Lotus on Rotarod Performance Time at Various Intervals

VI) were showing significant (P<0.001) reduction in forced motor activity when compared to control. But the standard drug continued to be more potent. Among the extract treated groups, group IV (red lotus seed extract @ 600 mg/kg) is showing maximum CNS activity. There was no significant difference (P>0.05) in activity of group III (red lotus seed extract @400 mg/kg) and group VI (white lotus seed extract @ 600 mg/kg) indicating similar potency.

After 180 minutes of drug administration, the mean time spent on rotarod by animals of group I to VI were 617.88± 12.56, 133.75± 19.82, 307.25± 16.01, 238.50± 4.85, 425.50± 11.22 and 316.88± 9.68 seconds respectively. Chlorpromazine continued to have the most potent activity. In between the extract comparison showed that groups III and VI are showing similar effects (P>0.05). Forced motor activity was seen improving in all the treated groups.

After 210 minutes of drug administration, the mean rotarod permanence time were 611.75± 17.73, 137.50± 9.52, 324.88± 14.18, 241.00± 4.23, 421.75± 4.13 and 325.00± 11.40 seconds respectively for groups I to VI. Similar results as that of 180th minute of study was observed and in all the treatment groups forced motor activity improved.

The mean time spent on rotarod by animals of group I to VI at the end of 240th minute were 577.63± 20.60, 144.50± 5.58, 326.00± 16.07, 250.38± 4.86, 432.13± 5.71 and 331.88± 11.80 seconds respectively. Forced motor activity is almost regained by the animals and the counts are almost similar to those after 30th minute of study. But a significant difference (P<0.001) in activity existed between the control and treatment groups. The standard drug continued to show a significant difference (P<0.001) in activity when compared with the extract treated groups.

4.4 HAEMATOLOGICAL PARAMETERS IN ANTIPYRETIC STUDY

The values are given in tables 8 –11.

4.4.1. Total Leukocyte Count at the Peak of Pyrexia

The results are given in table 8.

The mean total leukocyte count at the peak of pyrexia for groups I to VI were 6,294± 117.43, 6,875± 188.04, 7,238± 152.00, 7,713± 174.68, 7,950± 75.59 and 7,588± 164.14 per µl of blood

4.4.2. Total Leukocyte Count 5 hours After Drug Administration

The mean total leukocyte count 5 hours after drug administration for groups I to VI were 6,694± 187.90, 6,950± 139.19, 7,081± 73.76, 7,031± 77.88, 7,050± 75.00, and 7,225± 91.12 per µl of blood.

The mean total leukocyte counts were within the normal range. There was no significant difference between animals of different groups.

4.4.3. Differential Leukocyte Count at Peak of Pyrexia

The values are given in table 9.

The mean per cent Differential Leukocyte Count at peak of pyrexia for groups I to VI were 14.25± 0.90, 15.88± 0.97, 12± 0.87, 10.5± 0.94, 12.38± 0.50, and 12.50± 0.38, for neutrophil count, 84.5± 1.00, 83.5± 0.98, 88± 0.87, 89.13± 1.04, 87.12± 0.58 and 87.00± 0.46 for lymphocyte count and 1.25± 0.37, 0.63± 0.26, 0, 0.38± 0.18, 0.5± 0.19 and 0.5 ± 0.19 for eosinophil count.

4.4.4. Differential Leukocyte Count 5 Hours After Administration of the Drug

The values are given in table 9.

The mean Differential Leukocyte Count at peak of pyrexia for groups I to VI were13.755± 0.98, 15.38± 0.91, 12.38± 0.68, 11.88± 0.44, 13.00± 0.33 and 12.50± 0.50 per cent for neutrophil count, 85.25± 0.98, 83.63± 0.98, 87.13± 0.58, 87.5± 0.46, 86.50± 0.38 and 86.63± 0.38 per cent for lymphocyte count and 1.00± 0.27, 1.00± 0.19, 0.5± 0.19, 0.5± 0.19, 0.5± 0.19, and 1.00± 0.25 per cent for eosinophil count.

Table 9. Differential Leucocyte Count During the Antipyretic and CNS study, %

	Group I			Group II			Group III			Group IV			Group V			Group VI		
	N	L	E	N	L	E	N	L	E	N	L	E	N	L	E	N	L	E
At peak of pyrexia	14.25± 0.90	84.50± 1.00	1.25± 0.37	15.88± 0.97	83.50± 0.98	0.63± 0.26	12.00± 0.87	88.00± 0.87	0	10.50± 0.94	89.13± 1.04	0.38± 0.18	12.38± 0.50	87.12± 0.58	0.50± 0.19	12.50± 0.38	87.00± 0.46	0.50± 0.19
5 hours post drug administration	13.75± 0.98	85.25± 0.98	1.00± 0.27	15.38± 0.91	83.63± 0.98	1.00± 0.19	12.38± 0.68	87.13± 0.58	0.50± 0.19	11.88± 0.44	87.50± 0.46	0.50± 0.19	13.00± 0.33	86.50± 0.38	0.50± 0.19	12.50± 0.50	86.63± 0.38	1.00± 0.25
At peak of tranquillization	14.25± 0.98	84.50± 1.00	1.25± 0.25	15.50± 0.98	83.50± 0.98	1.00± 0.27	12.63± 0.68	87.00± 0.68	0.38± 0.18	11.88± 0.44	87.38± 0.42	0.75± 0.16	12.75± 0.37	86.50± 0.38	0.75± 0.16	12.63± 0.46	86.75± 0.41	0.63± 0.18

The mean values were within the normal range and there was no significant difference between the animals of different groups.

4.4.5. Total Erythrocyte count at the Peak of Pyrexia

The mean erythrocyte count at the peak of pyrexia for groups I to VI were 7.89± 0.09, 8.02± 0.12, 7.96± 0.03, 7.99± 0.07, 7.93± 0.06 and 7.97± 0.03 millions/mm^3 of blood (table 10).

4.4.6. Total Erythrocyte Count 5 hours After Drug Administration

The mean erythrocyte counts 5 hours after drug administration for groups I to VI were 7.89± 0.10, 8.01± 0.08, 7.93± 0.04, 7.98± 0.05, 7.92± 0.05, and 7.98± 0.04 millions/mm^3 of blood (table 10).

All the values were within the normal range.

4.4.7. Haemoglobin Concentration in Antipyretic Study

The mean haemoglobin levels of animals of all groups were 14± 0.13, 14.3± 0.19, 14.55± 0.21, 14.55± 0.23, 14.48± 0.19, and 14.38± 0.19 g/dl.

The mean haemoglobin levels of animals of all groups were within the normal range and the results are presented in table 11.

4.5 SERUM BIOCHEMICAL PARAMETERS IN ANTIPYRETIC STUDY

4.5.1. Serum ALT Levels in Antipyretic Study

The ALT levels are given in table 12.

The mean values for all the groups before induction of pyrexia were 23.00± 0.63, 22.62± 0.50, 23.25± 0.73, 23.38± 0.68, 22.63± 0.56 and 23.38 ± 0.50 U/l.

The mean values for all the groups during peak of pyrexia were 22.5± 0.5, 22.63± 0.60, 23.25± 0.67, 22.75± 0.45, 23.5± 0.46, and 22.88 ± 0.61U/l.

The mean values for all the groups 5 hours after administration of drug were 22.75± 0.53, 22.75± 0.45, 22.63± 0.56, 22.75± 0.45, 23.5± 0.46 and 22.63± 0.56U/l.

Table 10. Total Erythrocyte Count, millions/mm^3 of blood

	Group I	Group II	Group III	Group IV	Group V	Group VI
Peak of pyrexia	7.89± 0.09	8.02± 0.12	7.96± 0.03	7.99± 0.07	7.93± 0.06	7.97± 0.03
5 hours after drug administration	7.89± 0.10	8.01± 0.08	7.93± 0.04	7.98± 0.05	7.92± 0.05	7.98± 0.04
At peak of tranquillization in CNS study	7.89± 0.08	7.91± 9.03	7.94± 0.04	7.99± 0.05	7.92± 0.05	7.98± 0.05

Table 11. Haemoglobin Content During the Entire Study, g/dl

	Group I	Group II	Group III	Group IV	Group V	Group VI
During antipyretic study	14.00± 0.13	14.3± 0.19	14.55± 0.21	14.55± 0.23	14.48± 0.19	14.38± 0.19
During CNS study	14.1± 0.17	14.23± 0.18	14.25± 0.16	14.53± 0.14	14.4± 0.20	14.35± 0.20

Table.12. ALT Levels During Antipyretic Study, U/l

	Group I	Group II	Group III	Group IV	Group V	Group VI
Before induction of pyrexia	23.00± 0.63	22.62± 0.50	23.25± 0.73	23.38± 0.68	22.63± 0.56	23.38± 0.50
Peak of pyrexia	22.50± 0.50	22.63± 0.60	23.25± 0.67	22.75± 0.45	23.50± 0.46	22.88± 0.61
5 hours after administration of drug	22.75± 0.53	22.75± 0.45	22.63± 0.56	22.75± 0.45	23.50± 0.46	22.63± 0.56

The mean values for all the groups were within the normal range and no significant difference was seen between the different groups.

4.5.2. Serum AST Levels in Antipyretic Study

The results are given in table 13.

The mean AST values of all the six groups before induction of pyrexia were 197.25± 2.61, 203.5± 2.39, 198.5± 2.32, 203.75± 2.05, 198.75± 1.25 and 206± 3.80 U/l.

The mean AST values of all the groups at the peak of pyrexia were 198.75± 2.23, 203.25± 2.45, 197.75± 2.46, 202± 2.27, 198.5± 1.30 and 204.25± 2.30U/l.

The mean AST values of all the groups after 5th hour of treatment were 199± 2.3, 203.5± 2.64, 198± 2.56, 203.25± 2.39, 198.5± 1.3 and 202.25± 1.98U/l.

The mean AST values of all the six groups were within the normal range.

4.6. HAEMATOLOGICAL PARAMETERS IN CNS STUDY.

4.6.1. Total Leukocyte Count at the Peak of Tranquillization

The results are given in table 8.

The mean total leukocyte count at the peak of tranquillization for groups I to VI were 6,894± 148.64, 7,038± 102.10, 7,100± 73.80, 7,031± 69.40, 7,025± 71.34, 7,225± 72.58 per mm^3 of blood.

The mean values of total leukocyte count at peak of tranquillization were in the normal range.

4.6.2. Differential Leukocyte Count at Peak of Tranquillization

The values are given in table 9.

The mean Differential Leukocyte Count at peak of tranquillization for groups I to VI were 14.25± 0.98, 15.50± 0.98, 12.63± 0.68, 11.88± 0.44, 12.75± 0.37 and 12.63± 0.46 per cent for neutrophil count, 84.5± 1.00, 83.5± 0.98, 87± 0.68, 87.38±

0.42, 86.5± 0.38 and 86.75± 0.41 per cent for lymphocyte count and 1.25± 0.25, 1.00± 0.27, 0.38± 0.18, 0.75± 0.16, 0.75± 0.16, and 0.63 ± 0.18 per cent for eosinophil count.

The mean differential leukocyte counts for the animals of all the groups were within the normal range at the peak of tranquillization indicating a non toxic dosing.

4.6.3. Total Erythrocyte Count at the Peak of Tranquillization

The mean erythrocyte count at the peak of tranquillization for groups I to VI were and 7.89± 0.08, 7.91± 9.03, 7.94± 0.04, 7.99± 0.05, 7.92± 0.05, 7.98± 0.05 millions/mm^3 of blood (table 10).

All the values were within the normal range.

4.6.4. Haemoglobin Concentration at the Peak of Tranquillization

The results are presented in table 11.

The mean haemoglobin levels of animals of all groups were 14.1± 0.17, 14.23± 0.18, 14.25± 0.16, 14.53± 0.14, 14.4± 0.2, and 14.35± 0.20 g/dl

The mean haemoglobin levels of animals of all groups were within the normal range.

4.7 SERUM BIOCHEMICAL PARAMETERS IN CNS STUDY

4.7.1 Serum ALT Levels at the Peak of Tranquillization

The values are given in table14.

The ALT levels in CNS study, before drug administration were 22.75± 0.45, 23.38± 0.56, 23.25± 0.59, 22.75± 0.53, 22.5± 0.5, and 22.63± 0.56 U/l.

The mean ALT levels in CNS study, at the peak of tranquillization were 23± 0.53, 22.63± 0.53, 22.63± 0.53, 23.25± 0.58, 22.75± 0.58, and 22.75± 0.42U/l.

All the mean values were within the normal range and there was no significant difference between various groups.

4.7.2. Serum AST Levels at the Peak of Tranquillization

The results are given in tables 15.

The mean AST values of all the six groups before administration of drugs were 200.25± 2.02, 203.75± 2.49, 199.25± 2.23, 202.75± 2.10, 198.25± 1.33 and 202.75± 1.96 U/l.

The mean AST values of all the groups at the peak of tranquillization were 201.25± 1.73, 201.75± 1.87, 201.00± 2.00, 201.75± 1.70, 198.0± 1.96 and 202.25± 1.83 U/l.

The mean AST values of all the six groups were within the normal range.

Table.13. AST Levels During Antipyretic Study, U/l

	Group I	Group II	Group III	Group IV	Group V	Group VI
Before induction of pyrexia	197.25±2.61	203.5±2.39	198.5± 2.32	203.75±2.05	198.75± 1.25	206± 3.80
Peak of pyrexia	198.75±2.23	203.25± 2.45	197.75± 2.46	202±2.27	198.5± 1.30	204.25±2.30
5 hours after administration of drug	199± 2.3	203.5±2.64	198.00±2.56	203.25±2.39	198.5± 1.3	202.25±1.98

Table.14. ALT Levels During CNS Study, U/l

	Group I	Group II	Group III	Group IV	Group V	Group VI
Before drug administration	22.75± 0.45	23.38± 0.56	23.25± 0.59	22.75± 0.53	22.5± 0.50	22.63± 0.56
Peak of tranquillisation	23.00± 0.53	22.63± 0.53	22.63± 0.53	23.25± 0.58	22.75± 0.58	22.75± 0.42

Table.15. AST Levels During CNS Study, U/l

	Group I	Group II	Group III	Group IV	Group V	Group VI
Before drug administration	200.25±2.02	203.75± 2.49	199.25±2.23	202.75±2.10	198.25± 1.33	202.75±1.96
Peak of tranquillization	201.25±1.73	201.75± 1.87	201.00±2.00	201.75±1.70	198.00± 1.96	202.25±1.83

Discussion

5. DISCUSSION

The objectives of the present study were to detect the active principles present in alcoholic extracts of seeds of red and white lotus (*Nelumbo nucifera*), to assess and compare their antipyretic property and central nervous system activity.

The antipyretic study was conducted by inducing pyrexia in albino rats by subcutaneous injection of 20% suspension of Brewer's yeast. The central nervous system activity was assessed by measuring the forced and spontaneous motor activity using rotarod and actaphotometer.

5.1 ACTIVE PRINCIPLES PRESENT IN THE ALCOHOLIC EXTRACT FROM SEEDS OF RED AND WHITE LOTUS

The various active principles detected in *Nelumbo nucifera* seed extracts by different qualitative tests were steroids, alkaloids, flavonoids, glycosides, phenolic compounds, diterpenes, triterpenes and saponins. Flavonoids are phenols beneficial as powerful antioxidants, antipyretics, stress modifiers and antiallergic agents. Saponins are glycoside compounds and possess antioxidant effect (Batchelder, 1995; Lipkin, 1998).

The major phytoconstituents present in the seeds of *Nelumbo nucifera* are alkaloids like dauricine, lotusine, nuciferine, pronuciferine, liensinine, isoliensinine, roemerine, nelumbine and neferine. The seeds also contain saponins, phenolics and carbohydrates (Rai *et al.*, 2006). These observations are in agreement with the present study.

5.2 STUDY OF THE ANTIPYRETIC EFFECT OF ALCOHOLIC EXTRACT FROM SEEDS OF RED AND WHITE LOTUS

The antipyretic and antinociceptive activities are commonly mentioned as characteristic of drugs which have inhibitory effect on prostaglandin synthesis. Hence the experimental model of pyrexia which is due to production of prostaglandins, (yeast

induced hyperthermia) was employed to investigate the antipyretic effect of methanolic extracts of red and white lotus seeds.

All the animals showed peak pyrexia at 19th hour of yeast administration and the time of peak pyrexia was taken as the zeroth hour of study. Yeast induced pyrexia is called pathogenic fever which is due to the production of prostaglandins (PGE_2) which set the thermoregulatory center at a higher temperature (Howard, 1993). The standard drug used was aspirin which produced antipyretic effect by inhibition of prostaglandin biosynthesis (Roberts and Morrow, 2001). Sengupta (1999) reported that aspirin produced nonspecific inhibition of COX enzymes.

The results of the present study revealed that white lotus seed extract at the dose rate of 600 mg/kg body weight is the most potent of all the other extracts up to third hour of the study. But during the fourth hour of study red lotus (600 mg/kg) and white lotus (400 mg/kg and 600 mg/kg) were showing similar antipyretic effect. Seeds of red lotus at the dose rate of 400 mg/kg was having the least antipyretic effect. The antipyretic effect was comparable to that of standard drug aspirin.

A similar study conducted by Mukherjee *et al.* (1996a) indicated that *Nelumbo nucifera* rhizomes at dose rates of 200, 300 and 400 mg/kg possessed significant antipyretic effect and the antipyretic effect was comparable to that of standard drug paracetamol. The rosmarinic acid of *Rosmarinus officinalis* inhibited leukotriene and prostaglandin synthesis while COX-1 and COX-2 was inhibited by cirsilineol, cirsimartin, apigenin, rosmarinic acid and eugenol of *Ocimum sanctum* similar to Aspirin. Ursolic acid, a pentacyclic triterpene present in *Alstonia macrophylla* was found to inhibit 5-lipooxygenase and cyclooxygenase and thereby prostaglandin biosynthesis (Kelm *et al.*, 2000). The results of present study is in agreement with the results of the study conducted by Trongsakul *et al.* (2003) in which there is significant reduction in yeast induced pyrexia by hexane extract of *Diospyros variegata* and the antipyretic effect was similar to that of Aspirin. Furthermore Panthong *et al.* (2003) observed significant antipyretic activity of *Clerodendron petasites* in yeast induced

pyrexia in mice. They also explained that the mechanism of antipyretic action of plant extract was similar to that of other Non Steroidal Antiinflammatory Drugs. The results obtained in the present study suggested that the plant extract has some influence on prostaglandin biosynthesis because prostaglandin is believed to be a regulator of body temperature.

Mutalik *et al.* (2003) observed that dry residue of the fresh extract of *Solanum melongena* produced antipyretic effect in a dose dependent manner in yeast induced pyrexia in mice. They suggested that the antipyretic activity could be attributed to the presence of flavonoids. Many other previous studies also supported this observation. Presence of flavonoids in Dalbergia species was reported by Ramaswamy *et al.* (1985). Hajare *et al.* (2000) attributed the antipyretic effect to the flavonoids present in the same plant. The phytochemical analysis of the chloroform and methanolic extracts of *Vernonia cinerea* leaves revealed the presence of steroids, alkaloids, saponins, flavonoids and terpenoids which were responsible for the antipyretic effect of the plant (Iwalewa *et al.*, 2003). Hence the antipyretic effect of *Nelumbo nucifera* may be due to either any one of the active principles or combined effect of the active principles.

5.3 STUDY OF THE CNS ACTIVITY OF THE ALCOHOLIC EXTRACT FROM SEEDS OF RED AND WHITE LOTUS

5.3.1 Actaphotometer Counts

The spontaneous motor activity is measured by counting the spontaneous movement of the treated rats in an isolated cage. As the degree of tranquillization increases, the number of spontaneous movements decreases. Turner (1965) described one method to assess the tranquillization as counting of spontaneous motor activity, which is done by an actaphotometer, as number of light beam interruption which gave the number of spontaneous movements.

The methanolic extract of red and white lotus significantly decreased spontaneous motor activity in rats as evidenced by actaphotometer counts. The effect

was maximum from 90th minute to 120th minute, thereafter the counts continued to increase and reached normal by 240th minute. From the results it is evident that the onset of action is more with higher doses, but the time of peak effect remained the same. Locomotor activity is considered as an index of alertness and decrease in locomotor activity indicates sedation (Thakur and Mengi, 2005). Rabbani *et al.* (2005) demonstrated the sedative effect of *Salvia reuterana* using actaphotometer. Locomotor system is under the control of substantia nigra and basal ganglia where the predominant excitatory neurotransmitter is dopamine. Dopamine receptors are classified as D_1, D_2, D_3 and D_4 receptor sites. D_4 is responsible for hypolocomotion which is sensitive to small doses of dopamine agonists and antagonists. The D_2 receptor site is sensitive to larger doses of dopamine and mediate hyperlocomotion. D_3 receptors are involved in inhibition of dopamine release and locomotor activity. The activation of presynaptic dopamine receptors resulted in diminished dopamine output and so diminished locomotor activity (Booth, 2004). Hence it is inferred that the decrease in spontaneous motor activity observed in the present study may be due to either stimulation of D_4 receptors or activation of presynaptic dopamine receptors by the active principles present in the seeds of red and white lotus.

5.3.2. Rotarod Performance Time

Forced locomotor activity can be used as a method of assessing the degree of tranquillization (Turner, 1965) as it directly indicate the motor incoordination as an effect caused by the action of tranquillization on the motor centers of the brain. This is done with a rotarod. The time permanence on the rotarod of the test group was compared with a control group and analysed statistically for significance.

The results of the present study indicate that during the first thirty minutes red lotus at the dose rate of 400 mg/kg body weight and white lotus at the rate of 600 mg/kg body weight showed similar effect. Thereafter up to 240 minutes the extract of red lotus seed at a dose rate of 600 mg/kg induced maximum CNS activity in

inhibiting the forced motor activity. Furiuele *et al.* (1961) demonstrated that compounds which decrease spontaneous motor activity in doses lower than those required for affecting forced coordinated motor activity presumably act through sites other than the cortex. On the other hand, agents that are acting mainly on cerebral cortex, inhibit forced motor activity to a greater extent than spontaneous motor activity. The results of the present study are in agreement with the results of the similar work conducted by Mukherjee *et al.* (1996b). They observed a significant tranquillizing activity of methanolic extract of the rhizomes of *Nelumbo nucifera,* as it inhibited the forced motor activity in the rotarod at dose rates of 200, 300 and 400 mg/kg.

Kulkarni *et al.* (1988) also conducted similar work on extract of *Clitoria terneata* and found that there was a dose dependent reduction in forced motor activity. Nair (2001) also reported inhibition of spontaneous and forced motor activity by oral administration of alcoholic extract of *Clitoria terneata* at dose rates of 250 mg/kg and 500 mg/kg. A poly herbal formulation named Unmadnashak Ghrita induced decrease in spontaneous motor activity and antagonised hyperlocomotor activity induced by amphetamine in mice which indicated CNS depressant activity (Achliya *et al.*, 2004). They suggested that the drug acted by increasing the GABA concentration in the brain. Similar observations made by Achliya *et al.* (2005) by using Brahmi Ghrita in mice. Emamghoreishi *et al.* (2005) found that the flavonoids present in coriander seeds especially as in free state and glycosides were responsible for the CNS activity of the aqueous extract of coriander seeds. They suggested that the CNS activity may be due to the affinity of the flavonoids to the central benzodiazepine receptors. Methanolic extract of *Galphimia glauca* contain a triterpene named Galphimin B which possessed significant sedative action (Herrera-Ruiz *et al.*, 2006). The probable mechanism of action is the inhibition of dopaminergic receptors in the ventral tegumental area of rats. Saponins are known to have antagonistic property against amphetamine, sedative property and decreased forced motor activity in experimental animals.

Nelumbo nucifera seed also contain saponins. It is therefore possible that the saponin component of the extract may contribute to CNS sedative property of the plant. Hence the sedative property may be either due to its antidopaminergic action or direct action through central benzodiazepine receptors. The active principles like flavonoids, triterpenes or saponins may be responsible for producing this action.

5.4 EFFECT OF THE ALCOHOLIC EXTRACT FROM SEED OF RED AND WHITE LOTUS ON HAEMATOLOGICAL PARAMETERS

The results of the present study indicated that there was no significant effect of the plant extract on the various haematological parameters like Total Erythrocyte Count, Total Leucocyte Count, Differential Leucocyte Count and Haemoglobin Concentration as evidenced by the normal values during the entire course of the experiment (Schalm *et al.*, 1975). This could lead to a point that the extracts induced no haematological toxicity at the dose prescribed for the study. Hence it could be concluded that the plant extracts could be used safely for the Central Nervous System effects as well as antipyretic effects at the dose levels studied.

5.4 EFFECT OF THE ALCOHOLIC EXTRACT FROM SEEDS OF RED AND WHITE LOTUS ON SERUM ENZYMES

The serum biochemical parameters studied were Alanine Amino Transferase and Aspartate Amino Transferase. The levels of the enzymes showed no statistically significant variation in all the groups right from the beginning of the experiment till the end. All the values were within the normal range as documented by Hrapkiewics *et al.* (1998). This indicated that there was no hepatotoxicity. Hence it may be concluded that the dose selected for the study was non toxic and hence could be safely used for the antipyretic and CNS activity.

Many plant products have been found to exert antipyretic effect and sedation. The methanolic extract of the seeds of red and white *Nelumbo nucifera* exerted antipyretic and tranquillizing activity as evidenced by the results of the present study.

The extract of white lotus seed at the dose rate of 600 mg/kg produced maximum antipyretic effect. The extract of seed of red lotus at the dose rate of 600 mg/kg inhibited the forced motor activity to the maximum where as there was no difference between the red and white lotus seeds in inhibiting the spontaneous motor activity. The extracts at both dose rates induced no haematological as well as hepatotoxicity indicating its safety for therapeutic purpose. From this study, it can be concluded that the methanolic extract of *Nelumbo nucifera* possesses potent antipyretic and tranquillizing activity. Further research is needed to explore more about the role of each and every active principle of the seeds of red and white lotus in producing antipyretic and CNS activity.

Summary

6. SUMMARY

The present study was undertaken to detect the phytochemicals present in the methanolic extract of the seeds of red and white lotus and to assess their antipyretic and CNS activity. The effect of the extracts were compared at two different doses of 400 mg/kg and 600 mg/kg. The standard drugs used were aspirin and chlorpromazine in antipyretic and CNS study respectively.

The qualitative tests for the detection of phytochemical principles present in the extract were done to find out the active principles present in the extract. The test revealed the presence of steroids, alkaloids, flavonoids, glycosides, phenolic compounds diterpenes, triterpenes and saponins.

For antipyretic study 48 albino rats were divided into six groups of eight animals each. Pyrexia was induced by the subcutaneous injection of 20% yeast suspension at a dose rate of 1ml/100 g body weight. The first group served as pyrexia control. Second group was given aspirin at the dose rate of 100 mg/kg. The third and fourth groups were given the extract of seed of red lotus at the dose rate of 400 and 600 mg/kg respectively. The fifth and sixth group were given the extract of seed of white lotus at the dose rate of 400 and 600 mg/kg respectively. The body temperature was measured every hour from the peak of pyrexia continuously for five hours. The blood was collected at the peak of pyrexia and at fifth hour post drug administration for estimation of serum ALT, AST levels and haematological parameters.

For conducting CNS study, 48 albino rats were divided into six groups of eight animals each and the forced and spontaneous motor activity were tested using rotarod and actaphotometer respectively. Animals of the first group were kept as normal control, second group was given chlorpromazine at the dose rate of 7 mg/kg, third and fourth group were treated with the extract of seed of red lotus and fifth and sixth group with the extract of seed of white lotus at the dose rate of 400 and 600 mg/kg respectively. The count per ten minutes was taken in case of actaphotometer every 30

minutes till 240 minutes. The time spent by the animals on the rotarod was taken at every 30 minutes after drug administration for a period of four hours. Blood was collected at the peak of tranquillization for the estimation of ALT, AST levels and haematological parameters.

Among the extracts the maximum antipyretic activity was shown by the extract of seed of white lotus at the dose rate of 600 mg/kg (group VI) till the third hour of study. By the fourth hour, red lotus at the rate of 600 mg/kg (group IV) and white lotus 400 mg/kg (group V) showed same antipyretic effect as that of group VI. The antipyretic activity of the extract of seed of white lotus at the dose rate of 600 mg/kg was comparable to that of aspirin. The serum ALT and AST values were with in the normal range. The haematological parameters studied viz, Total Erythrocyte Count, Total Leucocyte Count, Differential Leucocyte Count and Haemoglobin Concentration were also within the normal range.

Spontaneous motor activity was assessed using actaphotometer. Eventhough the extract of red lotus seed at the dose rate of 600 mg/kg inhibited the spontaneous motor activity to the maximum initially, all the treatment groups were found to be equipotent after 120 minutes of drug administration. The standard drug was more potent inhibitor of spontaneous motor activity. On comparison of treatment groups maximum inhibition of forced motor activity was seen in group IV, followed by group VI and group III. Chlorpromazine inhibited the forced motor activity to the maximum when compared to the treatment groups. The serum ALT and AST values at the peak of tranquillization were within the normal range. The haematological parameters were also within the normal range. The results of the study indicate that the extract possessed tranquillizing property.

The results of the present study confirmed the antipyretic and tranquillizing properties of the seeds of red and white lotus. The presence of flavonoids, alkaloids, terpenes, saponins and glycosides in the methanolic extract of the seeds of *Nelumbo nucifera* can be attributed to the potent antipyretic and CNS activity of the extract. The

mechanism of antipyresis may be the inhibition of prostaglandin synthesis where as that of the CNS activity may be through potentiation of GABA mediated inhibition or through Dopamine receptors. Further study is needed to isolate the active principles responsible for the antipyretic and CNS activity of the methanolic extract of the seeds of red and white lotus.

References

REFERENCES

Achliya, G.S., Wadodkar, S.G. and Dorle, A.K. 2004. Evaluation of sedative and anticonvulsant activities of Unmadnashak Ghrita. *J. Ethnopharmacol.* 94: 77-83

Achliya, G.S., Wadodkar, S.G. and Dorle, A.K. 2005. Evaluation of CNS activity of Brahmi Ghrita. *Indian J. Pharmacol.* 37: 33-36

Adzu, B., Amos, S., Dzarma, S., Muazzam, I. and Gamaniel, K. S. 2002. Pharmacological evidence favouring the folkloric use of *Diospyros mespiliformis* Hoechst in the relief of pain and fever. *J. Ethnopharmacol.*82: 191-195

Agarwal, A., Malini, S., Bairy, K.L. and Rao, M.S. 2003. Effect of *Tinospora cordifolia* on learning and memory in normal and memory deficit rats. *Indian J. Pharmacol.* 34: 339-349

Ahmadiani, A., Javan, M., Semnanian, S., Barat, E. and Kamalinejad, M. 2001. Anti-inflammatory and antipyretic effects of *Trigonella foenum-graecum* leaves extract in the rat. *J. Ethnopharmacol.* 75:283-286

Al-Yousuf, M. H., Bashir, A. K., Ali, B. H., Tanira, M.O. M. and Blunden, G. 2002. Some effects of *Salvia aegyptiaca* L. on the central nervous system in mice. *J. Ethnopharmacol.* 81:121-127

Ambawade, S.D., Kasture, V.S. and Kasture, S.D. 2002. Anticonvulsant activity of roots and rhizomes of *Glycyrrhiza glabra. Indian J. Pharmacol.* 34: 251-255

Amos, S., Adzu, B., Binda, L., Wambebe, C. and Gamaniel, K. 2001. Neuropharmacological effect of the aqueous extract of *Sphaeranthus senegalensis* in mice. *J. Ethnopharmacol.* 78: 33-37

Anonymous. 1966. *The wealth of India.* Vol. II. CSIR. p.522

Asongalem, E. A., Foyeth, S., Ekobo. S., Dimo, T. and Kamtchouing, P. 2004. Antiinflammatory, lack of central analgesia and antipyretic properties of *Acanthus montanus* (Ness) T. Anderson. *J. Ethnopharmacol. 95*:63-68

*Batchelder, H.J. 1995. Pharmacognosy. *Herbal Res.* 23 : 11-16

Besra, S.E., Sharma, R.M. and Gomes, A. 1996. Antiinflammatory effect of petroleum ether extract of leaves of *Litchi chinensis* Gaertn. (Sapindaceae). *J. Ethnopharmacol.* 54:1-6

Bigoniya, P. and Rana, A.C. 2005. Psychopharmacological profile of hydroalcoholic extract of *Euphorbia nerifolia* leaves in mice and rats. *Indian J. Exp. Biol.* 43: 859-862

Booth, N.H. 2004. Tranquillizers, alpha-2 adrenergic agonists and related agents. *Veterinary Pharmacology and Therapeutics.* ed: Adams, H.R. Ninth edition. Iowa state University Press, Ames. pp. 311-318

Chindo, B. A., Amos, S., Odutola, A. A., Vongtau, H. O., Abbah, J., Wambebe, C. and. Gamaniel, K. S. 2003. Central nervous system activity of the methanol extract of *Ficus platyphylla* stem bark. *J. Ethnopharmacol.* 85: 131-137

Chopra, R.N., Nayar, S.L. and Chopra, A.C. 1958. *Glossary of Indian Medicinal Plants* CSIR, New Delhi. p.154

Devi, B.P., Boominathan, R., Subhash, C. and Mandal, S.C. 2003. Evaluation of antipyretic potential of *Cleome viscosa* Linn. (Capparidaceae) extract in rats. *J. Ethnopharmacol.* 87:11-13

Dewan, S., Kumar, S. and Kumar, V.L. 2000. Antipyretic effect of latex of *Calotropis procera. Indian J. Pharmacol.* 32: 252

Dhawan, K., Kumar, S. and Sharma, A. 2001. Anti-anxiety studies on extracts of *Passiflora incarnata* Linneaus. *J. Ethnopharmacol.* 78: 165-170

Emamghoreishi, M., Khasaki, M. and Aazam, M.F. 2005. *Coriandrum sativum*: evaluation of its anxiolytic effect in the elevated plus-maze. *J. Ethnopharmacol. 96*: 365-370

*Furiuele, A.R., Kinnard, W.J. and Buckley, J.P. 1961. Evaluation of certain psychopharmacological compounds. *J. Pharmacol. Sci.* 50: 252-254

Gilani, A. H., Aziz, N., Khan, M.A., Shaheen, F., Jabeen, Q., Siddiqui, B. S. and Herzig, J. W. 2000. Ethnopharmacological evaluation of the anticonvulsant, sedative and antispasmodic activities of *Lavandula stoechas. J. Ethnopharmacol.* 71: 161-167

Gupta, M., Mazumder, U.K., Kumar, R.S. and Kumar, T.S. 2003. Studies on antiinflammatory, analgesic and antipyretic properties of methanol extract of *Caesalpinia bonducella* leaves in experimental animal models. *Iran J. Pharmacol. Therap.* 2: 30-34

Gupta, M., Mazumder, U.K., Kumar, R.S., Gomathi P., Rajeshwar, Y., Kakoti, B.B. and Selven, V.T. 2005. Antiinflammatory, analgesic and antipyretic effects of methanol extract from *Bauhinia racemosa* stem bark in animal models. *J. Ethnopharmacol.* 98: 267-273

Hajare, S.W., Chandra, S., Tandan, S.K., Sharma, J., Lal, J. and Telang, A.G. 2000. Analgesic and antipyretic activities of *Dalbergia sissoo* leaves. *Indian J. Pharmacol.* 32:357-360

Harborne, J.B. 1991. *Phytochemical methods- guide to modern techniques of plant analysis.* Second edition. Chapman and Hall, India. p. 653

Hellion-Ibarrolla, M. C., Ibarrolla, D.A., Montalbetti, Y., Villalba, D., Heinichen, O. and Ferro, E.A. 1999. Acute toxicity and general pharmacological effect on central nervous

system of the crude rhizome extract of *Kyllinga brevifolia* Rottb. *J. Ethnopharmacol.* 66: 271-276

Herrera-Ruiz, M., Jimenez-Serrer, J.E., De- Lima, T.P.M., Aviles-Montes, D., Perez-Jarcia, D., Gonzalez-Portazar, M. and Tortoriello, J. 2006. Anxiolytic and antidepressant-like activity of a standarised extract from *Galphimia glauca. Phytomedicine.* 13: 23-28

Hogalin, D.C., Mosteller, F. and Tukey, J.W. 1991. *Fundamentals of exploratory analysis of varience.* M.A. Addisson Wesley. p. 448

*Howard, M. 1993. Fever: Causes and consequences. *Neuroscience Behavioural Review.* 17: 237-269

Hrapkiewics, K., Medina, L. and Holms, D.D. 1998. Appendix one. *Clinical Laboratory Animal Medicine.* Second edition. Iowa State University Press. p. 259

Hsieh, M.T., Peng, W.H., Yeh, F.T., Tsai, H.Y. and Chang, Y.S. 1991. Studies on the anticonvulsive, sedative and hypothermic effects of *Periostracum cicadae* extracts . *J. Ethnopharmacol.* 35: 83-90

Iwalewa, E.O., Iwalewa, O.J. and Adeboye. 2003. Analgesic, antipyretic and antiinflammatory effects of methanol, chloroform and ether extracts of *Vernonia cinerea* Ness leaf. *J. Ethnopharmacol.* 86: 229-234

Jain, S. C., Jain, R., Sharma R.A., and Capasso, F. 1997. Pharmacological investigation of *Cassia italica. J. Ethnopharmacol.* 58:135-142.

*Kashiwada, Y., Aoshima, A., Ikeshiro, Y., Furuikawa, H., Itoigawa, M., Fujioka, T., Mithashi, K., Cosentino, L.M., Morris-Natschke, S.L. and Lee, K.H. 2005. Anti-HIV benzyl isoquinoline alkaloids and flavonoids from the leaves of *Nelumbo nucifera* and

structure –activity correlations with related alkaloids. *Bioorg. Med. Chem.* 13 : 443-448.

Kelm, M.A., Nair, M.G., Starsburg, G.M. and Dewitt, D.L. 2000. Antioxidant and cyclooxygenase inhibitory phenolic compounds from *Ocimum sanctum* Linn. *Phytomedicine* 7:7-13

Kulkarni, C., Pattanshetty, J.R. and Amruthraj, G. 1988. Effect of alcoholic extract of *Clitoria ternatea Linn.* on central nervous system in rodents. *Indian J. Exp. Biol.* 26:957-960

Lanhers, M., Fleurentin, J., Cabalion, P., Rolland, A., Dorfman, P., Misslin, R. and Pelt, J. 1990. Behavioural effects of *Euphorbia hirta* l.: sedative and anxiolytic properties *J. Ethnopharmacol.* 29:189-198

*Ling, Z.Q., Xie, B.J. and Yang, E. L. 2005. Isolation, characterization, and determination of antioxidative activity of oligomeric procyanidins from the seed pod of *Nelumbo nucifera* Gaertn. *J. Agric. Food Chem.* 53: 2441-2445

Lipkin, R. 1998. Saponins Research Information. *Sci. News.* 148 : 2-5

Makonnen, E., Debella, A., Zerihun, L., Abebe, D. and Teka, F. 2003. Antipyretic properties of the aqueous and ethanol extracts of the leaves of *Ocimum suave* and *Ocimum lamiifolium* in mice. *J. Ethnopharmacol.* 88:85-91

Mazumder, U.K., Gupta,M., Pramanik,G., Mukhopadhyay, R.K. and Sarkar, S. 1992. Antifertility activity of seed of *Nelumbo nucifera* in mice. *Indian J. Exp. Biol.* 30: 533-534

Misar, A.V., Upadhye, A.S. and Mujumdar, A.M. 2004. CNS depressant activity of ethanol extract of *Luffa acutangula* Var. *amara* C. B. Clarke. fruits in mice. *Indian J. Pharmaceut. Sci.* 46: 463-467

Mora, S., Diaz-Veliz, G., Lungenstrass, H., Garcia-Gozalez, M., Coto-Motales, T., Poletti, C., De Lima, T.C.M., Herrera-Ruiz, M and Tortoriello, J. 2005. Central nervous system activity of the hydroalcoholic extract of *Casimiora edulis* in rats and mice. *J. Ethnopharmacol.* 97: 191-197

Mukherjee, P.K. and Wahile, A. 2006. Integrated approaches towards drug development from Ayurveda and other Indian system of medicines. *J. Ethnopharmacol.* 103: 25-35

Mukherjee, P.K., Das, J., Saha, K., Giri, S. N., Pal, M and Saha, B.P. 1996a. Antipyretic activity of *Nelumbo nucifera* rhizome extract. *Indian J. Exp. Biol.* 34: 275-276

Mukherjee, P.K., Saha, K., Balasubramanian, R., Pal, M. and Saha, B.P. 1996b. Studies on the psychopharmacological effects of *Nelumbo nucifera* Gaertn. rhizome extract. *J. Ethnopharmacol.* 54: 63-67

Mutalik, S, Paridhavi, K, Rao, C, M. and Udupa, N. 2003. Antipyretic and analgesic effect of leaves of *Solanum melongena* Linn. in rodents. *Indian J. Pharmacol.* 35: 312-315

Nadkarni, A.K. 1992. Dr. K.N. Nadkarni's Indian Materia Medica Vol I. Popular Prakashan, Bombay. p. 845

Nair, S.N. 2001. Tranquillizing property of *Clitoria ternatea* Linn. (Sankhupushpam), *Acorus calamus* Linn. (Vayampu) and *Vitex Leucoxylon* Linn. (Atta Nocchi) in rats. MVSC thesis, Kerala Agricultural University. p. 89

Nalini, K., Karanth, K.S., Rao, A. and Aroor, A.R. 1995. Effect of *Celastrus paniculatus* on passive avoidance performance and biogenic amine turn over in albino rats. *J. Ethnopharmacol.* 47:101-108

Narayanan, N., Thirugnanasambantham, P., Viswanathan, S., Vijayasekaran, V., Sukumar, E. 1999. Antinociceptive, anti-inflammatory and antipyretic effects of ethanol extract of *Clerodendron serratum* roots in experimental animals. *J. Ethnopharmacol.* 65:237-241

Ono, Y., Hattori, E., Fukaya, Y., Imai, S. and Ohizumi, Y. 2006. Anti-obesity effect of *Nelumbo nucifera* leaves extract in mice and rats. *J. Ethnopharmacol.* 106: 238-244

Panchal, G.M., Bhatt, V.H., Doctor, R.B. and Vajpayee, S. 1989. Pharmacology of *Acorus calamus* L. *Indian J. Exp. Biol.* 27: 561-567

Panthong, A., Kanjanapothi, D., Taesotikul, T., Wongcome, T. and Reutrakul, V. 2003. Antiinflammatory and antipyretic properties of *Clerodendrum petasites* S. Moore. *J. Ethnopharmacol.* 85: 151-156

Panthong, A., Kanjanapothi,D., Taesotikul, T., Phankummoon, A., Panthong, K. and Reutrakul, V.2004, Antiinflammatory activity of methanolic extracts from *Ventilago harmandiana* Pierre. *J. Ethnopharmacol.* 91:237-242

Peng, W., Hsieh, M., Lee, Y, Lin, Y., and Liao, J. 2000. Anxiolytic effect of seed of *Ziziphus jujuba* in mouse models of anxiety. *J. Ethnopharmacol.* 72: 435-441

Perianayagam, J.B., Sharma, S.K., Joseph, A. and Christina, A.J.M. 2004. Evaluation of antipyretic and analgesic activity of *Emblica officinalis* Gaertn. *J. Ethnopharmacol.* 95:83-85

Poli, A., Nicolau, M., Simoes, C. M. O., Nicolau, R.M.R. and Zanin, M. 1992. Preliminary pharmacologic evaluation of crude whole plant extracts of *Elephantopus scaber*. Part I: in vivo studies. *J. Ethnopharmacol.* 37: 71-76

Rabbani ,M., Sajjadi, S.E., Jafarian, A. and Vaseghi, G. 2005 Anxiolytic effects of *Salvia reuterana* Boiss on the elevated plus maze model of anxiety in mice. *J. Ethnopharmacol.* 101:100-103

Rai, S., Wahile, A., Mukherjee, K., Saha, B.S. and Mukherjee, P.K. 2006. Antioxidant activity of *Nelumbo nucifera* (Sacred lotus) seeds. *J. Ethnopharmacol.* 104: 322-327

Ramaswamy, S., Pillai, N.P., Gopalkrishnan, V., Parmar, N.S. and Ghosh, M.N. 1985. Analgesic effect of O(β-hydroxy ethyl) rutoside in mice. *Indian J. Exp. Biol.* 23: 219-220

Reitman,S. and Frankel, S. 1957. A colourimetric method for determination of serum glutamic oxaloacetate and glutamic pyruvic transaminase. *Am. J. Clinic. Pathol.* 28:56-63

Roberts, L.J. and Morrow, J.D. 2001. Analgesic, Antipyretic and anti-inflammatory agents and drugs employed in the treatment of gout. *Goodman and Gilman's The Pharmacological basis of therapeutics* (eds: Hardman, J.G., Limbird, L.E. and Gilman, A.G.) Tenth Edition. McGraw-Hill Medical Publishing Division, NewYork, pp. 687-727

Schalm, O.W., Jain, N.C. and Caroll, E.J. 1975. *Veterinary Haematology*. Third Edition. Lea and Febiger. p.1497

Sengupta, S. 1999. Cyclooxygenase-II: A new therapeutic target. *Indian J. Pharmacol.* 31:322-332

Shukia, B., Khanna, N.K. and Godhwani, J.L. 1987. Effect of Brahmi rasayan on the central nervous system. *J. Ethnopharmacol.* 21: 65-74

Simmons, D.L., Botting, R.M and Hla, T. 2004. Cyclooxygenase Isozymes: The biology of Prostaglandin synthesis and inhibition. *Pharmacol. Rev.* 56 (3): 387-437

*Sinha, S., Mukherjee, P.K., Mukherjee, K., Pal, M., Mandal, S.C. and Saha, B.P. 2000. Evaluation of antipyretic potential of *Nelumbo nucifera* stalk extract. *Phytother. Res.* 14: 272-274

So, H., Park, R., OH, H., Pae, H., Lee, J., Chai, K., Chung, S. and Chung, H. 1999. The methanol extract of *Spiraea prunifolia* var. *simpliciflora* root inhibits the generation of nitric oxide and superoxide in RAW 264.7 cells. *J. Ethnopharmacol.* 68:209-217

*Sohn, D.H., Kim, Y.C., Oh, S.H., Park E.J., Li, X. and Lee, B.H. 2003. Hepatoprotective and free radical scavenging effects of *Nelumbo nucifera. Phytomedicine* 10:165-169

Soulimani, R., Younos, C., Jarmouni, M. S., Bousta, D., Misslin, R. and Mortier, F. 1997. Behavioural effects of *Passiflora incarnata* L. and its indole alkaloid and flavonoid derivatives and maltol in the mouse *J. Ethnopharmacol.* 57: 11-20

Thakur, V.D. and Mengi, S.A. 2005. Neuropharmacological profile of *Eclipta alba* (Linn) Hassk. *J. Ethnopharmacol.* 122:23-31

Trongsakul, S., Panthong, A., Kanjanapothi, D. and Taesotikul, T. 2003. The analgesic, antipyretic and anti-inflammatory activity of *Diospyros variegata* Kruz. *J. Ethnopharmacol.* 85: 221-225

Turner, R.A. 1965. *Screening methods in Pharmacology.* Academic Press New York and London. p. 332

Viola, H., Wolfman, C., Stein, M.L., Wasowski, C., Pena, C., Medina J. H. and Paladini, A. C. 1994. Isolation of pharmacologically active benzodiazepine receptor ligands from *Tilia tomentosa* (Tiliaceae). *J. Ethnopharmacol.* 44:47-53

*Wu, M.J., Wang, L., Weng, C. Y. and Yen, J. H. 2003. Antioxidant activity of methanol extract of the lotus leaf (*Nelumbo nucifera* Gaertn.). *Am. J. Chin. Med.* 31: 687-698

*Wu, S., Sun, C., Cao, X., Zhou, H., Hong, Z. and Pan Y. 2004. Preparative counter chromatography isolation of liensenine and its analogue from embryo of the seed of *Nelumbo nucifera* Gaertn. using upright coil planet centrifuge with four multilayer coils connected in series. *J. Chromatogr.* 1041: 153-162

*Xiao, J.H., Zhang, J.H., Chen, H.L., Feng, X.L. and Wang, J.L. 2005. Inhibitory effects of isoliensinine on bleomycin induced pulmonary fibrosis in mice. *Planta Med.* 71: 225-230

Yeileda, E. and Kupeli, E. 2002. *Berberis crataegina* DC. root exhibits potent anti-inflammatory, analgesic and febrifuge effects in mice and rats. *J. Ethnopharmacol.* 79: 237-248

*Yu, J. and Hu, W.S. 1997. Effects of neferine on platelet aggregation in rabbits. *Yaoxue Xuebao.* 32: 1-4

Zia, A., Siddiqui, D.S., Begum, S., Siddiqui, S. and Suria, A. 1995. Studies on constituents of leaves of *Nerium oleander* on behavioural pattern in mice. *J. Ethnopharmacol.* 49: 33-39

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* Originals not consulted

ANTIPYRETIC AND CNS ACTIVITY OF SEEDS FROM RED AND WHITE TYPES OF LOTUS (*Nelumbo nucifera*) IN ALBINO RATS

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ABSTRACT

The antipyretic and CNS activity of the extract of the seeds of red and white *Nelumbo nucifera* was studied in albino rats. Qualitiative tests for the detection of phytochemicals showed the presence of steroids, alkaloids, flavonoids, glycosides, phenolic compounds, diterpenes, triterpenes and saponins. Pyrexia was induced by subcutaneous injection of 20% Brewer's yeast suspension. Group I served as pyrexia control, group II was administered aspirin at the dose rate of 100 mg/kg body weight, Group III and IV with extract of red lotus seed and group V and VI with extract of white lotus seed at the dose rate of 400 and 600 mg/kg body weight respectively. The body temperature was recorded from zeroth to fifth hour at one hour interval. Haematological parameters and Serum ALT, AST levels were estimated at peak of pyrexia and five hours after drug administration.

For CNS study group I was kept as normal control, Group II was administered chlorpromazine at the dose rate of 7 mg/kg body weight. Group III , IV V and VI were treated as in the case of antipyretic study. Actaphotometer and Rotarod were used to assess the spontaneous and forced motor activities respectively. Haematological parameters and serum ALT, AST levels were estimated at peak of tranquillization.

The extract of white lotus seed at dose rate of 600 mg/kg body weight showed maximum antipyretic effect followed by red lotus at 600 mg/kg body weight and white lotus at the rate of 400 mg/kg body weight among the treatment groups. By the fifth hour the effect of white lotus seed extract at the dose rate of 600 mg/kg body weight was comparable to that of standard drug aspirin.

Eventhough the extract of red lotus at the dose rate of 600 mg/kg body weight inhibited the spontaneous motor activity to the maximum initially, the extracts were found to be equipotent after 120 minutes of drug administration. But the activity was less when compared with the standard drug chlorpromazine. Comparison of the

treatment groups showed that group IV showed maximum inhibition of forced motor activity followed by group VI and group III.

The haematological and biochemical parameters assessed were within the normal range in both studies.

From the present study it can be concluded that the extracts of red and while lotus seeds possessed potent antipyretic and CNS effects. The extract from the seeds of white lotus showed more potent CNS activity where as both the extracts were showing equipotent tranquillizing property.