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RESEARCH ARTICLE

ANALGESIC AND ANTIPYRETIC ACTIVITIES OF HAMELIA PATENS LEAF ON ETHANOLIC EXTRACTION IN ANIMAL MODELS.

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Abstract

The present study investigates the analgesic and antipyretic effects of Hamelia patens leaves on ethanolicextraction.In order to assess the analgesic effects by Formalin induced writhing response model. However at the doses of 50-200 mg/kg analgesic activities were observed in the early and late phases of formalin induced paw licking test in rats. The latency in the hot plate test was increased from 2.2 + 0.3 to 5.7 + 0.3 second ($P < 0.05$). Likewise, the early and the late phases of formalin test were reduced from 7.4 + 9.4 and 57.8 + 8.4 to 36.6 + 4.4 and 29.2 + 4.4 second respectively.The result confirms the analgesic and antipyretic activities of Hamelia patens leaf extract. We recommended further research on this plant leaves for possible isolation and characterization of the various active chemical substances which has the toxic and medicinal values.

Introduction:-

Medicinal plants or their active components are used in the prevention and treatment of chronic diseases is based on experience from traditional systems of medicine from various ethnic societies. During the past decade, a large number of natural products and dietary components have been evaluated as potential chemopreventive agents (Sharma et al., 1994). The application of chemopreventive agents to cancer prevention and control is attractive because conventional therapy alone has not been fully effective in combating either the high incidence or the low survival rate of several forms of cancer (Boone et al., 1990; Rao et al., 1995).

Inflammation is a body defense reaction to eliminate or limit the spread of an injurious agent and is characterized by five cardinal signs, redness, swelling, heat, pain and loss of function. The inflammatory process involves a cascade of events elicited by numerous stimuli that include infectious agents, ischemia, antigen-antibody interaction and thermal or physical injury (Burke A, Smyth et.al, 2006).

Non-steroidal anti-inflammatory drugs (NSAIDs) are widely used in the treatment of acute and chronic inflammation, pain and fever. But the greatest disadvantage in presently available synthetic drugs is that they cause gastrointestinal irritation and reappearance of symptoms after discontinuation. Therefore, there is a dire need for screening and development of novel, but better anti-inflammatory drugs and indigenous medicinal plants could be a logical source to find these. Nonsteroidal anti-inflammatory drugs (NSAIDs) are of huge therapeutic benefit in the treatment of rheumatoid arthritis and various types of inflammatory conditions. The target for these drugs is cyclooxygenase (COX), a rate limiting enzyme involved in the conversion of arachidonic acid into inflammatory prostaglandins. The two isozymes of COX involved in prostaglandin biosynthesis are COX-1 and COX-2. COX-1 is

known as a housekeeping enzyme and constitutively expressed in all tissues, while COX-2 is constitutively expressed only in kidney, brain and ovaries. COX-2 is increasingly expressed during inflammatory conditions by pro-inflammatory molecules such as IL-1, TNF-a, LPS and agents such as carrageenan (Van Ryn et al, 2000).

Hamelia patens Jacq. (Rubiaceae) is a small to large tree found all over India. It is a large perennial shrub or small tree in the coffee family, Rubiaceae that is a native to the American subtropics and tropics. Common names such as Firebush, Hummingbird bush, Scarlet bush. It is also cultivated in garden as an ornamental tree (Fenster, Charles B, 1991).

Hamelia patens have been studied chemically. It is known to contain pentacyclic oxiindole alkaloids (Borges del Castillo et.al, 1984). Also, the plants are used in folk medicine against a range of ailments. number of active compounds have been found in firebush, such as apigenin, ephedrine, flavanones, isomaruquine, isopteropodine, maruquine, narirutins, oxiindole alkaloids, palmirine, pteropodine, rosmarinic acid, rumberine, rutin, seneciophylline, speciophylline, isopteropodine, stigmast-4-ene-3,6-dioneand tannin (Duke, 2007). It has been used in the indigenous system of medicine for the treatment of various ailments such as, dental disease, burning sensation, uterine disorders, ulcers, cardiac diseases, fever and the plant has been used as diuretic, astringent and aphrodisiac. Phytochemical evaluation showed the presence of alkaloids, tannins, ursolic acid, steroids, querrcitol, lupeol and mixtures of triterpenoidsaponins. However there have been no published reports on the anti-inflammatory, analgesic and antipyretic activities of Hamelia patens.

Thus the present study was undertaken to investigate the analgesic and antipyretic activities of alcohol extract of Hamelia patens leaf.

Materials and methods:-

The leaves of Hamelia patens (HP) were collected from mature trees grown locally. The leaves of the plant were authenticated in the Department of Botany, Andhra Loyola College, Vijayawada. Experiments were carried out on Wistar rats weighing 150-200 g atVasavi Institute of Pharmacy, Tadepalligudem. Animals were housed in standard polypropylene cages for one week to acclimatize to laboratory conditions before starting the experiment at a temperature of 24±2° and relative humidity of 30-70%. A 12:12 light: dark cycle was followed. They were given free access to standard rat feed and water ad libitum, but 12 h prior to an experiment; the rats were deprived of food but not water. The animal study protocols were approved by the institutional animal ethical committee.

Extraction and preparation of sample:-

Preparation of Hamelia patens leaf extract:-

The extracts were prepared with leaves of Hamelia patens (obtained from the Campus of the Andhra Loyola College, Autonomous Vijayawada Andhra Pradesh). To prepare the extracts, 25gm of the plant powder was weighed and transferred to a sterile beaker.125 ml of ethanol (1:5) was added to it and mixed well with shaker for 24 hours. When the powder mixed with ethanol thoroughly, it was filtered through What Mann No:1 filter paper. Then the solution was used for the experiments.

Analgesic studies by the hot plate test and Formalin induced paw licking test:-

The hot plate test:-

This was carried out by slightly modifying the method described by (Woolfe and Mac Donald, 1944). The rats were placed on a confined hot plate maintained at 55 ºC + 1 ºC. The time taken for the rats to respond to the thermal stimulus (usually by jumping) was noted as the latency (in seconds).The rats were divided into 5 groups (A to E), each made up of 5 rats. Rats in group B, C, and D were given extracts of HP orally after 12 hours fast. The doses were 50, 100 and 200 g / kg for the rats in groups B, C and D respectively; representing low medium and high doses. The rats in group A and E were given equivalent doses of normal saline (10 ml / kg) and Diclofenac sodium (5 mg / kg) respectively. Each of the rats was placed on the hot plate and the latency was recorded. The mean latency + standard error of mean (S.E.M) was determined for each group. This short lasting stimulus elicited from the hot plate surface causes little or no damage at all to paw tissues, so it can be followed immediately by the formalin test (Back-Rojecky, 2003).

The Formalin induced paw licking test:-
The formalin induced paw-licking test was carried out in accordance with the method described by (Hunskaar and Hole, 1997). 100 µl of 4 % formalin was injected subcutaneously into the plantar surface of the left hind paw of the rats, one hour after oral administration of the extract, Diclofenac sodium or saline. The same groups of rats used in the hot plate test and also, the same doses of extract, Diclofenac sodium or saline were also used in the test. The time spent by the rats in licking the injected paw as soon as the injection was given (early phase, 0-5 minutes post injection) and in the late phase (20-30 minutes post injection) was recorded. The mean time spent on licking the paw by each group was determined.

Antipyretic study:-
Brewer's Yeast Induced Hyperpyrexia: The test was carried out using the method described by (Adam. et al., 1968). The animals used for this study were fasted over night before the experiment but water was made available ad libitum. The rats were randomly divided into four groups (A to D) containing five rats per groups. Pyrexia was induced by subcutaneous injection of 20 % (w/v) brewer's yeast suspension (10 ml / kg) in the dorsum of the rats. 17 hrs.After injection, the rectal temperature of each rat was measured, using a clinical thermometer.Only rats that showed an increase in temperature of at least 0.7 °C were used for this study.

The rats in group A to C were given (orally) 50, 100,and 200 mg / kg of the extract respectively while those in group D weregiven Paracetamol (150 mg / kg). The initial rectal temperature of the ratswas measured and this served as the control. The temperatures weresubsequently measured at 60, 90 and 120 minutes post extract administrationand the mean temperature of each group was recorded.

Statistical analysis:-
All data was presented as mean ± standard error of mean (S.E.M). Statistical significance was determined by using student t-test; values with $P < 0.05$ compared with control were considered as significant.

Results and discussions:-

The analgesic and antipyretic properties of the ethanolic extract of Hamelia patens leaves were investigated in this study using three laboratory models.

1. **The hot plate test,**
2. **The formalin induced paw licking test and**
3. **The brewer's yeast induced pyrexia**.

The models chosen for the analgesic test were carefully selected based on the advantages and the disadvantages of each of the models. The hot plate thermal test is a form of acute (phasic) test which is important in determining the fast type of pain. It is mainly sensitive to strong analgesics and causes limited tissue damage (Prado et al., 1990; Hunskaar and Hole, 1997). However, this model has a short coming, because it last for a short time and it is difficult to access modulatory mechanism that may be triggered by the stimulus itself (Tjolsen et al., 1992).The formalin test differ from the hot plate test in that it mimic human clinical pain condition in which the pain last for longer period of time and tonic in nature due to the inflammation accompanying the formalin injection (Tjolsen et al., 1992; Back-Rojecky, 2003). It is sensitive to non-steroidal anti-inflammatory agents and other mild analgesic (Hunskaar and Hole, 1997).

Hotplate anti-nociceptive test:-
The result from this study shows that oral administration of Hamelia patens leaf extract (50-200 mg / kg) significantly($p < 0.05$) increased the reaction time of the animals to thermal stimuli in a dose dependent manner from 2.2 + 0.3 second to 5.9 + 0.3 seconds (Table 1).

Table 1: Effect of Ethanolic extracts of Hamelia patensleaves on hot plate test in rats**.**

Groups	Dose (mg / kg)	Reaction time (secs) a
Control	10 ml	2.2± 0.3
Hamelia patens leaf extract	50	2.7± 0.2
Hamelia patens leaf extract	100	3.4± 0.2*
Hamelia patens leaf extract	200	5.9± 0.3***
Diclofenac sodium	5	4.6± 0.7*

Each value is the mean + SEM of 5 rats* P< 0.05, ***P < 0.001 compares with control; student t-test.

Formalin induced paw licking test:-

In this model, oral doses (50-200 mg / kg) of the ethanolic extract of Hamelia patens leaf extract inhibited both the early and late phases of the licking response. The licking time was reduced from 77.4 + 9.4 s to 33.6 + 4.4 second in the early phase and from 57.8 + 8.4 to 29.2 + 4.4 s in the late phase (Table 2).

Table 2:- Effect of the ethanolic extract of Hamelia patens leaves on formalin-inducedpaw Licking test.

Group	Dose (mg / kg) Orally	Licking time (sec) a	
		Early phase	Late phase
Control (Saline)	-	77.4 ± 9.4	61.8 ± 7.58
Hamelia patens leaf extract	50	54.4 ± 3.2*	32.8 ± 4.3 *
Hamelia patens leaf extract	100	36.4 ± 2.8**	31.8 ± 7.0*
Hamelia patens leaf extract	200	33.6 ± 4.4**	29.2 ± 4.4*
Diclofenac sodium	5	40.4 ± 3.3*	37.5 ± 4.7 *

Each value is the mean ± S.E.M. of 5 rats.* P < 0.05; **p < 0.01, compared with control; student's t-test.

Brewer's yeast induced pyrexia:-

In the brewer's yeast induced hyperpyrexia model, artificial hyperthermia was induced by administration of exogenous pyrogens in the form of yeast. General reduction of the rectal temperature was observed 60 minutes, 90 minutes and 120 minutes after oral administration of the highest dose (200 mg / kg) of the extract. The observed antipyretic effect of the extract may be due to the flavonoids and alkaloids contents of the leaves. The result from this study was given in (Table 3).

Table 3:-Effect of ethanolic extract of Hamelia patens leafon yeast induced hyperpyrexia in rats.

Groups	Dose (mg / kg	Pre-Drug Temp (°C)	Post Drug Temp (60min)	Post Drug Temp (90min)	Post Drug Temp (120min)
Hamelia patens leaf extract	50	38.4± 0.3	38.3± 0.3	38.2± 0.3	37.9± 0.3
Hamelia patens leaf extract	100	38.2± 0.4	37.8± 0.4	37.6± 0.5	37.0± 0.2*
Hamelia patens leaf extract	200	38.5± 0.3	37.6± 0.2	37.1± 0.1**	36.6± 0.3**
Paracetamol	150	38.5± 0.3	37.5± 0.4*	37.1± 0.4*	36.9± 0.4*

Each value is the mean ± S.E.M. of 5 rats. * P < 0.05; **p < 0.01 compared with control; student's t-test.

The observed antipyretic effect of the extract may be due to the flavonoids and alkaloids contents of the leaves. These flavonoids and alkaloids may act by blockage of the synthesis of prostaglandins E2 (a peripheral fever mediator) through the inhibition of prostaglandins synthetase (Ramaswamy et al., 1985). Therefore the extract could be mediating it analgesic and antipyretic effects like the non-steroidal anti-inflammatory drugs (Vane, 1971, Zeil and Krupp, 1975).

In the present study, administration of the extract led to significant increase in the latency to thermal (hot plate) stimulus and also a significant reduction in the licking time in both the first and second phase of formalininduced paw licking tests. The observed analgesic activities of Hamelia patens leaf extract may be due to mainly of active compounds including maruquine, isomaruquine, pteropodine,isopteropodine, palmirine, rumberine, seneciophylline andstigmast-4-ene-3, 6-dione (Duke ,2007).

Fire bush contains 17.5percent crude protein and hasinvitrodigestibility of 61.6percent (Benavides, 2001). The extract of the H.patensrevealed the presence of alkaloids, carbohydrates, flavonoids, glycosides, phenols, proteins, quinones, saponins, steroids, coumarins, phytosterols and terpenoids. The plants are used in folk medicine against a range of ailments such as athlete's foot, skin lesions and rash, insect bites, nervous shock, inflammation, rheumatism, headache, asthma and dysentery (Liogier HA, 1990). Scarlet bush is rich in active phytochemicals including alkaloids and flavonoids.

It contains several of the same oxindole alkaloids as Cat's Claw (Uncaria tomentosa) including pteropodine and isopteropodine; both have been highly studied and even patented as effective immune stimulants. These two chemicals have also recently shown to have a positive modulating effect on brain neurotransmitters (called 5-HT(2) receptors) that are targets for drugs used in treating a variety of conditions including depression, anxiety, eating disorders, chronic pain conditions and obesity. Three new oxindole alkaloids have also been discovered in scarlet bush which have never been classified before; they've been named Hamelia patens alkaloid A, B and C. Scientists in

India discovered that scarlet bush leaves contain small amounts (00.05%) of ephedrine - a stimulant alkaloid that has received some negative press of late. In addition, the aerial parts of the plant have been found to contain rosmarinic acid, a phytochemical that has demonstrated immune modulating and antidepressant activity.

In the brewer's yeast induced hyperpyrexia model, artificial hyperthermia was induced by administration of exogenous pyrogens in the form of yeast. General reduction of the rectal temperature was observed 60 minutes, 90 minutes and 120 minutes after oral administration of the highest dose (200 mg / kg) of the extract. The observed antipyretic effect of the extract may be due to the flavonoids and alkaloids contents of the leaves. These flavonoids and alkaloids may act by blockage of the synthesis of prostaglandins E2 (a peripheral fever mediator) through the inhibition of prostaglandins synthetase (Ramaswamy et al., 1985). Therefore the extract could be mediating it analgesic and antipyretic effects like the non-steroidal anti-inflammatory drugs (Vane, 1971 Zeil and Krupp, 1975).

Conclusion:-

In conclusion this study has confirmed that the analgesic and antipyretic activities of Hamelia patens leaf extract. It also shows that the analgesic activityis more pronounced than the antipyretic activity since lower doses of the extract produced analgesia while the lower doses fail to reduce pyrexia.

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