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EVALUATION OF ANALGESIC ACTIVITY OF *LUDWIGIA PERENNIS* LEAVES ETHANOLIC EXTRACT IN WISTAR ALBINO RATS

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ABSTRACT:
Ludwigia perennis leaves were collected, washed and dried under shade for 24 hours. The dried leaves were grinded and passed through sieve no. 40 to obtain fine powder. The powder was subjected to soxhlet extraction using different solvents and the extracts obtained were dried in a rotary evaporator. Phytochemical screening was carried out for detection of phytoconstituents. The extracts showed the presence of alkaloids, flavonoids, carbohydrates, proteins. Analgesic activity was evaluated by using two models viz, Hot Plate test and Formalin pain induced test using Albino Wistar rats. The rats were divided into 5 groups of 6 animals each, i.e group I (control), group II (standard), group III (test I), group IV (test II) and group V (test III). Test extracts were taken as 50 mg/kg, 100 mg/kg, 200 mg/kg. Results obtained from test I, test II and test III were compared with that of standard and control. From the results obtained it was observed that Ludwigia Perennis has analgesic activity. Further studies can be carried out to establish mechanism of action of analgesic activity in this plant.
Keywords: *Ludwigia perennis*, Analgesic, Hot plate, Formalin Test, Wistar.

INTRODUCTION:

Plants have been recorded for their medicinal and economic use for a long time. Plants have shown the presence of various phytoconstituents(Sharmila et al., 2017) like alkaloids, flavonoids, carbohydrates, tannins, saponins and phenolic compounds. Ancient Chinese and Egyptian writings described the properties of medicinal plants.

Indian herbal medicine can be found in Rigveda. The Badianus Manuscript speaks of the story of the Aztecs ancient medical expertise. Hippocrates believed in natural causes for disease and employed a variety of herbal remedies in his therapies. Early Roman texts, particularly the works of Dioscorides, who amassed information on more than 600 kinds of plants with therapeutic significance in De Materia Medica, inspired the development of Western medicine. Many of the Greek and Roman herbal cures were excellent treatments that have since been adopted into contemporary medicine. (e.g., willow bark tea, the precursor to aspirin (Petrovska BB, 2012). For the following 1500 years, Dioscorides' work was the standard medical reference in most of Europe (Ferrini R et al.,1974) . Herbalists have used a variety of treatments that have proven to be successful. For pharmaceutical chemistry, studies of foxglove for the treatment of dropsy (congestive heart failure) established the gold standard for pharmaceutical chemistry. In the 19th century, morphine was isolated. Advances in pharmacology led to the creation of the first wholly synthetic therapeutic drugs based on natural ingredients in the mid-19th century(Krishnamurti C and Rao C SSC, 2019). Salicylic acid, for example, was discovered as an active ingredient in several plants in 1839 for its pain-relieving effects; in 1853, salicylic acid was synthesised, which led to the invention of aspirin(Miner J et al., 2007). Percentage based on active compounds that are semi-synthetic or totally synthetic and were initially extracted from plants(Greenway M and Wooley A, 1999) . Traditional medicine is a blend of generations of doctors' therapeutic experience with indigenous medicinal systems. Medicinal plants, minerals, and organic compounds, among other things, are used in the traditional recipe. Plants have medicinal qualities due to the existence of secondary metabolites such as phenols, flavonoids, alkaloids. These metabolites have antibacterial, antioxidant, anthelmintic, and anticancer properties(Abhipsa V, 2007). It dates back to 5000 years and was used by Indians, Chinese, Egyptians, Greeks, Romans, and Syrians. The discovery of new medications relies heavily on medicinal plants. Approximately 100 new herbal medications were introduced to the US market between 1950 and 1970(Biljana Bauerpetrovska, 2012). Deserpidine, reserpine, vinblastine, and vincristine are some of the higher plant-derived compounds. Etoposide, Guggulsterone, Teniposide, Nabilone, Plaunotol, lentinan, Artemisinin and Ginkgolide have occurred worldwide. 2% of the drugs were introduced between 1991 and 1995, including Paciltaxel, Topotecan, Gomisin, Irinotecan, etc. Herbal medicines have made a significant contribution to modern therapies, and medicinal plants have

played a key part in the development of effective therapeutic molecules. Plant-derived medications have been employed in contemporary medicine through folklore or traditional medical systems that utilised plant material as indigenous cures(Pan et al., 2014). More than 64 plants exhibit antibacterial activity, while more than 24 plants have anti-diabetic and antimicrobial activities(Salehi, Bahare et al., 2019). A variety of biological components in high yield, some of which have been found to have biologically beneficial properties, primarily phenols, flavonoids, and terpenoids(Sarmistha Rej et al.,2014). Flavonoids have recently attracted attention due to their diverse biological and pharmacological properties, which include antioxidant, anxiolytic, anticancer, analgesic, and antibacterial effects(Sushil Det al., 2016). Food additives, colours, pesticides, cosmetics, fragrances, and fine chemicals are all made from bioactive molecules derived from plants are secondary metabolites(Arun P et al., 2016). Different parts of the tree have been used in folkloric medicine for treatment of different diseases such as cancer, headache and pains (bark), fever, prenatal pains and oedema (leaves), diarrhoea (fruits).[14] Potential drug candidates can be found in medicinal plants with potential therapeutic efficacy. Non-steroidal anti-inflammatory medicines (NSAIDs), opioids, and other anti-inflammatory and analgesic medications have a wide range of negative effects(Beb S et al., 2011). The classical analgesic drugs, notably opiates and non-steroidal anti-inflammatory drugs, have their origins in natural products, but many synthetic compounds that act by the same mechanism have been developed and are associated with serious side effects such as ulceration, gastrointestinal bleeding, additive potential, respiratory distress, drowsiness, nausea, and other problems(Laurence DR et al., 2013 and Mate GS et al., 2008). As a result, developing novel active substances with minimal side effects is a top priority. The goal of this research is to give a complete review of analgesic plant species and plant chemicals. In-depth research into the discovery of novel analgesic medications from natural sources, followed by studies of their pharmacological characteristics and clinical applications, might lead to the creation of new drugs with improved therapeutic activity and selectivity in the future.

MATERIAL & METHODS:

Collection of plant material:

Ludwigia perennis leaves were obtained from a herbal medicine shop and authenticated by the Department of Pharmacognosy based on morphological criteria. The air dried leaves were ground into a powder. The powdered material was either kept in dry air tight bottles for the extraction procedure.

Chemicals and Drugs:

Diclofenac Sodium, Formalin, Petroleum Ether, Ethanol, Distilled Water was procured from Dhanalaxmi Pharmaceutical Pvt Ltd, Hyderabad, Telangana. All the chemicals and solvents were of analytical grades.

Experimental Animals:

Albino rats (Wistar strain) weighing between 150-200 grams were kept in appropriate cages at room temperature and 12/12 hours of light/ dark cycles. The animals had free access to standard pellets and water under strict hygienic conditions. The animals were habituated to laboratory conditions for 48 hours prior to experimental protocol to minimize any nonspecific stress(Bailey KR et al., 2009). The study was approved by institutional Animal Ethical Committee regulated under CPCSEA

Preparation of plant extracts:

The dried powder was extracted with ethanol using soxlet's apparatus(Zygler A et al., 2012). The extract was filtered and the filtrate obtained was concentrated using a rotavapor bath at a temperature of 30° C. It was stored in airtight glass vials for further dilutions.

Pharmacological Evaluation:

Preparation of Plant Extract Stock Solution:

The Ethanolic extract of Ludwigia perennis is suspended in distilled water. All the drugs were administered orally for experimental purposes. The drugs were administered at a constant volume of 10 ml/kg for each animal.

Preparation of Standard Stock Solution:

The standard solution of diclofenac was prepared by dissolving 1g of standard Diclofenac in 100 ml of distilled water(Junior D et al., 2019). [20]

Phytochemical screening:

The freshly prepared LPEE was subjected to Phytochemical screening tests for presence of various constituents as per standard methods(M. Sharmila et al., 2017).

Acute toxicity studies:

The acute toxicity research followed the Organization for Economic Cooperation and Development (OECD) recommendations No. 425(Iswar Hazarika et al., 2019). In separate groups of mice (n=3), LPEE was given orally in dosages of 100, 200, 400, 800, 1000, and 2000 mg/kg p.o., and % mortality was recorded for 24 hours. Based on the above toxicity study, a direct limit test was done(JB Corengle et al., 2017). Initially, a specific dose was administered to a single rat based on the above study, and the rat was observed for 48 hours with close surveillance up to the first 4 hours (same as the first rat); after 48 hours (of the second administration), the same dose was administered to two more rats, and observation was done similarly. The rats were watched for 14 days, with weight measurements taken on the 7th and 14th days.

Evaluation of Analgesic Activity:

The Ethanolic extracts of Ludwigia perennis leaves were tested for Analgesic Activity(Md. Rafikul Islam et al., 2011) Using Hot Plate Method and Formalin Induced Paw Licking Method(NB Eddy et al., 1953).

Experimental Model No. 1:

Hot Plate Method

The mice were split into five groups of six mice each at random. Prior to medication delivery, each animal was trained on the hot plate(Jim E Reviere et al., 2014); the latency duration spent on the hot plate before the drug was provided was also determined. Control (distilled water 10 ml/kg), LPEE (50, 100, 200 mg/kg body weight), and standard medicine Diclofenac (10 mg/kg P.O.) were given to the animals. The animals were placed on a hot plate (55 0.50 C) 30 minutes after being given the medication. When the animals licked their paws and tried to flee, the reaction time was observed at 0, 30, 60, 90, and 120 minutes. A cutoff time of 20 seconds was deemed acceptable.

Experimental Model No. 2:

Pain induced by Formalin test.

The formalin test(ANM Shafiuddin et al., 2018) is a valid and consistent nociception paradigm that results in two distinct periods of increased licking activity that can be linked to various nociceptive pathways(Stenar Hunskar et al., 1987). The early licking stage lasts five minutes, while the late phase lasts 15 to 45 minutes following the formalin injection. Formalin (20 L of a 1% solution) was administered subcutaneously into the dorsal surface of the right hind paw, as previously described. The animals were then put in front of a glass funnel on a glass tabletop with a 45-degree slanted mirror. The pain response time (licking time) was estimated in two phases: 0 to 5 minutes for the first phase, and 15 to 45 minutes for the second phase (inflammatory pain produced by release of inflammatory mediators). The animals were put into five groups at random. In the negative control group, animals were given 0.5 ml of Distilled Water. Diclofenac was injected to animals in the positive control group(Abdol Hossein Miri et al., 2015). Ludwigia perennis ethanolic extract (50, 100, 200 mg/kg) was given to the other groups at various dosages. All injections were administered intraperitoneally 30 minutes before the test.

Statistical Analysis:

The statistical programme GraphPad Prism version 5 was used to examine the data. The significance of differences in the periods of licking in the formalin test was determined using a one-way analysis of variance (ANOVA) test(Mesoume Rezaee-asl et al., 2014). Two-way repeated measure ANOVA test used to assay the differences in reaction time IN hot plate tests. Data are shown as mean ± S.E.M. All data were considered significant at $P<0.05$.

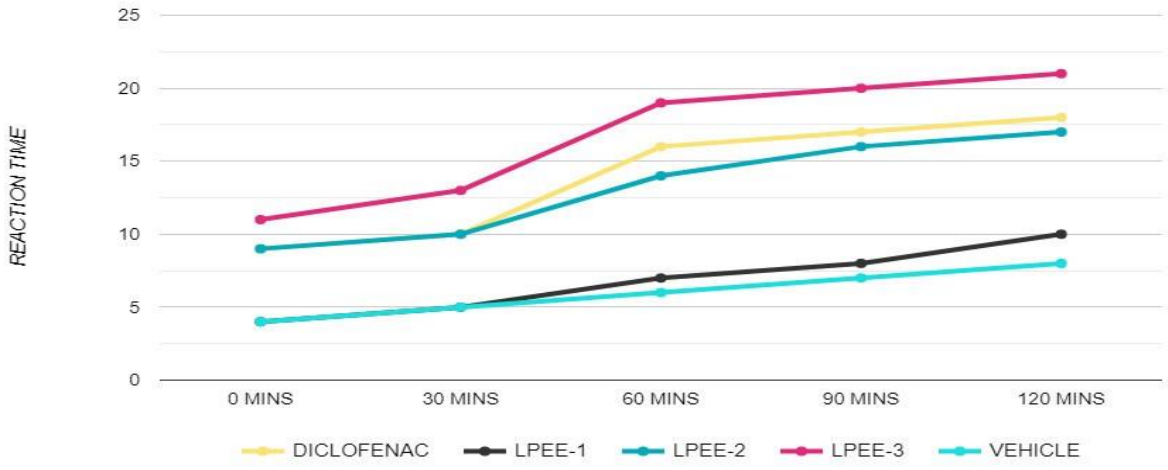
RESULTS:

ANALGESIC ACTIVITY BY HOT PLATE METHOD:

Analgesic Activity of Ethanolic extract of leaves of Ludwigia perennis was studied at a dose of 50, 100 & 200 mg/Kg, using Hot Plate Model. From the results of analgesic activity it was observed that in the hot plate model the test group I i.e LPEE at a dose of 50mg/kg showed an increase in the reaction time after 30 min of administration (5.53±0.256).when compared with the initial value(4.89±0.16) as shown in table. In 60 min the reaction time in the same group increased gradually (7.35±0.260) again when compared with the initial value. The Reaction

time slightly decreases in 120min (10.56±0.302). From the above results it was evident that LPEE Test I group which received 50 mg/kg of LPEE Showed a highly significant analgesic effect ($p<0.001$) in 90min and the effect was significant in 30 and 60 min. In the Test Il group i.e LPEE (100mg/kg) there was a gradual increase in reaction time again in 30min LPEE. (9.72±0.270) when compared with initial (9.02±0.192). In 60 min LPEE Test Il group showed an increase in the reaction time ie (14.95±+0.275) again when compared with the initial value (9.02±0.192). The reaction time slightly increase in 120 min in the same group (17.89±0.340). LPEE Test Il group showed a highly significant analgesic effect ($p<0.001$) in 90min and the effect was slightly significant in 30 and 60 min and significant in 15 min. There was also significant increase in reaction time in the standard group which was administered Diclofenac Sodium (10 mg/kg. p.o). In group III i.e LPEE at a dose of 200mg/kg showed an increase in the reaction time after 30min of administration (13.95±0.354) when compared with the initial value (11.52±0.302), In 60 min the reaction time of LPEE Test IIl group (19.56+0.436) increased and was at the peak, closer to the standard group (16.65±0.301). In 120 min the reaction time of LPEE (Test III) group increased (21.05±0.598) when compared with the initial (11.52±0.302). From the above results it was evident that the LPEE Test III group which received 200 mg/kg of FDLE showed a highly significant analgesic effect ($p<0.001$) in 120min and the effect was slightly significant in 30 and 90 min and significant in 15 min.

DATA OBTAINED FROM HOT PLATE EXPERIMENT:



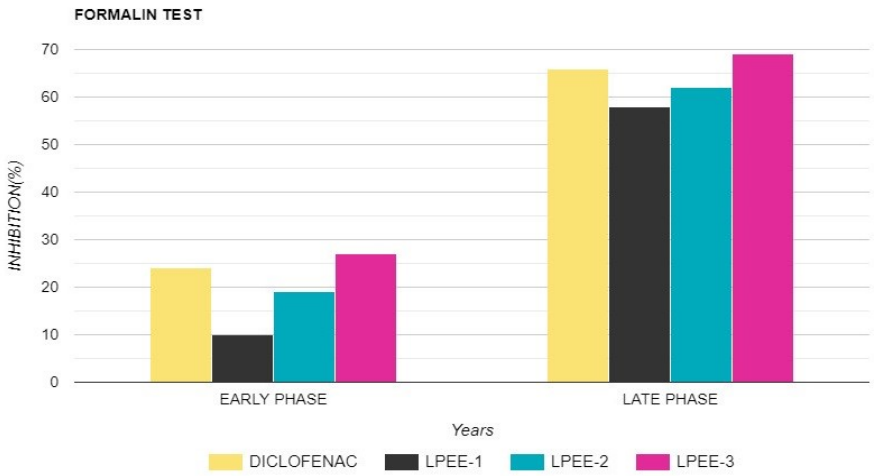
GROUP NO.	GROUP TREATMENT	DOSE	REACTION TIME IN SECONDS AT DIFFERENT TIME INTERVALS

			0 mins	30 mins	60 mins	90 mins	120 mins
1	**Distilled Water**	**10 ml/kg**	**4.5±0.05**	**4.8±0.07**	**4.9±0.09**	**5.1±0.11**	**5.0±0.09**
2	**Diclofenac**	**10 mg/kg**	**9.44±0.253**	**10.72±0.274**	**16.65±0.301**	**17.24±0.313**	**18.45±0.376**
3	**LPEE**	**50 mg/kg**	**4.89±0.186**	**5.53±0.256**	**7.35±0.260**	**8.21±0.199**	**10.56±0.302**
4	**LPEE**	**100 mg/kg**	**9.02±0.192**	**9.72±0.270**	**14.95±0.275**	**16.52±0.201**	**17.89±0.340**
5	**LPEE**	**200 mg/kg**	**11.52±0.302**	**13.95±0.354**	**19.56±0.436**	**20.01±0.493**	**21.05±0.598**

Data are presented as mean ± Sem.

ANALGESIC ACTIVITY BY FORMALIN TEST:

In the Formalin test, the ethanolic extract of leaves of Ludwigia perennis was studied at a dose of 50, 100 & 200 mg/Kg (p.o.). The ethanol extract of Ludwigia perennis (200 mg/kg, p.o.) significantly suppressed formalin-induced pain response in rats, with a more potent effect on the second than the first phase. In the late phase (15-30 min) of this test, the extract exerted 69.75% inhibition whereas 66.446% inhibition was obtained for diclofenac sodium against pain.



DATA OBTAINED FROM FORMALIN EXPERIMENT:

GROUP	TREATMENT	DOSE	EARLY PHASE (SEC)	INHIBITION (%)	LATE PHASE (SEC)	INHIBITION (%)vg66
1	**Distilled Water**	**10 ml/kg**	**1.53±0.75**	**-**	**3.034±0.298**	**-**

2	**Diclofenac**	**10 mg/kg**	**1.09±0.379**	**24.306**	**1.015±0.85**	**66.446**
3	**LPEE**	**50 mg/kg**	**0.95±0.236**	**10.673**	**0.987±0.556***	**58.628**
4	**LPEE**	**100 mg/kg**	**1.02±0.345**	**19.293**	**1.003±0.79**	**62.403**
5	**LPEE**	**200 mg/kg**	**1.95±0.420**	**27.456**	**1.245±1.12**	**69.755**

All values are expressed as mean ± STD (n=5); One way Analysis of Variance (ANOVA) followed by Dunnet's test. P<0.05, significant compared to control

DISCUSSION:

The analgesic efficacy of the ethanol extract was tested in a hot plate test and formalin test. In the hot plate model, the extract at all test doses(50 mg/kg, 100 mg/kg, 200 mg/kg) significantly($p<0.05$, $p<0.01$, $p<0.001$ respectively) elevated the pain threshold by increasing the reaction time starting from 30 min of observation onwards as compared to the negative control. The maximum analgesic effects of the extract were observed at 120 min of observation time with their respective percentage values of 58.62%, 62.40% and 69.75% as compared with diclofenac (10 mg/kg) which showed percentage analgesic value of 66.44% at this time. The analgesic value of the test doses were dose dependent. The possible mechanism of the central analgesic effects of the extract could be by activating the periaqueductal gray matter (PAG) to release endogenous peptides i.e endorphin or enkephalin. The endogenous peptides descend the spinal cord and function as inhibitors of the pain impulse transmission at the synapse in the dorsal horn orvia peripheral mechanisms involved in the inhibition of PG, leukotriene, and other endogenous substances that play a pivotal role in central pain transmission(Badole SL et al., 2012).

Formalin test technique distinguishes between central and peripheral activity. Due to the irritating impact of formalin on sensory C fibres, the early phase response is thought to constitute a direct chemical stimulation of pain (Arne Tjolsen et al., 1992). Inhibition of licking response of the test drugs in the early phase and late phase signifying the analgesic effect of the extract in the formalin test is the most Inhibition of licking response of the test drugs in the early phase and late phase signifying the analgesic effect of the extract in the formalin test. The hot plate approach proved helpful in clarifying centrally mediated analgesic responses, which focuses on alterations above the level of the spinal cord(Debases Mishra et al., 2011). Prolongation of the reaction time in the hot plate method indicates the involvement of supraspinal mechanisms(Sook-Ha Fan et al., 2014) These findings suggest

that Ludwigia perennis contains chemicals that are responsible for the plant's analgesic properties. The pharmacological characteristics of the ethanol extract of Ludwigia perennis found in this study show that it has analgesic properties, since it greatly reduced central and peripheral pain in rats. However, more research is being conducted to establish the exact phytoconstituents responsible for the neuropharmacological effects of Ludwigia perennis ethanol extract (Seetharaman Selvaraj, 2011).

CONCLUSION:

Ludwigia perennis has shown significant analgesic activity. Further isolation and characterization of bioactive compounds from the plant is in progress.

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