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EVALUATION OF ANTI-INFLAMMATORY ACTIVITY OF ELAGESIC LINIMENTS ON EXPERIMENTAL ANIMAL

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ABSTRACT

Objective: The main objective of the present research is to evaluate the anti-inflammatory activity of elagesic liniment on Wistar rats. **Materials and methods:** The elagesic liniment formulation were purchased, identified and Authenticated by Arpita Pharma, Nashik. After evaluation of various physical parameters like globule size, pH, viscosity, were selected for pharmacological evaluation. These formulations were subjected for evaluation of anti-inflammatory effect using carrageenan induced paw edema method. **Results:** The edema in paw was measured using plythesmometer. The inhibition obtained with liniment was 63.15%, oedema which was induced by carrageenan on sub-plantar administration when compared with untreated control groups. When compared to that of the standard group which clearly indicates the anti-inflammatory activity of elagesic liniment. **Conclusion:** Thus from the present study, it was concluded that the elagesic liniment shows significant anti-inflammatory activity.

KEYWORDS: Elagesic liniment, anti-inflammatory.

INTRODUCTION

Medicinal plants are believed to be therapeutically important as they are source of various phytochemical constituents, which treats many diseases. Several traditional folklore studies revealed the therapeutic importance of plants. Inflammation is a general protective mechanism in which the body reacts to any injury, infection or irritation which can be identified by some key features like redness, warmth and swelling at the site. It is considered to have different mechanisms for each type of pathogen (Abul and Andrew, 2009). As the traditional NSAIDS have some adverse effects like formation of ulcers, plant derived products are gaining importance (Mahesh et al., 2009). Topically applied plant preparations in the form of paste or liniment. The basis of the inflammatory responses generated, duration of the inflammation and the inflammatory cells present at the time of inflammation.

The Inflammatory response is triggered through two phases

1-Acute Inflammation

2-Chronic Inflammation

Liniment are made available which are useful in treating several ailments like inflammation (Lee, 2012). Liniment is a medicated topical preparation meant exclusively for application to skin. They are less viscous than lotions and are rubbed to create friction while applying (James and Adams, 2012). These are typically formulated from acetone, alcohol or suitable solvents and contain counter-irritant aromatic chemical compounds. Stability is the major hurdle, which is to be specifically considered while formulating a liniment. Inflammation happens when a physical factor triggers an immune reaction. Inflammation does not necessarily mean that there is an infection, but an infection can cause inflammation. When the body detects damage or pathogens, the immune system triggers a number of reactions: Exposure to a substance, such as a bee sting or dust an injury an infection. Tissues accumulate plasma proteins, leading to a buildup of fluid that results inswelling. The body releases neutrophils, a type of white blood cell, or leukocyte, which move toward the affected area. Leukocytes contain molecules that can help fight pathogens. Small blood vessels enlarge to enable leukocytes and plasma proteins to reach the injury site moreeasily.

MATERIALS AND METHODS

Indomethacin was a gift sample from M/s. Life Line Formulations, Vijayawada. Elagesic liniment were purchased from arpita pharma, nashik.. Carrageenan was procured from Om Pharma, Nashik. All other substances used were of analytical grade and commercially procured.

Experimental animals

Experiments are performed in accordance with committee for purpose of control & supervision of experimental animal(CPCSEA) guidelines after the

approval of the experimental protocol by Institutional animal ethical committee (IAEC).Wister rat weighing 150-250gm are obtained from animal house of Department of Pharmacology, Vidyabharti College of Pharmacy, Amravati. All the animal are acclimatized to the animal house prior to used. They are kept in cages in animal house with 12 hours light, 12 hours dark light at temperature (25±1°C) with 50±55% of relative humidity. Animal are fed on pellets and tap water ad libitum. The care and handling of animals in accordance with internationally accepted standard guidelines for use of animal (CPCSEA).

Evaluation of physical stability parameters

Determination of Globule Size

The globule sizes were estimated for Elagesic liniment using eye piece micrometer and stage micrometer. formulations, with oil to surfactant ratio of 1:0.5 were found to have optimised globule size with less standard deviation.

Determination of emulsion type

Using dilution method, the emulsion type was determined for the liniments. The liniments showed complete miscibility when mixed with water without any phase separation. This clearly indicates that the elagesic liniments are Oil in Water type of emulsion.

Estimation of pH

The pH of liniments was estimated using pH meter. The formulation showed a pH of 7.2 which is slightly alkaline.

Determination of viscosity

The viscosity of liniments was measured using Brookfield Viscometer. Liniment showed a viscosity of 2.15 Centi-poise

Qualitative chemical investigation

Test for Carbohydrates (Fehling's Test): preparations in the form of paste or liniment. The basis of the inflammatory responses generated, duration of the inflammation and the A small quantity of liniment was dissolved in 4 ml of double distilled and filtered. The filtrate was subjected to Molish test to detect the presence carbohydrate and further addition of fehlings reagent. It shows brick red colour comfirmed the presence of reducing sugar.

Test for Alkaloids

Alkaloids solutions produce white yellowish precipitate when a few drops of Mayer's reagents are added. Most alkaloids are precipitated from neutral or slightly acidic solution by Mayer's regent. The alcoholic extract was heated on a boiling water bath with 2% hydrochloric acid. After cooling, the mixture was filtered and treated with a few drops of Mayer's reagent. The sample was then observed for the turbidity or yellow precipitation.

Test for Glycosides

(Keller-Killani test) A portion of the liniment in glacial acetic acid, few drops of ferric chloride andconcentrated sulphuric acid are added, and observed for a reddish-brown coloration at the junction of two layers and the bluish green color in the upper layer showed presence of glycosides.

Test for Steroids (Liebermann-Burchard test)

0.5 mlliniment was dissolved in 2ml of acetic anhydride, cooled in ice bath, conc. Sulfuric acid was added. Color changed from violet to blue or green indicates presence of steroid compounds

Test for Terpenoids (Salkowski test)

0.5 ml each of the liniment was added to 2ml of chloroform. Concentrated sulphuric acid (3ml) was carefully added to form a layer. Reddish brown coloration of the interface indicates the presence of terpenoids.

Test forQuinones

1ml of liniment, 1ml of concentrated sulphuric acid was added. Formation of red color indicates presence of quinones.

Test for Phenols

1ml of the liniment, 2ml of distilled water followed by few drops of 10% ferric chloride was added. Formation of green color indicates presence of phenols.

Confirmatory Test

4ml of liniment solution was treated with 1.5ml of 50% methanol solution. The solution was warmed and metal magnesium was added. To this solution, 5-6 drops of concentrated Hydrochloride acid was added and red colour was observed and flavonoids is confirm.

Anti-Inflammatory Activity

***In-vivo* Anti-Inflammatory activity by Carrageenan induced Paw Oedema method**

The liniments were tested for anti-inflammatory activity by carrageenan (inflammagen) induced paw oedema method in rats (Kulkarni, 2002). The animals were grouped into four with six animals in each group. The control group was treated with normal saline and the standard group was treated with Indomethacin at a dose of 10 mg/kg orally. Whereas the test groups were treated with elagesic liniment separately to paw. The animals were pre- treated with vehicle/Indomethacin 30 minutes before the injection of 0.1 ml of 1% carrageenan into the s.p (sub-plantar) region in right hind paw to the rats. Paw volumes were measured by the dislocation of water column in a plythesmometer at 1, 2, 3 and 4 hours after the administration of test materials. The liniments were rubbed to rat paw. Reduction in the paw volume compared to the control animals was considered as the anti- inflammatory response. Finally, the anti-inflammatory activity was calculated by using the percentage inhibition of oedema which can be given by

the formula:

RESULT

In-vivo Anti-inflammatory activity using Carrageenan induced paw oedema

Carrageenan-induced inflammation is most commonly equipped experimental model for evaluation of anti-inflammatory property of compounds because it produces reproducible results (Winter et al., 1962). Development of oedema in the paw of the rats is due to the release of histamine, serotonin and prostaglandin like substances (Umesh et al., 2011). The effect of liniment on carrageenan-induced paw oedema in albino rats is shown in the table 1 and figure 1. The results obtained indicate that the liniments had significant anti-inflammatory activity in albino rats when compared with that of the control groups. The potency was found to be inversely proportional to the time taken for reduction in paw volume. The inhibition obtained with elagesic liniment was 63.15%, this clearly indicates that elagesic liniment has anti-inflammatory activity when compared to that of control group. Based on the reports, it can be inferred that the inhibition effect of Liniments on carrageenan induced inflammation on rats may be due to inhibition of mediators that are responsible for inflammation.

Table 1: Efect of elagesic liniment on Carrageenan Induced Paw Oedema in rats.

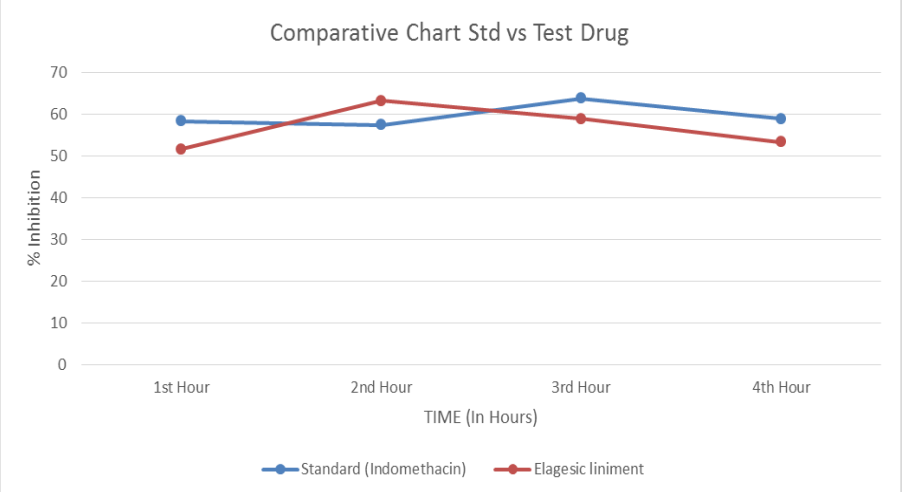
Treatment group	Dose	Paw Oedema (ml) Mean ± S.E.M (% Inhibition)			
		1st Hour	2nd Hour	3rd Hour	4th Hour
Control	Saline	0.5 ± 0.421	0.85 ± 0.214	1.02 ± 0.785	0.9 ± 0.104
Standard (Indomethacin)	10 mg/Kg	0.25 ± 0.332 (58.33)	0.31 ± 0.012 (67.36)	0.37 ± 0.003 (63.72)	0.30 ± 0.106 (58.88)
carragenan	0.1 ml 1% carragenan	0.34	0.41	0.53	0.39
Carragenan +Elagesic liniment	0.1 ml 1% carragenan+EL	0.29 ± 0.042 (51.66)	0.35 ± 0.842 (63.15)	0.42 ± 0.071 (58.82)	0.37 ± 0.006 (53.33)

Table 2: Phytochemical screening.

Sr. No	Phytochemical Analysis	Test performed	Result
1	Carbohydrates	Fehling's Test	(+)
2	Flavonoids	Ammonium test, Aluminum chloride solution test	(+)
3	Alkaloids	Mayer's test	(+)
4	Steroids	Liebermann-Burchardtest	(+)
5	Terpenoids	Salkowski test	(+)
6	Glycosides	Keller-Killani	(+)
7	Anthraquinones	Borntrager test	(+)
8	Phenols	Ferric Chloride test	(+)
9	Proteins	Copper sulphate test	(-)
10	Phlobatannins	HCL test	(-)

- Indicates absence
+ Indicates presence

Graph representing Anti-inflammatory activity effect of Elagesic



DISCUSSION

Elagesic liniment were investigated for it's anti-inflammatory activities in the present study. *Elagesic Liniment* were subjected to phytochemical screening of the extracts. These extracts were found to contain anti-inflammatory phytochemical constituents, Flavonoids, Alkaloids, Steroids, Terpenoids, Carbohydrates, Glycosides, Anthraquinones, and Phenols. Henceforth, the in vivo studies and research carried out on the elageic linimentwere found to be flavonoidrich. In current study we observed the anti-inflammatory potential in carragenan induced paw edema. The present study result showed that treatment with EL effectively inhibited the inflammation**.** Phytochemical analysis of this el has mainly demonstrated the presence of flavonoids, steroids alkaloids, phenols, tannins and saponins. Flavonoids and alkaloids have been reported to have anti-inflammatory activities. Carragenen induced paw edema model is one of the most commonly employed models in animal research to screen for anti-inflammatory activity of drugs. In this model, the carragenan is injected by subplanter way in right hind paw of rat edema was measured with the help of plythsmometer. Elagesic liniment is rubbed on that and observed the percentage of inhibition of edema.it can be compared with standard drug indomethacine 10mg/kg body weight. The results demonstrated that EL has the ability to treat inflammatory diseases. Hence, it needs further detailed pharmacological and clinical investigations to prove it as an effective therapeutic agent for inflammation. Thus our finding suggests that EL could be a potential agent in the treatment of chroni inflammatory disorders.

CONCLUSION

In conclusion, the data obtained in this study demonstrate that elagesic liniment possess different secondary metabolites, which by acting through array of possible different mechanism are effective in treatment of both acute and chronic inflammatory condition. our finding suggest that elagesic liniment has anti inflammatory effect which are associated with inhibition of pro-inflamatory cytokines/receptor and mediators in activated macrophages. As chronic inflammation and activated macrophages are the central players of pathogenesis of inflammatory disease. Our finding support and validate the use of elagesic liniment in the treatment of acute and chronic inflammatory disorder in the traditional system of medicine.

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