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EVALUATION OF ANTI-INFLAMMATORY ACTIVITY OF LALAB PURPUREOUS METHANOLIC LEAVES EXTRACT ON WISTER ALBINO RATS

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ABSTRACT

This work has been done for the investigation of the anti-inflammatory activity of solvent extracts of dried leaves of Lalab purpureous linn. by oral administration at dose of 200 and 400 mg/kg body weight in healthy albino rats. Extracts were studied for its anti-inflammatory activity by using formalin and egg white albumin induced right hind paw edema in albino rats and the mean increase in paw volume and % inhibition in paw volume were measured plethysmometrically at different time intervals after 20µl of (2.5% solution) injection. The methanol extract showed significant ($P < 0.001$) reduction in the formalin and egg white induced paw edema in comparison to aqueous extracts. Petroleum ether and hexane extracts showed no reduction in paw edema. The methanol extract showed anti-inflammatory effect in dose dependent manner when compared with the control and standard drug, Diclophenac sodium (10mg/kg, p.o). The human red blood corpuscular membrane is similar to lysosomal membranes that influence inflammatory process. Result obtained using methanolic extract of Lalab purpureous Linn. have better acceptance as it shows good response in inhibiting haemolysis (33.15%) at highest concentration These inhibitions were statistically significant ($p < 0.05$). Thus our investigation suggests a potential benefit of methanol and aqueous extracts of leaves of Lalab purpureous linn in treating conditions associated with inflammation. This study illustrates about the presence of some active polar compounds in the leaves extracts which might be responsible for anti-inflammatory activity.

KEYWORDS: Lalab purpureous linn. , Anti-inflammatory activity, Methanol extract, Paw volume, Diclophenac sodium.

INTRODUCTION

Lalab purpureous linn.[1,2] is a species of the plant genus lalab, belongs to the family Fabaceae. The phytochemical analysis of Dolichos lablab showed that it contained sugar, alcohols, phenols, steroids, essential oils, alkaloids, tannins, flavonoids, saponins, coumarins, terpenoids, pigments, glycosides, anthnanoids, wide range of minerals and many other metabolites. The preliminary pharmacological studies revealed that Dolichos lablab possessed antidiabetic, anti-inflammatory, analgesic, antioxidant, cytotoxic, hypolipidemic, antimicrobial, insecticidal, hepatoprotective, antilithiatic, antispasmodic effects and also used for the treatment of iron deficiency anaemia. This study therefore, intends to investigate the anti-inflammatory activities of the leaves of lalab purpureus linn by studying the effects[3-5] of methanol extracts of the plant on formalin and egg white induced inflammation in experimental animal models, in order to confirm the medicinal properties of the plant. Inflammation is one common and major cause of sufferings now and every time past.[6] Those drugs that are available are known as NSAID, i.e. non-steroidal anti-inflammatory drugs, act by inhibiting the function of prostaglandin. Prostaglandin is an autocoid that release extracellularly and initiate pain. Anti-inflammatory agents either block this autocoid synthesis by inhibiting COX enzyme or protecting lysosomal membrane from break down.[7-9]

MATERIALS AND METHODS

Plant material

The fresh plant of Lalab purpureus Linn. leaves were collected from Amaravati, Guntur, Andhrapradesh, India. The plant leaves dried under room temperature for seven days and chopped in to small pieces. The powdered plant was used for the preparation of methanolic leaves extract.

Preparation of *Lalab purpureous linn*. Leaves extract by Soxhletion Method

Methanolic leaves extraction

The plant material was dried under shade and powdered mechanically. The 50 gm of powder sample was extracted with methanol by using soxhlet apparatus. The extraction was continued till a few drops of the last portion of the extract left no residue on drying. The solvent was removed by concentrated *in vacuo* in a rotary evaporator and dried under reduced pressure. The yield of the methanol extract was 9.4%. The dried extract was stored in refrigerator until further studies.

Primary phytochemical screening

Phytochemicals of the selected plants were carried out by using aqueous and powdered form of the plant following Harborne (1973) Trease and Evans (1989).

Drugs

Diclofenac sodium, Formalin (Merck Specialities, Mumbai), Sodium chloride (Merck Specialities, Mumbai). Egg white albumin, alsever solution.

Animals

Adult Wistar Albino male rats (150-180g) were procured from the laboratory animal model house, Hindu College of Pharmacy, Guntur, Andhra Pradesh, India and used in the study. The animals were kept under standard environmental conditions of room temperature ($22^0 \pm 2^0$C), relative humidity (50%±5%) and 12h light and dark cycle. The animals were housed in the colony cages (three rats per cage) and provided feed (commercial pellets contain a balanced ration obtained from the Sri Venkateshwara Enterprises, Banglore) and water ad libitum.

All the animals were acclimatized to the laboratory environment 5 days prior to experiment. The animal were fasted overnight just prior to the experiment but allowed free access to drinking water.

Statistical analysis

The results are expressed as mean ± S.D. (n = 6). Statistical significance was determined by analysis of variance and subsequent followed Turkey's tests. *P*values less than 0.05 were considered as indicative of significance. The analysis was performed using INSTAT statistical software.

RESULTS AND DISCUSSION

Formalin induced paw edema model

The procedure was similar to that described previously by Hunskaar and Hole, 1987 and consisted of the injection of 20 µl of 2.5% solution of formalin (0.92% formaldehyde) made up in phosphate buffer (pH 7.3) in the dorsal surface of the left hind paw of the mice. Immediately, the animals were placed individually in an observation chamber made of acrylic transparent; beneath the floor, a mirror was mounted at a 45° angle to allow clear observation of the paws of the animals. The amount of time that the animal spent licking the injected paw, considered as indicative of pain, was recordedduring 30 min following formalin injection. The initial nociceptive scores normally peaked 5 min after formalin injection (early phase) and 15-30 min after formalin injection (late phase), representing both the neurogenic and inflammatory pain responses, respectively. Animals were treated with the methanol extract of l. purpureus (at dose of 200 and 400 mg/kg p.o.) 1 h before the formalin injection. Control animals received only the vehicle used to dilute the substances (NaCl solution 10 ml/kg).

Table 1: Effect of Methanolic extract of Lalab Purpureous in formalin induced edema model.

Group	Paw oedema volume in ml (% Inhibition of paw edema)				
	0 hr	1 hr	2 hr	3 hr	4 hr
Vehicle control	1.20±0.02	1.40±0.05	1.55±0.03	1.65±0.06	1.84±0.04
Test Group I (MELP; 200 mg/kg p.o)	1.00±0.05	0.94±0.07	0.88±0.04	0.77±0.07	0.66±0.06
Test Group - II (MELP; 400mg/kg p.o)	0.95±0.05	0.88±0.04	0.76±0.06	0.65±0.08	0.58±0.4**
Diclofenac sodium (10mg/kg, p.o)	0.89±0.03	0.79±0.01	0.68±0.01	0.59±0.02	0.40±0.03***

Values are expressed as mean ± SEM (n=6). Values were statistically significant at **P<0.01, ***P, 0.001 Vs control using one way ANOVA followed by turkey test.

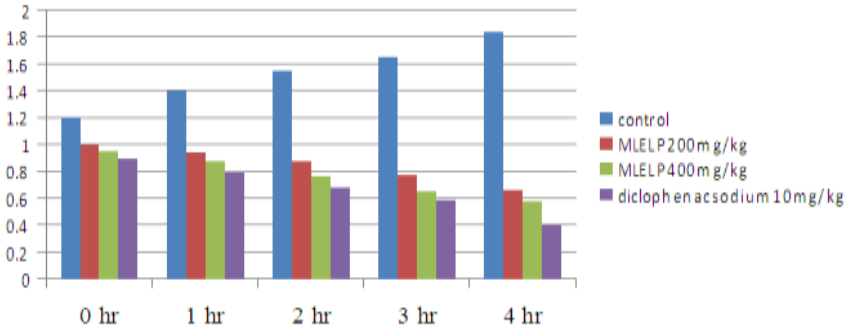

Fig.1: Effect of methanolic extract of Lalab purpureous in formalin induced edema model.

Egg white albumin induced edema model

Wister Albino rats were divided into 4 groups each group containing 6 animals. First group served as a control, second group received as a dilocuslalab plant methanolic extract 200 mg/kg, third group served as extract 400 mg/kg , and fourth group served as Diclofenac sodium 10 mg/kg. Edema was induced by administration of 0.5 ml of undiluted fresh egg white in the sub plantar region. The paw volume is measured at 0hr - 3hr after the injection of undiluted fresh egg white using Plethysmograph then % inhibition of edema is calculated by using formula:

$$\% \text{ inhibition of edema} = \frac{\text{Mean of control-Mean of test}}{\text{Mean of control} \times 100}$$

Table 2: Effect of Methanolic Extract of Lalab purpureous in Egg white albumin induced edema.

S. No	Treatment and Dose	Time interval				
		0hr	1hr	2hr	3hr	4hr
1.	Control	1.60±0.00	1.50±0.00	1.30±0.00	1.00±0.00	0.70±0.00
2.	MLELP200mg/kg	1.40±0.00	1.30±0.00	1.10±0.00	0.90±0.00	0.50±0.00
3.	MLELP400mg/kg	1.20±0.00	1.10±0.00	0.90±0.00	0.70±0.00	0.30±0.00
4.	Diclophenac sodium 10mg/kg	1.00±0.00	0.90±0.00	0.70±0.00	0.50±0.00	0.10±0.00

Values are expressed as mean ± SEM(n=6). Values were statistically significant at **P<0.01***P, 0.001 Vs control using one way ANOVA followed by turkey test.

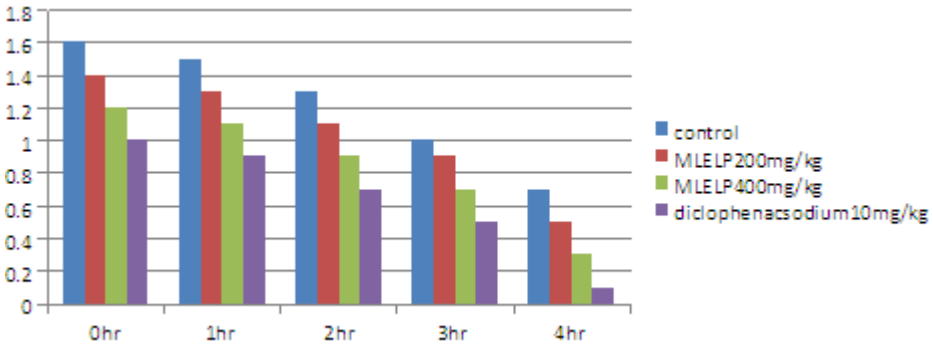

Fig. 2: Effect of methanolic extract of Lalab purpureus in Egg white albumin induced edema.

Study of anti-inflammatory activity by membrane stability assay

Anti-inflammatory activity of methanolic extract of lalab purpureus linn. was evaluated by using *in vitro* human red blood cell stability method. Blood sample was collected from a fresh volunteer, who doesn't have anti-inflammatory or contraceptive drugs at least since a week. The collected blood was mixed with sterilized Alsever solution. Alsever solution was prepared by 2% dextrose, 0.8% sodium citrate, 0.05% citric acid and 0.42% sodium chloride dissolved in distilled water. Blood sample was centrifuged at 3000rpm and packed cell was washed with isosaline and a 10% (V/V) suspension of isosaline was made. Three different solution of lalab purpureus linn. were mixed with 1ml phosphate buffer, 2ml hyposaline and 0.5ml HRBC suspension. Aspirin was used as contrastable drug and instead of hyposaline 2ml water was used as control. The hemoglobin content in supernatant was calculated using Spectrophoto-meter at 560nm spectrum.

The result was estimated by following equations:

$$\% \text{ of Hemolysis} = \frac{\text{OD of test} \times 100}{\text{OD of control}}$$

The percent of membrane protection was calculated by the following equation:

$$\% \text{ of Membrane protection} = \frac{\text{100- OD of test} \times 100}{\text{OD of control}}$$

Table 3: Anti-Inflammatory Activity of Methanolic Extract of *L.*purpureus leaves by using HRBC Membrane Protection Testing.

S.No.	No. of samples	Concentration		
		100µg/ml	200µg/ml	300µg/ml
1.	MLELP200mg/kg	22.16±0.63%	26.64±0.35%	29.25±0.73%
2.	MLELP400mg/kg	27.54±0.84%	30.75±0.74%	33.15±1.01%
3.	Diclophenac Sodium 10mg/kg	29.15±1.06%	33.84±1.65%	35.78±0.54%

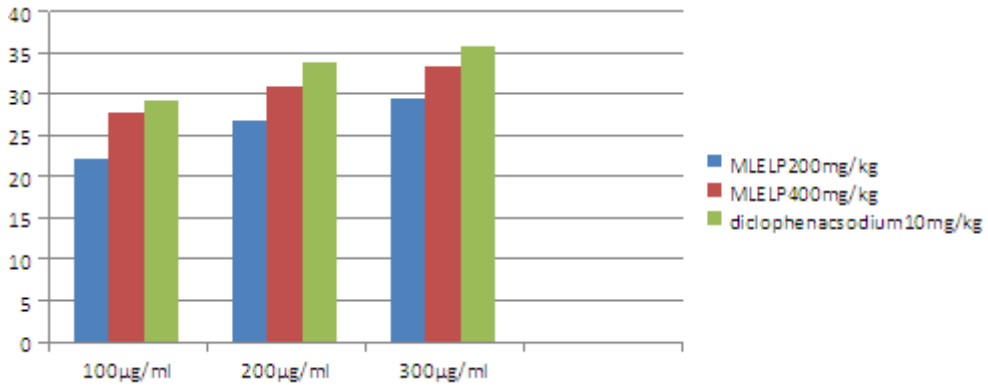

Fig. 3: Anti-Inflammatory Activity of Methanolic Extract of L. purpureus Leaves by using HRBC Membrane Protection Testing.

CONCLUSION

Methanolic extract of lalab purpureous linn. Showed significant anti inflammatory activity in Wister Albino Rats in a dose dependent manner. The formalin and egg white albumin induced paw edema model is used to evaluate the anti inflammatory activity of rodents. Results obtained on the formalin and egg white induced paw edema model after treatment with MLELP (200mg/kg and 400mg/kg) revealed anti inflammatory activity. Pre-treatment with MLELP (200mg/kg and 400mg/kg) concentration dependent percent of membrane protection. As the HRBC membrane is likely to the membrane of lysosome therefore the stabilizing ability of HRBC will be implied as its ability to protect the lysosomal membrane as well. In this test, Diclophenac sodium as standard, has 35.78% of protection at 300µg/ml where extract 200mg/kg has 33.15% and 400mg/kg has 29.25% at the same concentration. It seems the extract has significant activity on antiinflammatory functioning. The results of present study provide the evidence for the anti inflammatory activity of lalabpurpureous as claimed in the traditional use. The flavonoids present in the extract may be responsible for the above activity. Further studies on the exact mechanism of action and isolation of the active constituents are needed and different types of extracts can be evaluated for anti inflammatory activity.

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