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THE PRESENT STUDY ON EXTRACTS OF *DOLICHOS LABLAB* STUDIED FOR ITS ANTI INFLAMMATORY ACTIVITY IN ANIMAL MODELS OF INFLAMMATION

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ABSTRACT

Background and objectives: *Dolichos lablab* has been refered in Indian traditional medicine system (Ayurveda) for treatment of various disorders. In the present study extracts of *Dolichos lablab* studied for its anti inflammatory activity in animal models of inflammation. **Materials and methods:** Methanolic extracts of complex product prepared from dried leaves of Dolichos *lablab* plant. In the present study, the anti inflammatory effect of MLELP was examined using two behavioural models, the formalin induced edema model and egg white induced edema model in wister albino rats and one *in-vitro* model human red blood cell membrane protection assay. **Results:** The *in-vitro* anti-inflammatory studies such as HRBCMP assay shows satisfactory results, the concentration of extract increases the percentage of membrane protection, so they shows the dose dependent action. In FIE and EWIE demonstrated a dose dependent, statistically reduction in paw edema thatwas comparable to Diclophenac sodium (10mg/kg). The effect of 400mg/kg of MLELP was better than 200mg/kg of MLELP. The effect of 400mg/kg MLELP was significant when compared to vehicle treated group. *In vitro* study, MLELP in the dose of 200mg/kg and 400mg/kg showed decreased inhibition of edema when compared to that of normal. Plant extract at dose of 400mg/kg showed increases human red blood cell membrane protection, which is nearly equal to that of standard. **Conclusion:** The methanolic extract of *Dolichos lablab* leaves possessed significant *in-vitro* and *in-vivo* anti inflammatory activity in animal models.

KEYWORDS

Dolichos lablab, Animal models and MLELP.

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INTRODUCTION

Importance of Indian herbal plants

The role of natural products as remedies has been recognized since ancient times. A medicinal plant is any plant used in order to relieve, prevent or cure a disease or to alter physiological and pathological process or any plant employed as a source of drugs

or their precursors. 80% of the world`s population till relies upon plants for primary health care. Even today in western medicine and despite in synthetic chemistry 25% of prescription medicines are still derived either directly or indirectly from plants[1]. Nearly 50,000 species of higher plants have been used for medicinal purposes. They are also used in food, cleaning, personal care and perfumery. In systems of traditional healing, major pharmaceutical drugs have been either derived from or patterned after compounds from biological diversity[2].

Role of plants in inflammation regulation

Unlike modern Allopathic drugs which are single active compounds that can specifically target one pathway, herbal remedies work in a way that depends on orchestral approach. A plant contains a multitude of several molecules that synergistically act on targeted elements of the cellular complex pathway[3]. Medicinal herbs have been source of wide range of biologically active compounds for many centuries and they have been used extensively as crude drugs or as pure components for treating varieties of disease conditions. When compared to synthetic ones, natural remedies are having less side effects and toxicity. So, now days the usages of herbal remedies are increased when compared to allopathic drugs[4]. In the development of potential therapeutic agents, medicinal plant plays an important role. There are over 1.5 million practitioners of traditional medicinal system using medicinal plants in preventive, promotional and curative applications[5]. India with its biggest repository of medicinal herbs in the world may maintain an important position in the production of raw materials either directly for crude drugs or as the bioactive components in the formulation of pharmaceuticals and cosmetics etc[5].

Acute Inflammation

Acute inflammation is a short procedure, lasting from minutes to a few days, and its major features are leakage of plasma proteins or fluid and movement of leukocytes into an extra vascular area. These cellular and vascular reactions are intermediated by chemical factors produced from cells or plasma and are responsible for the classic clinical symptoms ofinflammation such as swelling, redness, pain, warmth, and loss of function. Even though an inflammatory response can happen in any injurious stimulus, the characteristic of this process is the reaction of vascularized connective tissue[3]. There are three main steps in acute inflammatory responses which include enhanced blood flow to inflame area, followed by vasodilatation and enhanced vascular permeability with leakage of plasma from the microcirculation and phagocytic leukocyte migration to the surrounding tissue.

MATERIAL AND METHODS

Preparation of *dolichos lablab linn.* Leaves extract by soxhletionmethod

Methanolic leaves extraction

The plant material was dried under shade and powdered mechanically. The 50gm of powder sample was defatted with petroleum ether (60-80°C), and then 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.

Phytochemical analysis

The following chemical tests were carried out for the extract of *Dolichos lablab* to identify the presence of various chemical constituents.

Evaluation of anti- inflammatory activity of methanolic extract of *dolichos lablab linn*

Experimental animals

Adult Wistar Albino male rats (150-180g) were procured from the laboratory animal model house. The animals were kept under standard environmental conditions of room temperature ($22^{o}\pm2^{o}$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 vyas labs 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.

Experimental design

Anti-inflammatory activity

In-vivo studies

Formalin test model

The procedure was similar to that described previously by Hunskaar and Hole, 1987 and consisted of the injection of 20µl 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 rat.

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 recorded during 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 *Dolichos lablab* (at dose of 200 and 400mg/kg p.o.) 1 h before the formalin injection.

Control animals received only the vehicle used to dilute the substances (NaCl solution 10ml/kg).

A total of 24 rats were divided into 4 groups, each containing 6 animals.

Group 1: Control (normal saline solution 10ml/kg; p.o)

Group 2: MLELP (200mg/kg; p.o)

Group 3: MLELP (400mg/kg; p.o)

Group 4: Diclophenac sodium (10mg/kg.)

Egg white albumin induced edema model

Male Wister Albino rats were divided into 4 groups, each group containing 6 animals.

First group served as a control, second group recived as a *Dolichos lablab* plant methanolic extract of 200mg/kg b.w.p.o, third group served as extract of 400mg/kg b.w.p.o and fourth group served as Diclophenac sodium 10mg/kg.

Edema was induced by administration of 0.5ml of undiluted fresh egg white in the subplantar 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:

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

A total of 24 rats were divided into 4 groups, each containing 6 animals.

Group 1: Control (normal saline solution 10ml/kg; p.o)

Group 2: MLELP (200mg/kg; p.o)

Group 3: MLELP (400mg/kg; p.o)

Group 4: Diclophenac sodium (10mg/kg)[50].

In-vitro studies

Human red blood cell membrane protection assay

Blood sample was collected from a fresh volunteer, who doesn't have anti inflammatory action or contraceptive drugs at least since a week.

Later the collected blood sample 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.

Plant extract sample was mixed with 1ml phosphate buffer, 2ml hyposaline and 0.5 mlHRBC suspension.

Diclophenac drug used as a standard drug and instead of hyposaline 2ml water was usedas control.

Finally the hemoglobin content in supernatant was calculated using spectrophotometer at 560nm spectrum[51].

Statistical data was estimated by following equation:

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

RESULTS AND DISCUSSION

Percentage yield after extraction with Methanol

Plant name: *Dolichos lablab linn.*

Parts used: Leaves

Solvent used: Methanol (95%)

Preliminary phytochemical screening

The revealed results of the preliminary phytochemical screening of the methanolic extract ofleaves of *Dolichos lablab linn* were shown below in Table No.2.

Acute toxicity studies

2000mg/kg b.w.p.o. doses of MLELP have no toxicity on observation for 14 days. The therapeutic doses selected for the assessment of anti inflammatory activity of MLELP were 200 and 400mg/kg b.w.p.o.

Anti-inflammatory activity Formalin induced edema model

After 15 days of treatment with MLELP in Wister Albino Rats there was significant decrease in the paw edema volume at dose levels of 200mg/kg of MLELP and 400mg/kg of MELP when compared to control. Further treatment with 400mg/kg the results were comparable with that of the standard drug Diclophenac sodium10mg/kg, the results were shown in the Table No.3.

Effect of methanolic extract of *Dolichos lablab* in 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 *Dolichos lablab* plant methanolic extract 200mg/kg, third group served as extract 400mg/kg and fourth group served as Diclofenac sodium 10mg/kg. Edema was induced by administration of 0.5ml 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:-

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

Human red blood cell membrane stabilization

Blood sample was collected from a fresh volunteer, who doesn't have anti inflammatory action or contraceptive drugs at least since a week. Later the collected blood sample 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 is washed with isosaline. Plant extract sample was mixed with 1ml phosphate buffer, 2ml hyposaline and 0.5ml HRBC suspension. Diclophenac drug used as a standard drug and instead of hypo saline 2ml water was used as control. Finally the hemoglobin content in supernatant was calculated using spectrophotometer at 560nm spectrum.

Statistical data was estimated by following equation:

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

Discussion

The fresh leaves of *Lalab purpureus* was collected from local habitat after authentification were shade dried and powdered to course powder size. The powdered material was subjected to successive hot extraction (soxhlet) with various solvents in increasing order of polarity from petroleum ether and methanol. After complete extraction, the solvent was distilled off and concentrated on water bath.

The preliminary phytochemical screening of extract of *Lalab purpureus* shows presence of alkaloids, flavonoids, carbohydrates, tannins, saponins, and phenolic compounds. Thus these activities of lalab purpureous could be due to alkaloids, flavonoids, and triterpenoids.

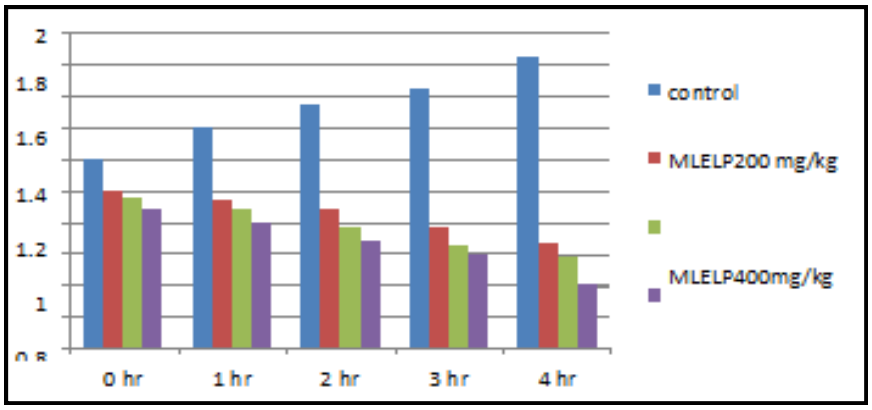
Albino rats, Wister strain, of weighing 150-180gm were used for acute model.

Acute toxicity study was carried out according to OECD guideline. The extracts were given to rats by oral route at dose level 500, 1000, and 2500mg/kg body weight, to groups of 4 animals. No death occurred within 24hrs of dose of 500, 1000mg/kg but at a dose of 2500mg/kg 50% mortality was observed. As a dose was increased further up to 5000 mg/kg, at that dose all the animals were died. Hence 2500mg/kg dose were considered as LD50.

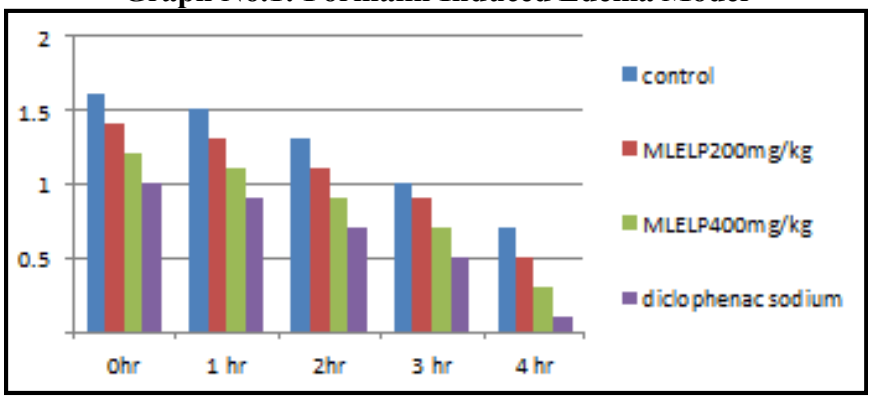
The formalin induced edema model and egg white albumin induced edema has been a popular inflammatory model to investigate the anti inflammatory effect of compounds. It hasa biphasic effect. The first phase is due to release of histamine and serotonin (5HT) (0-2hr), Plateau phase is maintained by kinin like substance (3hr) and second accelerating phase swelling is attributed to P.G release (>4hr).

In this study methanolic extract of *Lalab purpurous* (200 and 400mg/kg p.o) significantly reduces the oedema induced by formalin and egg white albumin in all the phases.

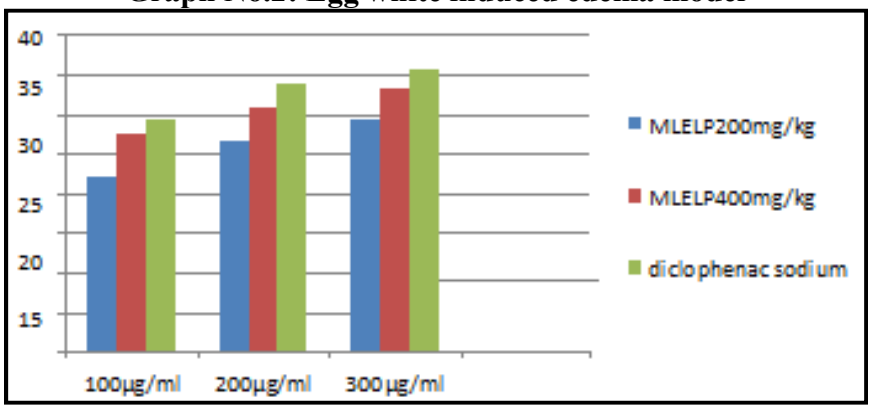
Hence it can be concluded that methanolic extract of *Lalab purpureus* possess anti inflammatory activity that may be mediated by alkaloids, flavonoids, and triterpenoids.



Graph No.1: Formalin Induced Edema Model



Graph No.2: Egg white induced edema model



Graph No.3: Human red blood cell membrane protection assay

Table No.1: Percentage yield of extract

S.No	Weight of powder	250gms
1	Yield	19gms
2	Percentage yield	7.6%

Table No.2: Preliminary phytochemical screening

S.No	Phytochemical Tests	Results
1	Test for carbohydrates	+
2	Test for alkaloids	+
3	Test for glycosides	+
4	Test for tannins	+
5	Test for steroids	-
6	Test for saponins	+
7	Test for flavonoids	+
8	Test for triterpenoids	+

(+) Indicates Presence of that Compound

(-) Indicates absence of that Compound

Table No.3: Formalin Induced Edema Model

S.No	Group	Paw edema volume in ml (% inhibition of paw edema)				
		0hr	1hr	2hr	3hr	4hr
1	Vehicle	1.20±0.02	1.40±0.05	1.55±0.03	1.65±0.06	1.84±0.04
2	MLELP200mg/kg	1.00±0.05	0.94±0.07	0.88±0.04	0.77±0.07	0.66±0.06
3	MLELP400mg/kg	0.95±0.05	0.88±0.04	0.76±0.06	0.65±0.08	0.58±0.4**
4	Diclofenalic sodium 10mg/kg	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.

Table No.4: Egg white induced edema model

S.No	Treatmentand 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	D.S, 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.

Table No.5: Human red blood cell membrane protection assay

S.No	Treatment	Concentration		
		100mg/kg	200mg/kg	300mg/kg
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	D.S,10mg/kg	29.15±1.06%	33.84±1.65%	35.78±0.54%

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.

CONCLUSION

Dolichos lablab Linn. Is used in traditional medicine remedy against many diseases. The other dilocus species like *Monoscus purpureus* was found to elicit anti inflammatory activity. Despite the numerous scientifically proven pharmacological activities of *Dolichos lablab linn*. There is no data on its potential as anti inflammatory activity. Hence the present study is done to evaluate anti inflammatory activity of methanolic extract of *Dolichos lablab linn.* In Wister albino rats.

Anti inflammatory studies provided significant evidence that the groups treated with the MELP at two dose levels 200mg/kg and 400mg/kg, in animals cause significant decrease in paw edema volume compared with control.

In formalin induced edema model study, results showed that duration of right hind pawlicking in animals was significantly reduced by pre-treatment with MELP at two dose levels 200 and 400mg/kg compared with control. In egg white albumin induced edema model study, results showed that right hind paw licking in animals was significantly reduced by pre- treatment with MELP at two dose levels 200 and 400mg/kg compared with control.

In Human red blood cell membrane stabilization assay method shows the anti-inflammatory action of *Dolichos lablab* methanolic extract. This was only a preliminary testing where we have such kind of 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 29.25% and 400mg/kg has 33.15% at the same concentration. It seems that the extract has significant activity on anti-inflammatory functioning.

The results of present study provide the evidence for the anti inflammatory activity of methanolic extract of *Dolichos lablab linn*. As claimed in the traditional use. Safety and effectiveness in the anti inflammatory activity were established.

The flavonoids namely quercetin present in the extract and it may be responsible for its anti inflammatory activity.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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