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Investigation of wound healing activity of lablab purpureus L. sweet seed extract on rats

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ABSTRACT

Aim: The present study was formulated to investigate the wound healing activity of lablab purpureus seed extract in rats.

Objectives: The objective of the present study are :

- To identify, authenticate and collection of Lablab purpureus seeds.
- To extract lablab purpureus seeds using ethanol by maceration extraction.
- To carry out preliminary qualitative phytochemical screening to identify phytochemical constituents.
- To conduct acute oral toxicity studies of the seeds extract as per OECD 425 guidelines[1].
- To evaluate the wound healing activity of Lablab purpureus beansusing the following models.

Materials and Methods: The seeds of lablab purpureus were collected, dried, powdered and kept for extraction(maceration) with ethanol. Preliminary phytochemical evaluation and acute toxicity studies were carried out. The invivo wound healing was carried out by Excision, Incision and Dead space methods. The parameters like wound contraction, tensile strength and formation of collagen fibres.

Result: In wound healing activity extract showed faster contraction, increase tensile strength and increased dry and wet tissue weight compared to the control group.

Conclusion: Based on the result it is confirmed that the ethanolic extract of lablab purpureus shows wound healing activity in a dose-dependent manner. The high dose(400mg/kg) shows statistically significant activity.

Keywords: Wound healing, Lablab purpureus, tensile strength.

I. INTRODUCTION

Lablab purpureus L. sweet is a bean species belongs to the family Fabaceae, which found in the parts of Africa. Wound healing is a common issue which should be givenmore importance. There are many studies have been conducted regarding woundhealing[2]. The other parts of lablab purpureus L. sweet have already undergone theanalysis of wound healing. Lablab purpureus L. sweet seed is used to analyse thewound healing capacity and it is edible too. The locally used plant has othertraditional uses which are discussed in other articles and journals. The wound is adisruption of the cellular and anatomic structure of the skin. Based on their location,aetiology, presenting symptoms or type of injury, wound depth and tissue loss it isdivided into several types. The process of wound repair is accompanied byanatomical changes and followed by collagen production. In involves three steps likeinflammation, cellular proliferation and remodelling[3]. [referto figure no.1].

II. MATERIALS AND METHODS

Methods of collection of Data

By animal experiment and laboratory investigations. Data will be collected from different in vivo and in vitro pharmacological experiments. The following experimental protocol is made to fulfil the maximum bio-statistical requirement. Animal experiments will be carried out as per the CPCSEA guidelines.

Intended out a plan of the work

- Collection of plant materials.
- Authentication by a taxonomist and preserving the specimen sample in the herbarium.
- Preparation of ethanolic extracts of Lablab purpueus.
- Evaluation of wound healing activity of aerial parts Lablab purpureus
- Statistical analysis

Collection of Plant Material and Extraction

The beans of Lablab purpureus will be collected from the local markets. The material will be shade dried, powdered and will be extracted(maceration). The extract obtained will be concentrated and evaporated under reduced pressure and controlled temperature and stored in desiccators until further use.

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Evaluation of LD50 by toxicity studies

Acute oral toxicity study

The acute oral toxicity study will be carried out in adult female albino rats weighing about 180-200gm, by up and down method as per OECD 425 guidelines [14]. Animals will fast (food but not water withheld overnight) before dosing. The fasted body weight of each animal will be determined, and the dose will be calculated according to the body weight[15]. **Based on the acute toxicity study 1/10th of the maximal dose as X i.e. the middle dose, 50% of the X as the maximum dose will be selected for the extract.**

Pharmacological Evaluation

1.Animals

Male albino rats Wistar strain, weighing (180g-200g) will be obtained from central animal house NUCARE. The animals will be grouped and housed in cages and maintained under standard laboratory conditions (temperature 25 ± 2°) with dark and light cycle (12h/12h). They will be allowed free access to a standard dry pellet diet and water ad libitum. The experiment will be carried out according to the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi, India and will be approved by the Institutional Animal Ethics Committee (IAEC).

2. Experimental design[4]

Screening Of Wound healing activity will done by using the following models; Excision wound model, Incision wound model, Dead space wound model.

Excision wound model

Male rats weighing 180-200g was taken and anaesthetized and the dorsal surface of the animal was shaved properly and the area in which the wound has to be created were marked using a permanent marker and the wound of a circular area of 4.9cm^2 and the depth of 0.2cm was done using a surgical blade and wound was left open. Soon after the creation of the wound the group I was untreated and kept as control. Group II was the reference standard and was treated with silver sulfadiazine ointment and was topically applied once in a day. Group III, IV, V was the test group of ethanolic extract of Lablab pupureus seed extract low, moderate and high dose and was administered orally to for 14 days. The wound was checked for 1st, 4th, 8th, 11th and 14th days by tracing the wound over the transparent paper and area was measured using 1mm^2 graph paper. Changes in the wound area were observed and wound concentration was calculated by using the formula[4].

%Wound contraction = Healed area/Total wound area *100

Healed area = original wound area – present wound area

[refer to table no 1 for materials and methods]

[refer to table no 2 for treatment protocol of excision wound model]

Incision wound model

Male rats weighing 180-200g were taken and anaesthetized and the dorsal surface of the animal was shaved properly and a longitudinal paravertebral incision was done about 6cm long using surgical blade through the skin and cutaneous tissue on the back. Once the long incision was done they parted skin was sutured using surgical thread and curved needle about 1cm apart.the wound was left open and undressed. Group Ⅰ was untreated and kept as control. Group II was the reference standard and was treated with silver sulfadiazine ointment and was topically applied once in a day. Group III, IV, V were the test group of ethanolic extract of Lablab purpureus low, moderate and high dose were administrated orally to all the animals respectively. Breaking strength was measured using tensiometer[5].

Dead spacewound model

Male rats weighing 180-200g was taken and anaesthetized and the dorsal surface of the animal was shaved properly and an incision wound was created and implanting the sterilized cotton pellets of weight 10mg were implanted on the lumbar region on the dorsal surface of the rat. The wound was left open and undressed. Group, I was untreated and kept as control. Group II was the reference standard and was treated with silver sulfadiazine ointment and was topically applied continuously and the Group III, IV, V was the test group of ethanolic extract of Lablab purpureus seed low, medium and the high dose was given orally[6].

Statistical analysis

The data will be expressed as Mean ± SEM and will be analyzed by one-way analysis of variance (ANOVA), followed by usingspss computer software. A P-value is less than 0.05 will be considered statistically significant.

Result: In wound healing activity extract showed

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faster contraction, increase tensile strength and increased dry and wet tissue weight compared to the control group.

Data
The percentage yield of the ethanolic extract is calculated.
[refer table no 3 for percentage yield]

- Preliminary phytochemical analysis of lablab purpureus L. sweet seed[7][8].

To detect the presence of various chemical constituents in the extract, the extract of dried lablab purpureus L. sweet seeds had to go through preliminary qualitative phytochemical tests. The results of various chemical tests were recorded as below.
[refer table no 4 for chemical tests]

Acute toxicity studies:

The ethanolic extract of lablab purpureus L. sweet seed was found to be safe up to 2000mg/kg body weight by the oral route. After 24hrs the animals were found to be showing no effects of toxicity. There were no signs of mortality, thus the extract was found to be safe.

Wound healing Activity-
i. Excision wound model
Effect of ethanolic extract of lablab purpureus L. sweet seed on the excision wound model.
[refer table no 5 for results]
[refer figure no 2 for graph]
[refer figure no 3 for picture]

ii. Incision wound model
Effect of ethanolic extract of lablab purpureus L. sweet seed on the incision wound model.
[refer table no 6 for results]
[refer figure no 4 for graph]
[refer figure no 5 for picture]

iii Dead space wound model
Effect of ethanolic extract of lablab purpureus L. sweet seed on dead space wound model.
[refer to table no 7 for results]
[refer to figure no 6 and figure no 7 for graph]

III. DISCUSSIONS

Excision woundmodel

The wound healing potential of ethanolic extract in the excision wound model was studied in terms of the reduced area of the wound, causing contraction of wound. High dose of Lablab purpureus L. Sweet seed extract increased the percentage of wound contraction and completed wound healing by indicating rapid epithelization and collegenation.

Incision wound model

The wound healing property of plant extract can also be determined by measuring the tensile strength of the wound closure by tensiometer. The result shows that the high dose of extract was able to increase the splitting strength of the tissue covering the wound. This may be due to enhanced collagenformation.

Dead space wound model

Given the increase in the weight of granulation tissue, the effect of oral administration of the extract lablab purpureus L. sweet seed on dead space wound model was studied. The extract of lablab purpureus L. sweet seeds and also the high dose showed significant activity when compared to standard (silver sulfadiazineointment).

IV. CONCLUSION

→ The current research was undertaken to examine the healing of the wound in lablab purpureus L. Sweet seed. Ethanolic extract of lablab purpureus L.sweet seed was found to be virtually non-toxic, as it has no lethal effect even at a dose of 2000mg / kg when administered orally. At the high dose, the ethanolic extract of lablab purpureus L.sweet seed was found to be a significant factor in wound healing.

→ Preliminary phytochemical screening of ethanolic lablab purpureus seed extractL. Alkaloids, glycosides, phenols, saponins, flavonoids, triterpenoids, steroids, and tannins was found to be nice. The present study revealed the wound healing ability of extract lablab purpureus L.sweet seed peel as demonstrated by improved woundcontraction, increased wound tensile strength, cotton pellets dry and wet in excision, incision, and dead space woundpattern.

→ The healing effects resulting from faster college deposition, and the formation of other constituents such as connective tissue. Such findings include pharmacological evidence andhelp on conventional use as a wound-healing agent of lablab purpureus L.sweet seed. This work

maybe extended in the future by adding more models of wound healing to confirm the wound healing potencyof lablab purpureus L. Sweet seed can also be tried to isolate and characterize the

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phytoconstituents responsible for pharmacological activity. Toxicological studies can also be done to get more information on the drug's toxicity profile.

Acknowledgement

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FIGURES AND TABLES:

Figure 1 lablab purpureus L. sweet seed

Table no 1: Materials and methods

Animals	Albino wistar strain rats
Age and sex	Male
Body weight	180-200g
No of animals in each group	6
Number of groups	5
Route of administration	Oral route
Water and food	ad.Libitum

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Table no 2: Treatment protocol

Groups	**Treatment**
Group 1 (control)	No Drug treatment
Group 2 (standard)	Silver sulfadiazine
Group 3 (test-low dose)	Test drug of 100mg/kg
Group 4 (test-medium dose)	Test drug of 200mg/kg
Group 5 (test-high dose)	Test drug of 400mg/kg

Table no 3:Percentage yield

Extract	Colour	Consistency	Percentage yield
Ethanolic	Dark brown	Semisolid	40%

Table no 4 Phytochemical evaluation

Sl.no	Tests	Ethanolic extract of lablab purpureus L. sweet seed
1.	Alkaloids:	+
	a. Dragendroff'stest	+
	b. . Hager'stest	+
	c. Wagner's test	+
	d. Mayer's test	+
2.	Carbohydrates:	
	a. Molish'stest	+
	b. Benedicttest	+
	c. Fehling's test	+
3.	Flavonoids:	
	a. Shinoda test	+
4.	Steroids:	
	a. Libermanburchardtest	+
	b. Salwoskitest	+
5.	Triterpenoids	
	a. Liebermann Burchard's test	+
6.	Proteins	

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	a. Biurette test b. Millon's test	+ +
7.	Saponins	+
8.	Tanins	+
9.	Glycosides a. Keller killiani test	 +
10.	Phenols a)Ferric chloride test	 +

Table no 5 Resutls of excision wound model

Groups	Treatment	wound area in				% wound contraction
		4^{th}	8^{th}	11^{th}	14^{th}	
I.	Control	56.10.79	56.60.86	56.00.01	55.50.15	56.60.93
II.	Standard	50.00.66	52.00.33	63.60.42	62.20.28	65.60.78
III.	100mg/kg Test drug	57.330.89	57.160.46	56.160.20	58.160.06	57.50.09
IV.	200mg/kg test drug	51.660.94	52.161.71	51.660.94	52.830.02	52.000.11
V.	400mg/kg test drug	48.330.80	47.000.82	56.200.80	57.400.66	56.40.46

The values are expressed as mean ±SEM (n=6) $p < 0.05$ is considered as statistically significant.

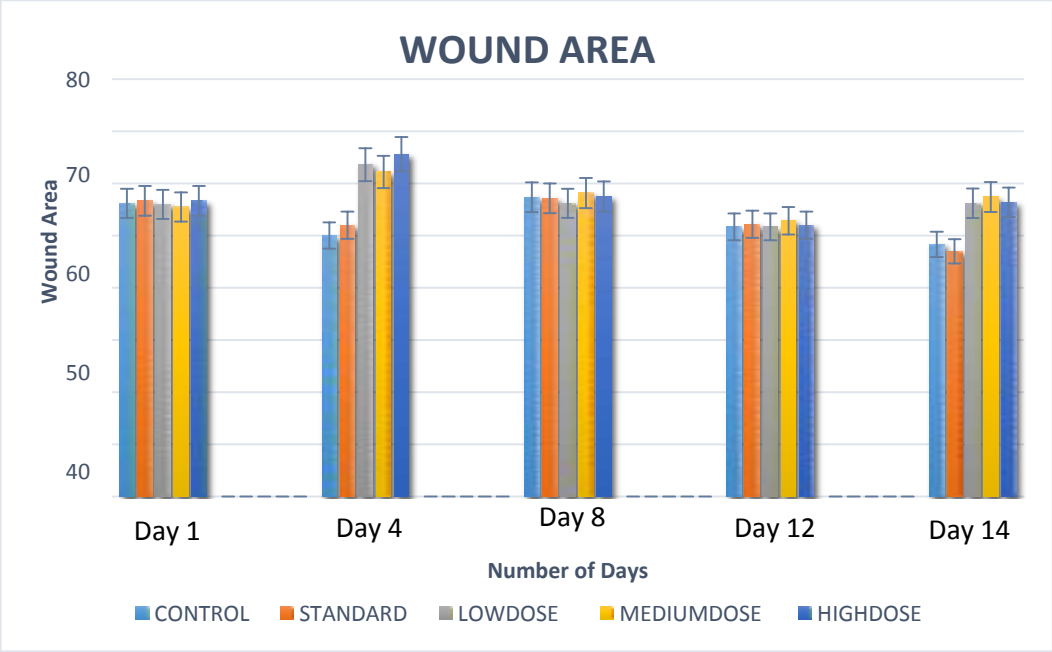

Figure 2: Graph of excision wound model

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Figure 3: excision wound

Table 6 Results of incision wound model

Parameter	Control	standard	100mg/kg	200mg/kg	400mg/kg
Wound breaking strength	643.319.0	320.713.6	457.613.5	342.216.07	192.09.2

The values are expressed as mean ±SEM (n=6) $p < 0.05$ is considered as statistically significant.

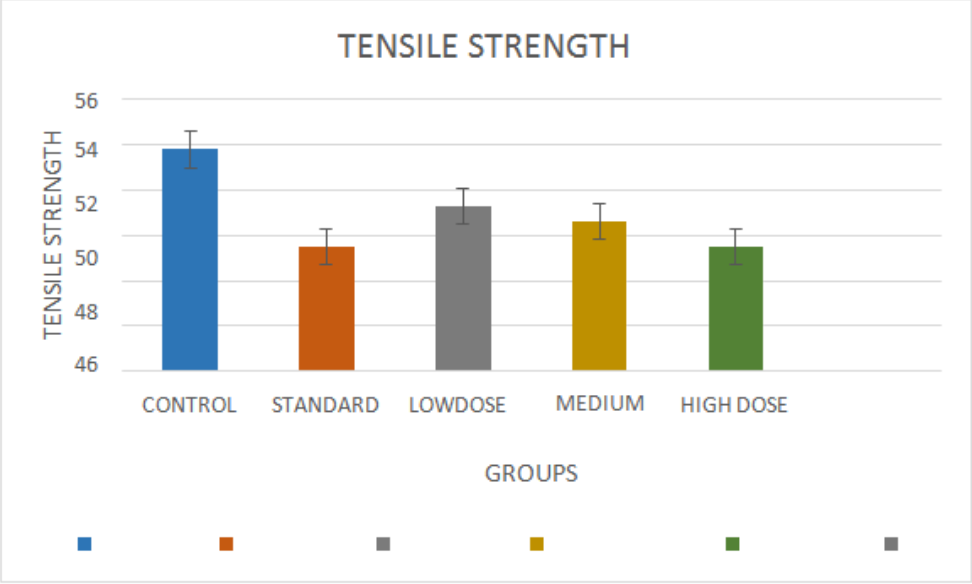

Figure 4: graph of incision wound model

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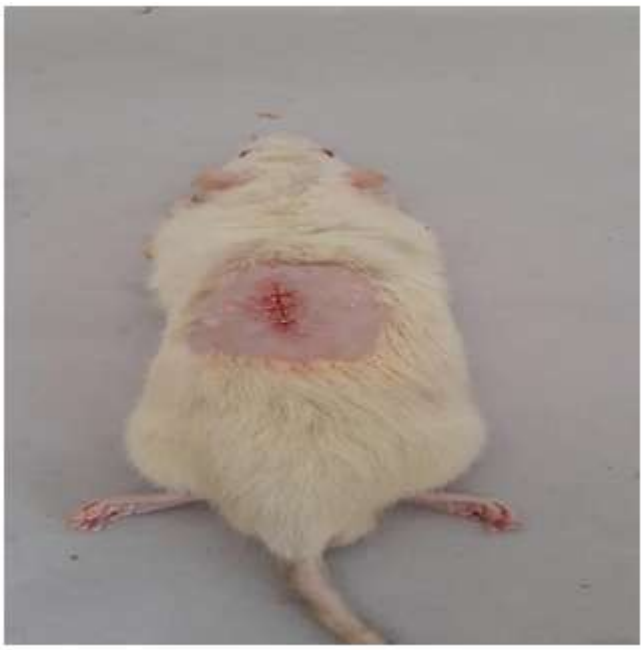

Figure 5: incision wound

Table 7 Results of dead space wound model

Parameter	Control	Standard	100mg/kg	200mg/kg	400mg/kg
Wet weight	3000.06	470.51.6	4061.34	4120.76	4580.21
Dry weight	1011.0	1741.2	1140.89	1421.34	1651.77

The values are expressed as mean ±SEM (n=6) . a=p<0.05

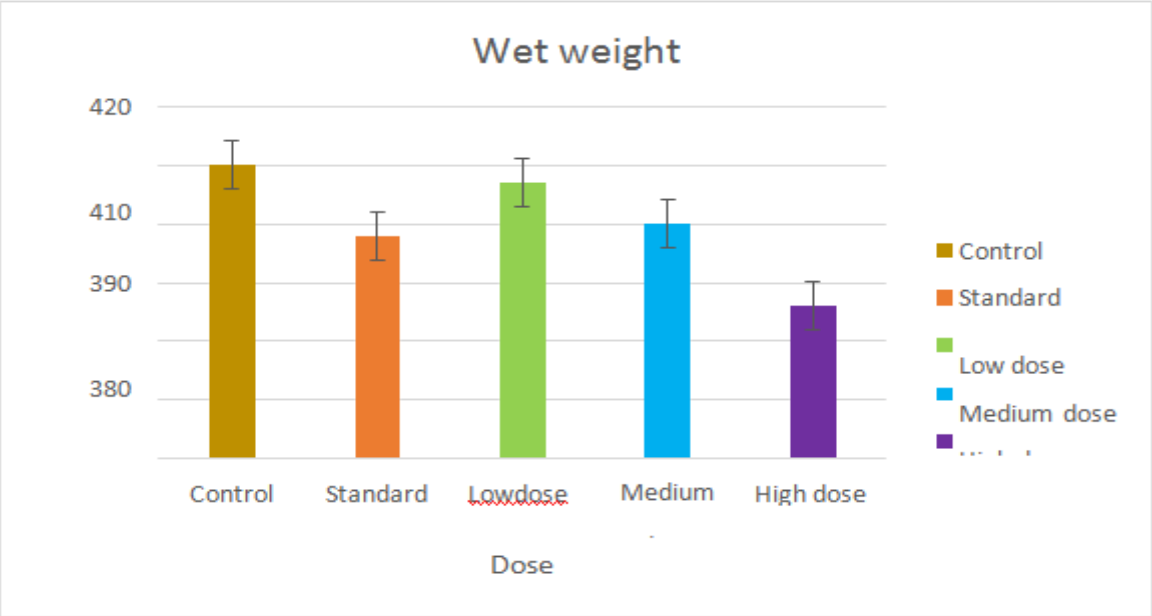

Figure 6: graph of dead space wound model

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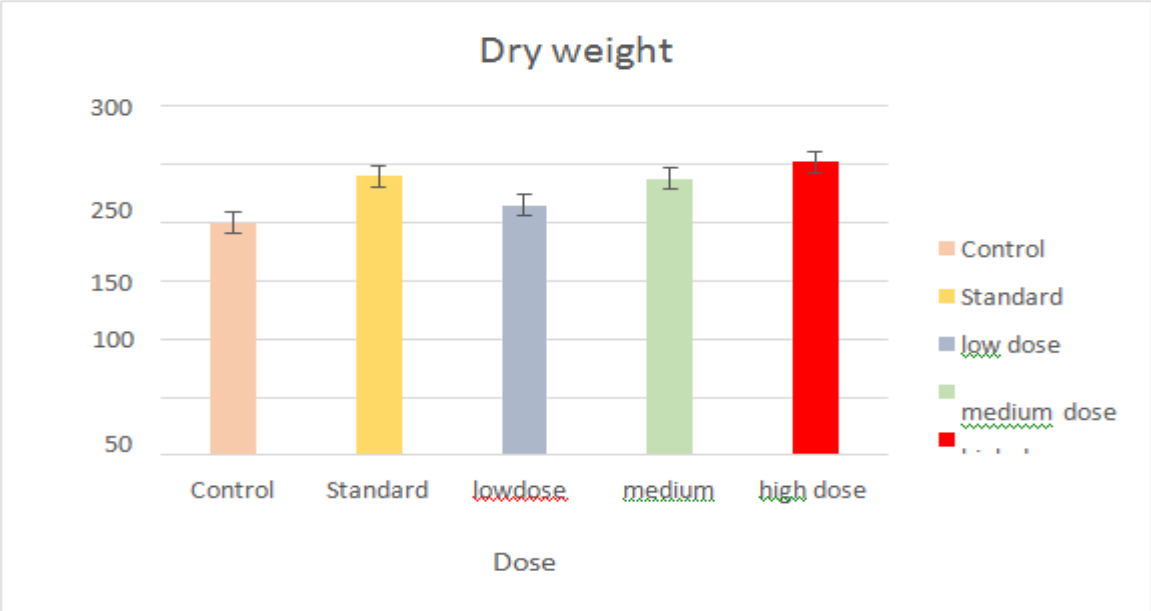

Figure 7: graph of dead space wound model