EVALUATION OF GEL OF *CARICA PAPAYA LATEX* AND *BOERHEVIA DIFFUSA LINN.* LEAVES FOR WOUND HEALING ACTIVITY IN EXPERIMENTAL ANIMALS

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ABSTARCT

The wound is the result of damage to the skin tissue and exposure of this wound to external environment leads to the wound infection. Various systems of medicines are available in market for wound healing but still there is requirement of another best treatment. In ayurveda adverse variety of plants are available for the wound healing which shows grateful results. It is a best way to use the combination of the drug than using the single drug to treat the infection which will give better results. *Carica papaya latex* reported to have antibacterial activity and *Boerhevia diffusa*showed antioxidant activity long with antibacterial effect. We found that the combination of these two herbal plants can leads to great effect in treating wound. The current study aimed out in vivo onWister rats by excision and incision model of wound. The gel of the *Carica papaya latex and Boerhevia diffusa leaves* showed to promotes the cell proliferation, increase the wound contraction by the development of new blood vessels and formation of the collagen. This study will lead to development of newer medicine therapy for wound healing activity.

Keywords – *Carica papaya latex, Boerhevia diffusa linn* leaves, Antioxidant, Antimicrobial, Wound healing.

Abbreviations

GOCB-1 – Gel of *Carica papaya* latex and *Boerhevia diffusa linn* -1

GOCB-2 - Gel of *Carica papaya* latex and *Boerhevia diffusa linn* - 2,

CP – *Carica papaya*

BD – *Boerhevia diffusa*

HPC – Hyroxyproline content

STD – Standard

1. INTRODUCTION

Wound is a loss or breaking of cellular and anatomic of functioning continuity of living tissues. Wounds result in damaged skill call that lose their function and need time and simple treatmentto heal.[1] Chronic wounds afflict many patients and significantly lower their quality of life, making them a major concern for both patients and clinicians. An initial phase of inflammation is followed by re-epithelialization of the wound site, the development of granulation tissue, neovascularization, and wound contraction. Both the development of a chronic wound and the creation of a hypertrophic scar might occur if one or more of these stages are out of balance.[2]

Wounds are often categorized based on the underlying factor that led to their development- Acute wound, Closed wound, Open wound, Gunshot wound, Chronic wound, Tear wound, Puncture wound, Penetration wound etc. Acute wound is the anatomical and functional integrity is usually sustainably restored in acute wounds after tissue damage/injury through an ordered and time-reparative phase. Cuts or surgical incisions frequently result in acute wounds[3]. Chronic wounds are those that have not gone through the typical stages of healing and have instead progressed to a level of pathologic inflammation. They require more healing time[4]. Deep soft tissue damage from gunshot wounds is known as penetrating trauma. Carers frequently deal with the difficulties brought on by sluggish and poor wound healing.[5]

Carica papaya belongs to family *Caricaeae*, cultivated in tropical and subtropical regions around whole world. [6] Papaya fruit is easy to digestion and nutritive value. *Carica papaya* is most commonly used in the diuretic, anthelmintic, diarrhoea, pain of burns, bleeding haemorrhoids, stomachic wohhing cough.[7] *Carica papaya* contains proteolytic enzyme which soothes the stomach and aides in digestion.[8] Papaya latex is used for ringworm, and removal of cancerous growth. Antifungal (fluconazole) shows the synergistic effect in partial cell wall degeration.[9]

Boerhevia diffusa is commonly known as Punarnava in Indian traditional medicinal system. Boerhevia diffusa belongs to family Nyctaginaceae (Four o' clock).[10] *Boerhevia diffusa* is growing perennial creeping herb found in India. It is spreading vine widely distribute in the tropical and subtropical region of world.[11] Leaves of *Boerhevia diffusa* are thick, fleshy, hairy, simple, arranged in unequal pairs and green in coloured. Shape of leaves is oval, round. B. *diffusa linn*. Is rich source of minerals, vitamin, protein and carbohydrate.[12]The leaf juice is

used in jaundice and the present study was under taken to investigate wound healing activity of Ethanolic extract of *Boerhevia diffusa Linn* leaves.[13]

Carica papaya latex and Boerhevia diffusa Linn. Leaves posses' health benefits, nutritional benefits, therapeutic benefits, several bioactive compounds. *Carica papaya latex* an *Boerhevia diffusa linn* leaves exhibits various pharmacological properties including antimicrobial, antioxidant, antiviral, anti-inflammatory which are due to richness of alkaloid and flavonoid bioactive compounds like papain, peptidase[14] A and peptidase B, Lysozymes, Chemopapain, Oxalic acids, Purnarnavine, Lignan etc. Therefore, the present study evaluates the gel of *carica papaya latex and Boerhevia diffusa* linn leaves for wound healing activity in rats.[15]

2. MATERIALS AND METHODS –

2.1Collection and Authentication of plant –

Carica papaya unripe fruit were collected from Pune region of Maharashtra state, India in month of October 2022.The plant was identified and authenticated by D.L.Shirodkar, Botanist of Botanical Survey of India, Pune, and a voucher specimen no AACP-1 was deposited in the herbarium for future reference.

Boerhevia diffusa linn leaves were collected from Bhakti shakti road side area Pune region of Maharashtra state, India in month of October 2022.The plant was identified and authenticated by D.L.Shirodkar, Botanist of Botanical Survey of India, Pune, and a voucher specimen no AABD-2 was deposited in the herbarium for future reference.

2.2 Extraction of phytoconstituents

2.2.1Preparation of Papain Course

Samples were taken from papaya fruit varieties are aged between 2.5 and 3 months. Latex carried out early morning at 6:00 am to 08:00 am. Papaya fruit is cleaned with the help of water then scratched fruit from base with the needle as deep as 1-2 mm. Latex dripped out in stainless steel container. Latex collected and dried using vacuum oven. Papain is crushed in a powder form[16].

2.2.2 Preparation of plant extract of *Boerhevia diffusa linn* leaves -

The leaves of *Boerhevia diffusa* were dried in the shade and ground to a coarse powder by using a dry grinder. The 200g of coarsely ground plant material was soaked with a 25% ammonia solution and left to stand the next day. A Soxhlet extractor was then used to do a thorough extraction with 95% ethanol. Vacuum distillation was used to concentrate the extract.

Following shaking and filtering, sections of benzene were used to wash the acidic filtrate, pieces of ammonia solution were used to make it basic (pH 10), and portions of chloroform were used to extract the material. In order to get the alkaloidal fraction (2g, 1% w/w), the mixed chloroform extracts were washed with water, dried over anhydrous sodium sulphate, and evaporated.[17]

2.4 Formulation of Gel

Table 1Formulation of gel

FORMULA	CONCENTRATION
Carbapol 920	1%
Tragacanth	1%
Carica papaya latex	2.5%,5%
Boerhevia diffusa linn. Leaves extract	5%, 10%
Isopropyl myristate	1%
Methyl paraben	0.25%
Water	QS

Weigh Carbopol and add it in 20ml of water and leave it for overnight 24hrs to get swell. Add tragacanth in water after that add this dispersion in Carbopol and mix it well with the help of mechanical stirrer. Stirring is continued then add both the extract slowly. Gel mass form is formed, after that isopropyl myristate as a skin penetration enhancer and finally add the methyl paraben as a preservative.[18]

2.5Experimental animals and Model -

2.5.1 Experimental animals-

Twenty-four adult Wistar rats (180-210 g) were procured from Crystal Biological Solutions, Pune. The rats were kept in standard polypropylene cages. Animals were maintained under standard laboratory conditions of temperature (25±2°), relative humidity (50±15%), 12 h light-dark cycle, standard diet, and water. The care and handling of the animals were in accordance with the internationally accepted standard guidelines for use of animals, and the protocol was approved by the Institutional Animal Ethical Committee (IAEC) of Modern College of Pharmacy in accordance with the regulations of CPCSEA (MCP/IAEC/01/2022).

2.5.2 Experimental Model -

2.5.2.1.Circular excision wound model

Rats were divided into four groups containing six animals in each group. In this model circular wound of 500mm^2 was made using the surgical scissor and forceps. Wound is formed from 1cm away from the vertebral column of the rat. Fastly, the skin is sterile with the help of 90% alcohol solution. After that circle of 500mm^2 is drawn and skin was cut to form a circular excision wound.[19] The wound area was measured on 4th, 7th, 10th, 13th, 16th, 19th, 21st day after surgery. The measurement was done by placing a transparent tracing paper over the wound and the borders of wound was traced by using a pointer marker. The healed area was counted and recorded. Percentage wound contraction was determined. In this study independent animal experiment were formed for model of wound healing i.e. Excision wound model. For circular excision twenty-four rats were randomly assigned in four groups of six rats in each group i.e. Disease control group, Standard group (Silver colloid gel), GOCB-1(*Carica papaya latex* 2.5%and *Boerhevia diffusa linn* leaves 5%), GOCB-2(*Carica papaya latex* 5% and Boerhevia diffusa linn leaves 10%). Disease control group was untreated, Standard group was treated with Silver colloidal gel, GOCB-1 group was treated with *Carica papaya latex* 2.5% and *Boerhevia diffusa linn* leaves 5%, GOCB-2 group was treated with *Carica papaya latex* 5% and *Boerhevia diffusalinn* leaves 10%.

$$\% \text{ Wound contraction} = \frac{\text{Initial wound size} - \text{Specific day wound size}}{\text{Initial wound size}} \times 100$$ [20]

Figure 1Circular excision wound model

2.5.2.2 Linear incision wound model

In this model tensile strength of the tissue was measured. The animals were divided into four groups. Incision wound is made 2cm away from the vertebral column of length of 5cm. Firstly, skin is sterile with the help of the 90% alcohol. Then skin is stretched so as to get perfect linear incision cut. Using surgical blade and forceps deep cut was made up to the visceral layer of skin. Excess of blood loss was cleared using surgical cotton. For stiching needle holder, forcep and stiching material i.e thread and needle was used i.e vicryl. Required number surgical sutures were made for closing the wound. Then this closed wound was left for 10days. On 10th day this suture was cut using the scissor and tensile strength of the closed wound was measured using suitable instrument i.e. tensilometer. [21]In this study independent animal experiment were formed for model of wound healing i.e. Incision wound model. For Linear incision twenty-four rats were randomly assigned in four groups of six rats in each groups Disease control group was untreated, Standard group was treated with Silver colloidal gel, GOCB-1 group was treated with *Carica papaya latex* 2.5% and *Borhevia diffusa Linn* leaves 5%, GOCB- 2 group was treatedwith *Carica papaya latex* 5% and *Borhevia diffusa Linn* leaves 10%.

Figure 2 Linear incision wound model

2.6 MEASUREMENT OF WOUND

Daily wound was traced using tracing paper and randomly three diameters are selected and taking the average of it. Using the formula of the circular area of wound is calculated.[22] Daily wound photographs were taken of wound using a digital camera and wound contraction was observed.

2.7 HISTOPATHOLOGY

In excision wound models, a sample of skin tissues from the Control, Standard, GOCB-1, and GOCB-2 groups were removed from the animals' healed wounds for histological analysis. The thin slices were cut, preserved in 10% formalin, and examined under a microscope for histopathological alterations such tissue granulation, collagen deposition, and fibroblast proliferation. [23]

2.8 STATISTICALANALYSIS

The results were expressed as mean ± SEM. Statistical analysis was performed using GraphPad Instat software, version 3.00 for Windows 95, San Diego, California USA.The values were analyzed by a one-way analysis of variance (ANOVA) followed by the Tukey multiple comparison test at a significance level of $p < 0.05$.

3. RESULTS –

3.1 Evaluation parameters of gel

3.1.1.PH

Sr.no	Standard PH range	PH
1	4 to 6	5.6

3.1.2. Viscosity

Sr.no	Standard viscosity range (cps)	Viscosity (cps)

1	2000-4000	3412

3.1.3. Spreadability

Sr.no	Standard Spredability range (cm)	Spredability (cm)
1	5 - 7	5.9

3.1.4. Diffusion Test

Sr no	Time (min)	Absorbance
1	15	0.004
2	30	0.04
3	45	0.07
4	60	0.046

3.2 *Boerhevia diffusa linn* leaves extract

Boerhevia diffusa leaves are kept on alcohol for period of 1 week. The extract was obtained by the Soxhlet extraction process, having dark greenish colour.

3.3 Phytochemical screening

Phytochemical screening showed presence of following constituents

Table 2 Phytochemical screening showed presence of test

Sr no	Phytochemical test	Results
1	Test for flavonoids	+
2	Test for alkaloids	+
3	Test for glycosides	+

4	Test for tannins	+

3.4 Wound healing activity of *Carica papaya* latex and *Boerhevia diffusa* gel

3.4.1Circular Excision wound model

3.4.1.1PERCENTAGE WOUND CONTRACTION

The below table shows the outcomes of the excision wound model for wound healing activity. The data in the table show the healing of a wound after 4, 7, 10, 13, 16, 19, and 21 days for the GOCB -1, GOCB-2 groups of two concentration of *Carica papaya* (CP) and *Boerhevia diffusa* (BD) was used i.e., *Carica papaya*-2.5%, *Boerhevia diffusa*-5% and *Carica papaya*-5%, *Boerhevia diffusa*-10%. Standard group is treated with gel of colloidal silver (1%) and Disease control (DC) group is untreated. Wound contraction shows good results from 4th day of the wound. It is observed that group treated with standard gel (Colloidal silver), GOCB (Gel of *Carica papaya* latex and *Boerhevia diffusa* linn)i.e., CP-2.5%, BD-5% and CP-5%,BD-10% shows significant results than that of disease control group. Out of all groups GOCB treated shows more percentage of wound contraction than that of std and DC treated groups.

Table 3. Effect of topical application of Herbal gel containing extract of Carica papaya latex and Boerhevia diffusa Linn. on wound contraction of excision wound

DAYS	**Control group**	**Standard (Silver colloidal gel)**	**GOCB -1**	**GOCB-2**
4	1.67±0.34	28.76±1.66*	32.57±0.31*	35.02±1.42**
7	2.43±0.12	46.07±3.79*	41.73±3.12*	43.17±3.11**
10	4.01±0.03	66.38±2.31*	63.38±1.34*	65.43±3.07**
13	14.35±0.84	74.08± 5.68*	77.01±4.56*	79.32±2.71**
16	28.56±1.75	84.51±7.01*	87.82±6.44*	89.05±4.01**
19	42.12±3.71	91.88±1.93*	95.07±1.06*	96.21±0.83**
21	72.39±7.09	98.31±2.70*	98.97±0.43*	99.56±0.62**

Values are expressed as mean± SEM; n = 6; Data analyzed by One-way ANOVA test followedby Tukey's

multiple tests for comparison. #p<0.05, as compared to C; *p<0.05, **p<0.01

Note - GOCB 1- Gel of *Carica papaya* latex 2.5% and *Boerhevia diffusa Linn* 5%, GOCB 2-Gel of *Carica papaya* latex 5% and *Boerhevia diffusa Linn* 10%

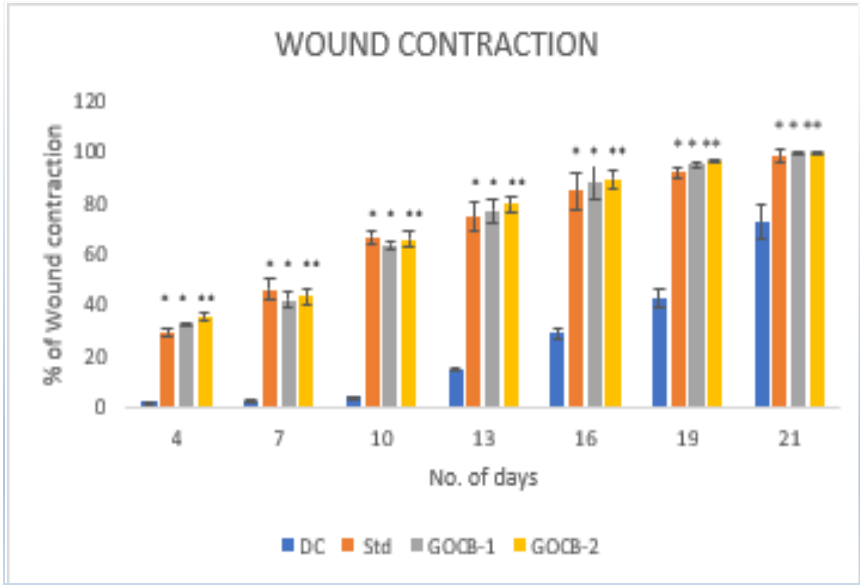
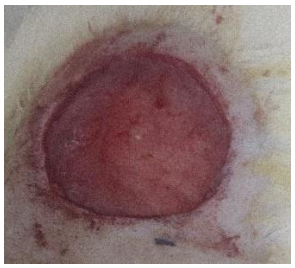


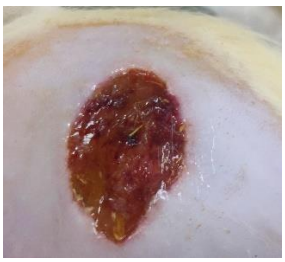
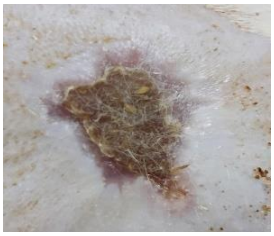
Figure 3. Decrease in percentage area of wound contraction in circular excision wound model.

Values are expressed as mean± SEM; n = 6; Data analyzed by One-way ANOVA test followedby Tukey's multiple tests for comparison. #p<0.05, as compared to C; *p<0.05, **p<0.01

Note - GOCB 1- Gel of *Carica papaya* latex 2.5% and *Boerhevia diffusa Linn* 5%, GOCB 2- Gel of *Carica papaya* latex 5% and *Boerhevia diffusa Linn* 10%

Table 4Wound contraction in circular excision wound model

Days	**Control**	**Standard (colloidal silver gel)**	**GOCB 1**	**GOCB 2**
Day 1				

Day 5				
Day 11				
Day 17				
Day 21				

Changes occurs in area of wound when treated with standard (colloidal silver gel) base, GOCB-1and GOCB-2 extracts were prepared. Control group remained untreated.

3.3.1.1 Complete Epithelization period

Table 5 Complete epithelization period in circular excision wound model

Groups	Complete epithelization period (days)
Control	23
Standard (colloidal silver gel)	20
GOCB 1	18
GOCB 2	17

Note - GOCB 1- Gel of *Carica papaya* latex 2.5% and *Boerhevia diffusa Linn* 5%, GOCB 2-Gel of *Carica papaya* latex 5% and *Boerhevia diffusa Linn* 10%

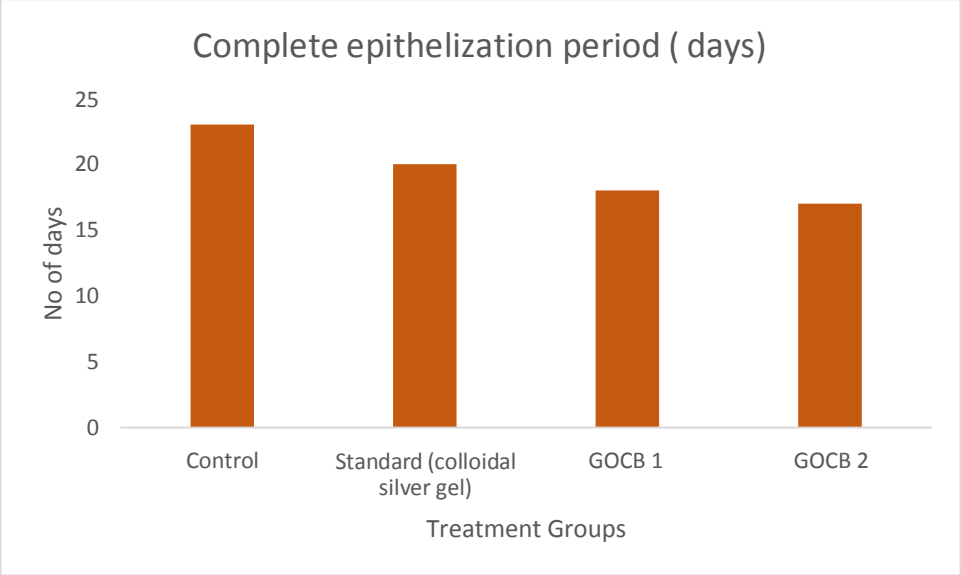


Figure 4. Complete epithelization period of circular excision wound model

Note - GOCB 1- Gel of *Carica papaya* latex 2.5% and *Boerhevia diffusa Linn* 5%, GOCB 2-Gel of *Carica papaya* latex 5% and *Boerhevia diffusa Linn* 10%

The Complete epithelization period was found to be least of GOCB- II which is 17 days, than that of other treated groups.

3.3.1.2 . Hydroxyproline content

Table 6 Hydroxyproline level in circular excision wound model

Sr no	Groups	Absorbance
1	HP	0.587
2	DC	0.361
3	Std (colloidal silver gel)	0.546
4	GOCB 1	0.443
5	GOCB 2	0.498

Note – HP – Hydroxyproline content, DC- Disease control, STD – Standard (Silver colloidal gel), GOCB 1- Gel of *Carica papaya* latex 2.5% and *Boerhevia diffusa Linn* 5%, GOCB 2-Gel of *Carica papaya* latex 5% and *Boerhevia diffusa Linn* 10%

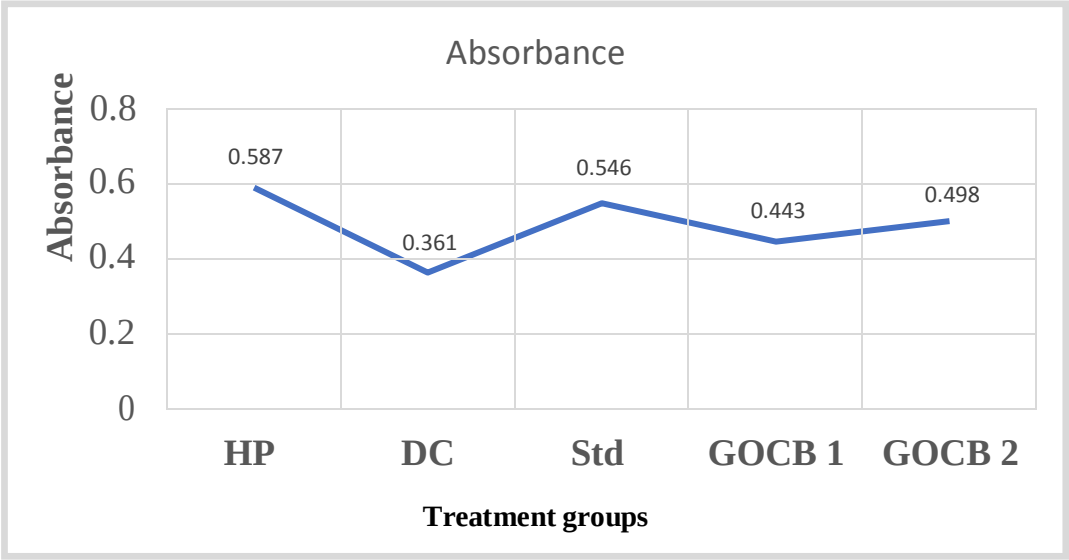


Figure 5. Hydroxyproline level in circular excision wound model

Note – HP – Hydroxyproline content, DC- Disease control, STD – Standard (Silver colloidal gel), GOCB 1- Gel of *Carica papaya* latex 2.5% and *Boerhevia diffusa Linn* 5%, GOCB 2-Gel of *Carica papaya* latex 5% and *Boerhevia diffusa Linn* 10%

All STD, GOCB -1,GOCB-2 treated groups showed more amount of HPC than that of disease control group. STD treated group shows higher amount of HPC than that other groups.

3.3.1.3 Histopathology

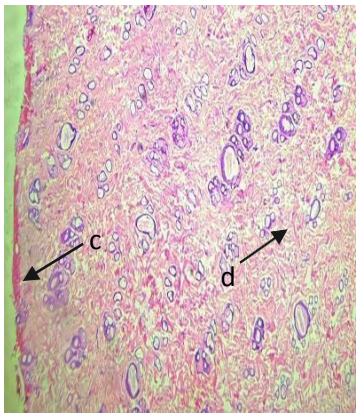


Figure 6.a DC

Figure 6.b STD

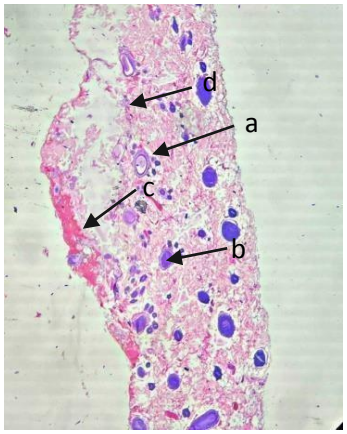


Figure 6.c GOCB-1

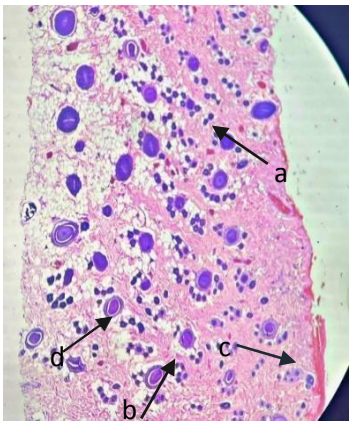


Figure 6 .d GOCB-2

Figure 6 : **Histopathological investigation of DC, STD, GOCB-1 and GOCB-2 groups**

Note –DC- Disease control, STD – Standard (Silver colloidal gel), GOCB 1- Gel of *Carica papaya* latex 2.5% and *Boerhevia diffusa Linn* 5%, GOCB 2-Gel of *Carica papaya* latex 5% and *Boerhevia diffusa Linn* 10%

Histological reports of STD, GOCB -1,GOCB-2 treated groups showed significant wound healing i.e. tissue granulation (a), new blood vesicles (b), fibroblast cells (c), and collagen fibres (d) than DC group.

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Table 7 Evaluation parameters of Histopath

Sr no.	**Histopath evaluation Parameters**	**DC**	**STD**	**GOCB - 1**	**GOCB- 2**
1	Tissue granulation (a)	_	+	++	++
2	Neovascularization (b)	_	+	++	+++
3	Fibroblast Proliferation (c)	+	+++	++	+++
4	Collagen Deposition (d)	+	+++	++	++

Note - DC- Disease control, STD – Standard (Silver colloidal gel), GOCB 1- Gel of Carica papaya latex 2.5% and Boerhevia diffusa Linn 5%, GOCB 2- Gel of Carica papaya latex 5% and Boerhevia diffusa Linn 10%

3.3.2 . Linear Incision wound

3.3.2.1 Tensile strength

TENSILE STRENGTH OF LINEAR INCISION WOUND MODEL

Table 8. Tensile strength of linear incision wound model

GROUPS	STATISTICAL MEAN
DC	215.41± 0.35
STD(colloidal silvergel)	453.54± 0.65***
GOCB 1	390.62± 1.08***
GOCB 2	431.04± 1.42***

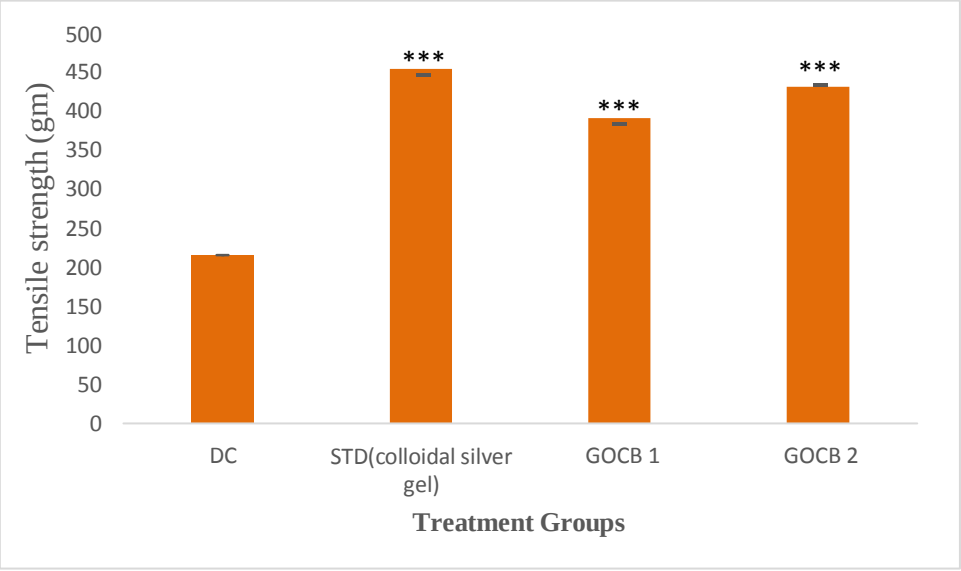

Figure 6. Tensile strength of linear incision wound model

Note - DC- Disease control, STD – Standard (Silver colloidal gel), GOCB 1- Gel of Carica papaya latex 2.5% and Boerhevia diffusa Linn 5%, GOCB 2- Gel of Carica papaya latex 5% and Boerhevia diffusa Linn 10%

Values are expressed as mean± SEM; n = 6; Data analyzed by One-way ANOVA test followedby Tukey's

multiple tests for comparison. #p<0.05, as compared to C; *p<0.05, **p<0.01

Results of linear incision model of wound healing shows in table .Stiches of the linear incision wound. Tensile strength is the main evaluation parameter for linear excision wound. STD, GOCB- 1, and GOCB -2 treated groups show significant tensile strength than that of control group STD treated group shows more tensile strength than that of other groups.

3. DISSCUSION –

Wound is the damage to the skin tissue. If it is not treated in well manner it leads to infection .Healing of wound is followed by the various events like inflammation, cell migration and proliferation, tissue granulation, angiogenesis, collagen synthesis, re-epithelization fibre formation is occurring due to the aggregation of fibroblast, platelets, collagen and neovascularization.[24]

In ayurveda diverse variety of herbal plants are used for wound healing. Herbal plants possess various chemical constituents mainly secondary metabolites like Flavonoid, alkaloid, glycosides, tannins and other which have therapeutic value. According, to earlier literature study *Carica papaya* latex and *Boerhevia diffusa Linn*. Leaves possess antibacterial, as well as antioxidant activity.[25]

Carica Papaya latex contain the papain which is the flavonoid have antimicrobial,anti-inflammatory, antifungal, antioxidant activities which are responsible for wound healing. Antioxidant activity of the papain is helpful to reduce the oxidative stress which helps in wound healing. [26]

Boerhevia diffusa Linn contain the Punarnavine which is alkaloid it is reported to have antimicrobial activity it also contains flavonoid that posse's antioxidant activity.Therefore combination of the antimicrobe of the *Carica papaya* latex and *Boerhevia diffusa* leadsto the better wound healing.[27]

Circular excision wound model in present study is widely used to check the wound contraction activity of the drug. As mentioned above antibacterial activity of the *Carica papaya latex and Boerhevia diffusa Linn* leaves helps wound to stay away from microbial infection while their antioxidantactivity reduces the oxidative stress at the site wound.
Therefore, we can propose that combineaction of *Carica papaya latex and Boerhevia diffusa*

Linn can leads to the better wound contraction in circular excision model. The result of excision model shows significant increase in wound contraction from 4th days after wound contraction. Both the test gel (*Carica papayalatex* 2.5% and 5%) and (*Boerhevia diffusa Linn* leaves 5%,10%) of plant extract and standard

treated group shows very fine wound contraction ability which increase wound healing as compare to disease control. The complete wound closure of Disease control occurs in 23 days, Standard in 20days, GOCB-1 in 18days, GOCB-2 in 17days respectively.

Linear incision wound model was used to determine the tensile strength of regenerated tissue. The aggregation of the collagen and fibroblast increase the strength of the tissue. Antioxidant and antibacterial activity of *Carica papaya* is due to the papain which is the flavonoid and *Boerhevia diffusa linn* is due to the punarnavine which is alkaloid which helps to increase collagen synthesis. Gel of *Carica papaya latex* and *Boerhevia diffusa linn* increase the tensile strength as compare to disease control group.[28]

Hydroxyproline is the acid involve in the formation of the collagen. Increase level of hydroxyproline amino increase the production of the collagen. In the study it is found that the regenerated tissue of the wound has increase hydroxyproline level in standard, GOCB-1 and GOCB-2 group of circular excision model as compare to disease control group.[29]

In a histological analysis of the skin, regeneration of the epidermis, proliferation of fibroblastic and mononuclear cells, deposition of collagen fibres, lack of ulceration. Absence of necrotic cells, and inflammatory cells are signs of successful wound healing. The histological analysis of the tissue samples revealed enhanced skin regeneration, with results for all of the aforementionedcriteria in the circular excision model being positive.[30] According to the study, flavonoids and alkaloids may be the responsible necrotic phytoconstituents, and the antioxidant and antibacterial properties of leaves from *carica papaya* and *boerhevia diffusa linn* may be the primary mode of action for wound healing. Carica papaya and Boerhevia diffusa leaves also have anti-inflammatory properties, which may promote wound healing by lowering inflammation in the vicinity of the lesion. The combined effects of these activities and the presence of phytochemicals in *carica papaya latex* and *boerhevia diffusa linn* leaves have been discovered to be advantageous for wound healing activities.

4. CONCLUSION –

The research study gives the idea about the combined potential of *Carica papaya* latex and *Boerhevia diffusa* leaves extract in wound healing activity. The main highlight of this research is for the first-time wound healing potential of this combination is checked. Gel of this herbal plant promotes great wound concentration by accelerating angiogenesis, enhancing epithelization and increase collagen deposition, this gel might be used in future clinical application animal study gives the valuable information, it can be helpful to add one more drug combination in treatment of wound infection.

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