Research Article ISSN: 2349-2678

Contents lists available at www.ijpba.in

International Journal of Pharmaceutical and Biological Science Archive

NLM (National Library of Medicine ID: 101738825)

Index Copernicus Value 2019: 71.05

Volume 11 Issue 4; July-August; 2023; Page No. 01-11

Pharmacological Evaluation of *Bauhinia Acuminata* Leaf extract For Antipyretic and Wound Healing Activity.

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Article Info: Received: 20-04-2023 / Revised: 02-05-2023 / Accepted: 15-06-2023

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Conflict of interest statement: No conflict of interest

Abstract

The methanolic extract is rich in tannin, glycoside, phenol, flavonoid, steroid and saponins etc. The dried leaf's methanolic extract exhibit significant quantities of flavonoid, tannin, phenols, saponins, steroid and glycosides. *Bauhinia acuminata* methanolic leaves extract was studied for phytochemical characterisation and in-vivo antipyretic and wound healing activity.*Bauhinia acuminata* traditionally used to treat different type of disease like skin infection, diabetes mellitus, cancer respiratory infection &acute and chronic pain.Because there were no scientific studies on the uses of *Bauhinia acuminata* for antipyretic and wound healing activity, the current study was undertaken to test the effects of a methanolic extract.In wistar rats, antipyretic efficacy was assessed using brewer's yeast doses (250 mg/kg and 500 mg/kg), and wound healing activity was assessed using an excision test using methanolic extracts at two different dose levels (250 mg/kg oral and 5% ointment topically). The antipyretic effect started as early as 1h and the effect was maintained for 4h, after its administration. The standard drug paracetamol 150mg/kg and tested drug *Bauhinia acuminata* leavesextract were significantly reduced the yeast elevated rectal temperature at 2nd, 3rd and 4th hour compared to control group. The wound healing effect started as day 0 and the effect was maintained after it's orally & topically administration in rats. The standard drug povidone iodide and test drug *Bauhinia acuminata* leaves extract ssignificantly decrease in period of wound contraction was observed in all the treatment groups when compared to control.

Keywords: Phytochemical, Antipyretic, Wound Healing

Introduction

Herbal medicines even though unrecorded in science, are being used in some developing countries. About 80% of population in some developing countries rely on traditional folk medicines [1]. Traditionally used to treat skin infection, diabetes, cancer, acute and chronic pain, respiratory disease [2]. The leaves are a rich source of various beneficial compounds, including flavonoid, steroid, glycoside lignin, kaempferol, phenol quercetin and tannins more [3]. *Bauhinia acuminata* Linn is widely cultivated for their ornamental values require very less space to grow. The species occurs widely in deciduous forests and scrub. *Bauhinia acuminata* Linn is scattered tropical to subtropical & warm temperature Asia, and

tropical regions of Africa and America. *Bauhinia acuminata* Linn leaves found that the upper Miocene Xiaalongtan deposits from China, Wenshan, and Southeast Yunnan [4]. *Bauhinia acuminata* L grow to 2-3 meters tall. *Bauhinia* species, leaves are shaped like ox hoof and bilobed, 6-15 cm long and broad, with the apical cleft up to 50mm deep, and petiole is 15mm to 40mm long. The fruit pod is 7.5-15 cm in length and 1.5-1.8cm wide. The flowers are aromatic, with 5 white petals, green stigma and a 10 yellow tipped stamen and the diameter across 8-12cm [5]. The chemical compound of flavonoids shows the more antipyretic & wound healing activity. Hence the present research work was aimed to evaluate antipyretic and wound healing activity methanolic extract of *Bauhinia acuminata* Linn.

Pharmacological Activity

- **Antioxidant Activity**
- **Cytotoxicity Activity**
- **Antinociceptive Activity**
- **Antidiarrheal Activity**
- **Anti-diabetic Activity**
- **Membrane Stabilizing Agent**
- **Antibacterial Activity**
- **Anti-inflammatory**
- **Antimicrobial Activity**
- **Antifungal Activityy**

1. Material and methods

2.1.Chemical and drug

CMC (Carboxymethyl cellulose), petroleum jelly was used in the study, and PCM (Paracetamol), povidone iodide used as a standard drug.

2.2. Collection and authentication of plant material

The fresh leaves of *Bauhinia acuminata* Linn. Were collected in the month of November from Dineshpur, District (Udham Singh Nagar) Uttarakhand. The botanical identity of plant specimen (No.118883) was authenticated by Dr. Ambrish Kumar (Scientist-D) Botanical Survey of India, Dehradun. A voucher specimen of the leaf has been deposited in the department for future reference. The leaves were thoroughly cleaned and shade dried properly. The leaves were coarsely powdered and further utilized for preparation of methanolic extracts.

2.3. Preparation of methanolic extract

The leaves were washed with water, shade dried at room temperature for a period of 24 day for proper removal of moisture. The plant material converted to coarse powder and properly stored in an air-tight container before extraction. A know amount of sample was extracted by soxhlation method using 95% methanol. Further the extract has been concentrated by using rotary vacuum evaporator to obtain the crude extract. After complete removal of the solvent, the extract (MEBA) was obtained, stored in air tight container at 4ºC till further.

Figure 1.1: *Bauhinia acuminata* Figure 2.1: Soxhlet extraction
Picture taken by oppo F1, 8 mega pixels from Dineshpur (Rudrapur)

2.4. Formulation of ointment

The formulation was crafted by combining a plant extract with a petroleum jelly base. Additionally, another formulation was created by blending the same plant extract with an ointment base.

For the first formulation, the whitest soft petroleum jelly wasmelted, and then meticulously mixed with plant extract using a triturator at a concentration of 5%. Similarly, for the second formulation, the white soft petroleum jelly was melted, and after that, it was combined with the plant extract and triturated at a concentration of 5%.

The evaluation parameters were performed for both formulations [6].

Table 2.4: Composition of formulations

Ingredients	Formulation 1 (5%)
Petroleum jelly	95 gm
Extract	5gm

2.5. Evaluation of ointment

2.5.1. Appearance

Appearance was evaluated by colour, odour and smoothness.

2.5.2. pH

a. The ointment was analyses by using pH meter.

b. The pH meter was calibrated by using standard buffer solution.

1gm of ointment was diluted in CMC solution and then pH meter was immersed incontainer. Reading was taken [7].

2.5.3. Spreadability

a. Spreadability is a term to express the donator extends of area to which the ointment radially spread on application to skin or affected part.

b. It expressed in terms of time in seconds taken by two slides from to slip off from the ointment.

c. Lesser the time taken for separation of two slides, results the better Spreadability.

d. It was calculated by using the formula (S= M.L. / T.), where, Spreadability, M- weight tied to upper slid, L-length of glass slide, T-time taken to separate the completely from each other [6].

2.5.4. Extrudability

a. The formulation was filled in collapsible tube.

b. The extrudability of different formulation was determined in terms of weight in grams to require extrude an ointment in 10 seconds [8].

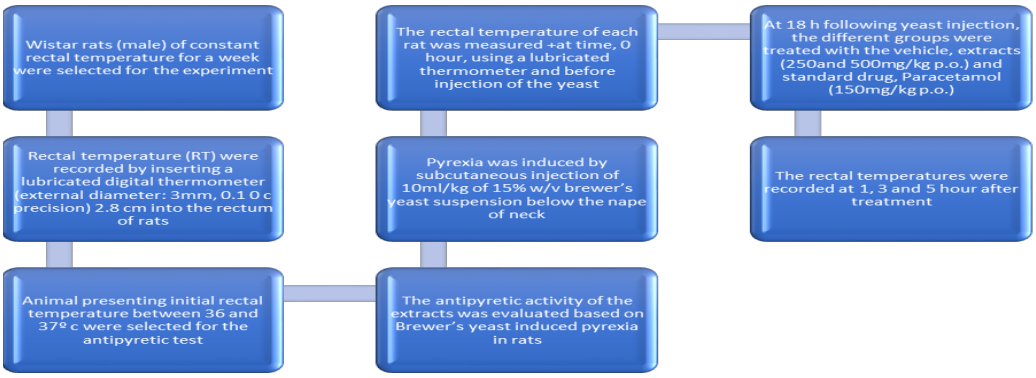
2.6. Experimental animals

Animals Wistar rats (150-200gm) weighing 150-200gm were housed under laboratory condition in animal facility at relative humidity 75 ± 5% temperature 22 ± 2° C, and a 12 h light/dark cycle. The animal had free access to water and food ad libitum. Animals were allowed to acclimatize to the environment for seven days before start of the experiment. The experimental protocol was approved by Institutional Animal Ethics Committee. All the animal experimental procedures were performed according to the National Institutes of Health (NIH) guidelines on handling of experimental animals.

2.6.1. Antipyretic activity

The antipyretic activity of methanolic extract activity was evaluated using the brewer's yeast induced pyrexia method. Prior to conducting the study, acute toxicity tests were performed to determine safe dosage levels, and subsequently, one-fifth and one-tenth of the established safe doses were selected. The animal used for testing the antipyretic activity was Brewer's yeast induced pyrexia.

2.6.2. Brewer's yeast induced pyrexia method.



Percentage reduction in rectal temperature = Y-X X 100 Y-Z

Where Z = Initial rectal temperature ºC, Y = Rectal temperature 18 hour after yeast administration, X =Rectal temperature after extract administration [9].

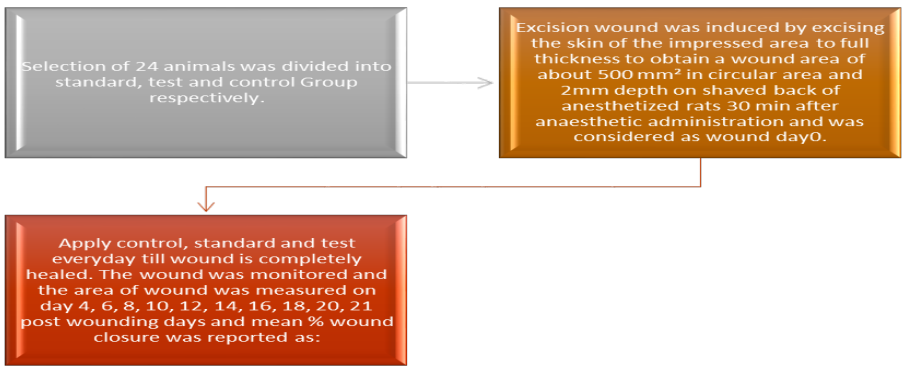
Table 2.6.2: Animal composition of groups

S.NO.	Treatment Groups	Dosing
1	Control	0.5%w/v CMC
2	Standard	150 mg/kg PCM
3	MEBA Test 1	250 mg/kg
4	MEBA Test 2	500 mg/kg

2.7. Wound healing

The wound healing activity was evaluated using the Excision wound method. The dosage for the experiment was determined based on the results of acute toxicity studies, specifically 1/5 & 1/10th of the standard dose. The methanolic extract's wound healing potential was assessed in animal models, specifically through the excision wound model. The selection of one-fifth and one-tenth doses of methanolic extracts was informed by outcomes of the acute toxicity study.

2.7.1. Excision wound



[% wound closure= wound area on day 0-wound area on day n/ Wound area on day 0 X100][10].

Table 2.7.1: Animal composition of groups

S.NO.	Treatment of Groups	Dosing
1	Control	0.5% w/v CMC
2	Standard	Povidone iodide
3	MEBA (Oral)	250 mg/kg
4	MEBA (Topical)	5% ointment
5	MEBA (Oral, Topical)	250mg/kg, (5% ointment)

2.8. Physical Evaluation

2.8.1. Wound size measurement

Any contraction in wound size was measured by tracing the wounded margin on a tracing paper and calculated percentage reduction in wound area post treatment area of the wound at day zero was considered as 100% for the calculations of percentage reduction in wound area. The percentage wound contraction was calculated using the following equation-

%Wound contraction= Healed area / Total area x 100.

3. Results

3.1. Leaf powder of Bauhinia acuminata Lwas extracted with the help of soxhlation apparatus using methanol as solvent.

Table 3.1: Extraction requirement for Bauhinia acuminata L

Plant	Solvent	Temperature	Amount of powder	Amount of extract
Bauhinia acuminata L.	Methanol	60°C	100 gm	15.2 gm

3.2. Effect of Pyrexia

Table 3.2: Effect of Bauhinia acuminata L leaves extracts on pyrexia

Plant	Solvent	Temperature	Amount of powder	Amount of extract
Bauhinia acuminata L.	Methanol	60°C	100 gm	15.2 gm

3.2. Effect of Pyrexia

Table 3.2:Effect of *Bauhinia acuminata* L leaves extracts on pyrexia

Groups	Dose	Time					
		0 hours	18 hours	1 hours	2 hours	3 hours	4 hours
Control	0.5% w/v CMC	35.683±0.1169	38.999±0.000483	38.998±0.00081	38.992±0.0034	38.573±0.0193	38.212±0.0064
Standard	150mg/kg PCM	35.65±0.10**	38.999±0.00054***	38.997±0.000816***	37.989±0.00116***	37.621±0.030***	37.367±0.046***
Test 1	250mg/kg MEBA	35.667±0.1211*	38.98±0.00053***	38.999±0.00054**	38.998±0.00089**	37.881±0.010**	37.478±0.0062**
Test 2	500mg/kg MEBA	35.917±0.1262**	38.998±0.00048***	38.998±0.00075***	37.998±0.000894***	37.683±0.0068***	37.398±0.0408***

Antipyretic activity values are expressed as mean ± SEM (n=6), data was analyzed by one way analysis of variance (ANOVA) followed by (Tukey's multiple comparisons test) P<0.001***; P<0.001*** compared with the control group.

Effect of the extract on brewer's yeast suspension induce pyrexia

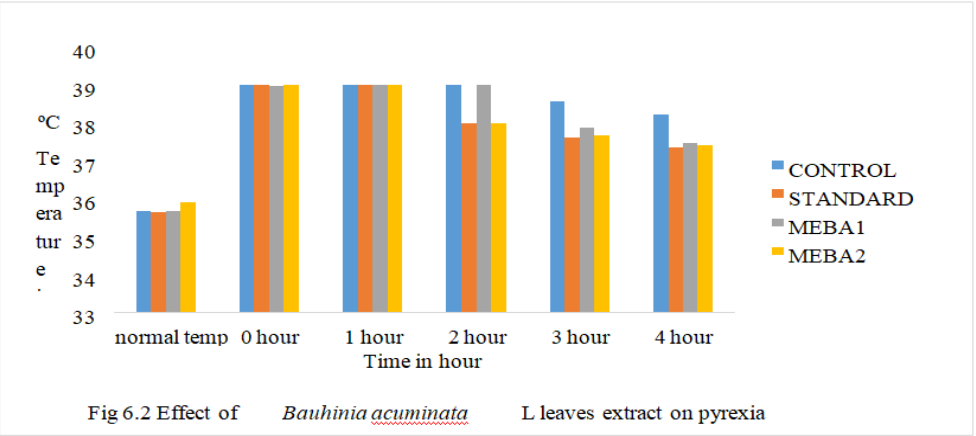


Fig 6.2 Effect of *Bauhinia acuminata* L leaves extract on pyrexia

Standard drug Paracetamol 150 mg/kg and both the test drugs (T1 and T2) orally showed statistically significant (P<0.001) difference in 1hr, 2hr and 3hr respectively.

3.3. Evaluation of ointment

3.3.1. Appearance

a. Appearance of the ointment was dark greenish due to presence of leaf extract.
b. Odor of all formulation was pungent.

3.3.2. pH

a. The ointment was analysed by using pH meter.
b. The pH was found to be 5.32 and 5.45 which is considerable.
c. This will not cause any skin irritation**.**

Table 3.3.2: pH of ointments

Formulation	pH
F1	5.32
F2	5.45

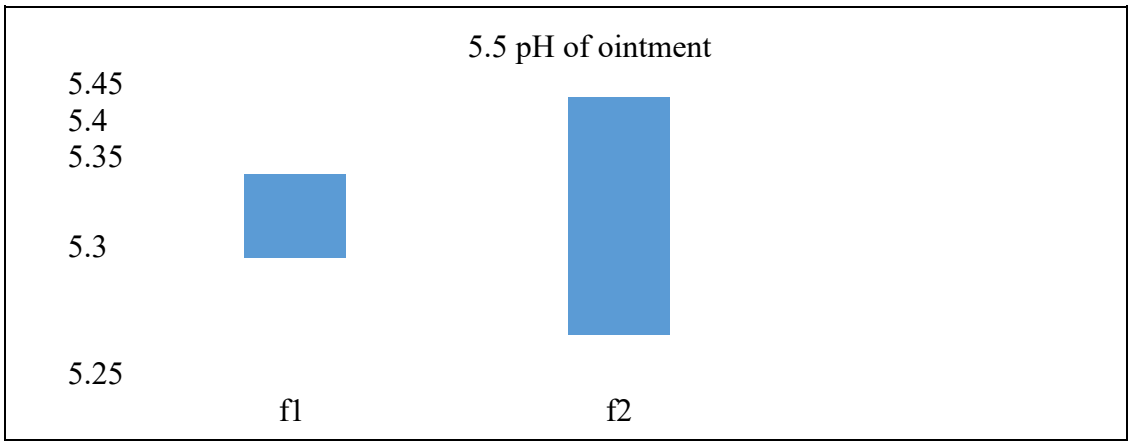


Figure 3.3.2: pH of ointment

3.3.3. Spreadability

a. Spreadability of ointment was tested to evaluate the ease of application of ointment on skin.
b. Spreadability of an ointment increase with decrease in viscosity.
c. Both formulations showed good Spreadability because they take lesser time so spread to the skin.

Table 3.3.3: Spreadability of ointments

Formulation	Spreadability (sec)
F1	16
F2	16

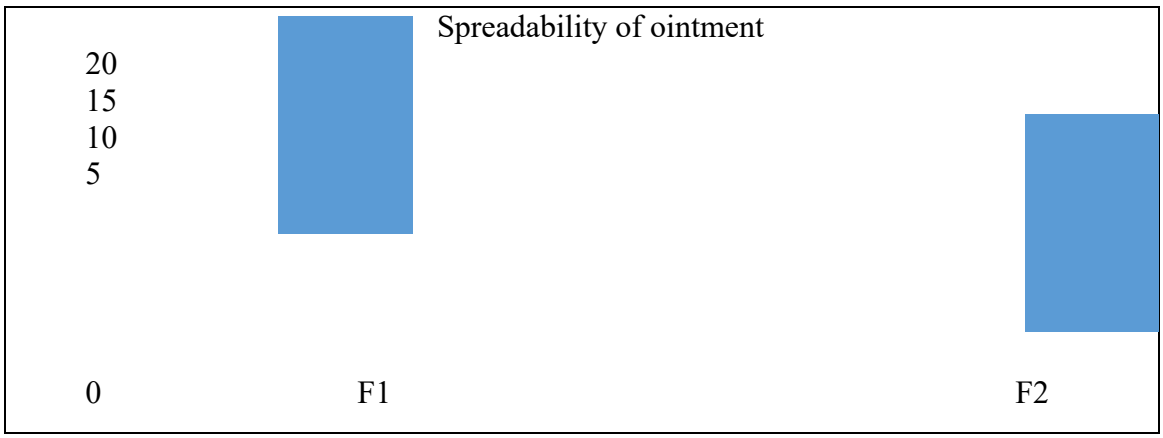


Figure 3.3.3: Spreadability of ointment

3.3.4. Extrudability

a. The formulations were filled in collapsible tube.

b. The extrudability of each formulation was determined in terms of weight in grams to require extrude an ointment in 10 seconds.

Table 3.3.4: Extrudability of ointments

Formulation	Extrudability (gm)
F1	25
F2	27

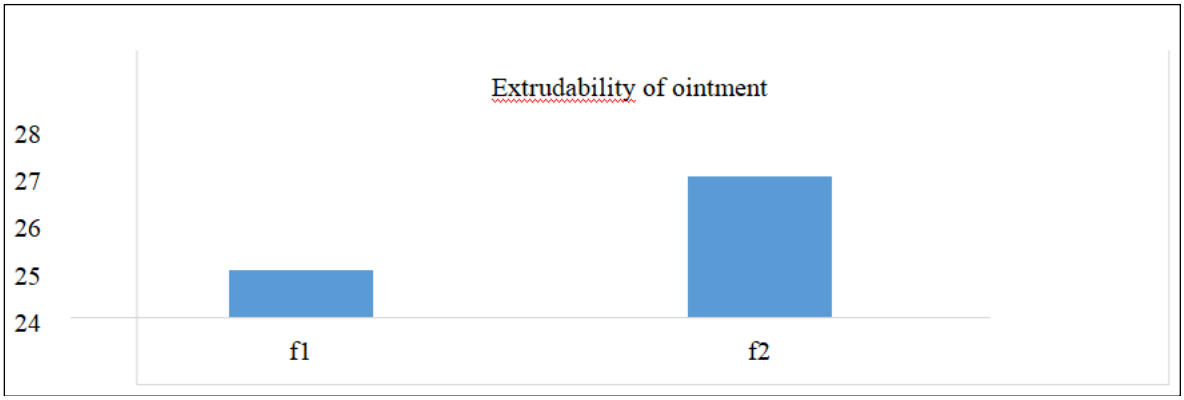


Figure 3.3.4: Extrudability of ointment

Table 3.4: Effect of Wound

S. No.	Treatment group	Period of epithelization (mean±SEM)			
		Day 0	Day 4	Day 6	Day8
1	Control	1.417±0.556	1.433 ±0.56	1.41±0.55	1.31±0.55
2	Standard	1.400±0.70***	0.983±0.60***	0.516±0.325***	0.266±0.242***
3	Test 1 (oral)	1.633± 0.3077*	1.450±0.459**	1.360.48**	1.25±0.45*
4	Test 2 (topical)	11.850 ±0.3937*	1.683±0.617**	1.75±0.393**	1.58±0.386**
5	Test 3 (oral /topical)	1.567±377**	1.567±0.377**	1.467±0.377**	1367±0.37***

Table 3.4: Effect of *Bauhinia acuminata* L extract ointment and standard drug on wound healing.

S.No.	Treatment group	Period of epithelization (mean ±SEM)				
		Day10	Day12	Day14	Day16	Day 18
1	Control	0.933±0.3724	0.75 ±0.38	0.46±0.20	0.31±0.17	0.08±0.07
2	Standard	0.0000±0.00	0.0±0.00	0.0±0.0	0.00±0.00	0.00±0.00
3	Test 1 (oral)	1.033± 0.186**	0.66±0.19***	036±0.12**	0.25±0.05***	0.00±0.00
4	Test 2 (topical)	1.267 ±0.242***	0.90±0.14***	0.63±0.12***	0.25±0.05**	0.00±0.00
5	Test 3 (oral /topical)	1.167±0.3***	1.03±0.27***	0.75±0.21***	0.08±0.03**	0.00±0.00

Values are mean ± SEM (n = 6) statically significant difference in comparison with control group: P < 0.05* Once a day, for 18 day; control, MEBA (Oral dose 250 mg/kg), MEBA (Tropical dose 5% ointment) MEBA (Oral/Tropical dose 500 mg/kg, 5% ointment) treatment considered with statistically significant using one way ANOVA test.

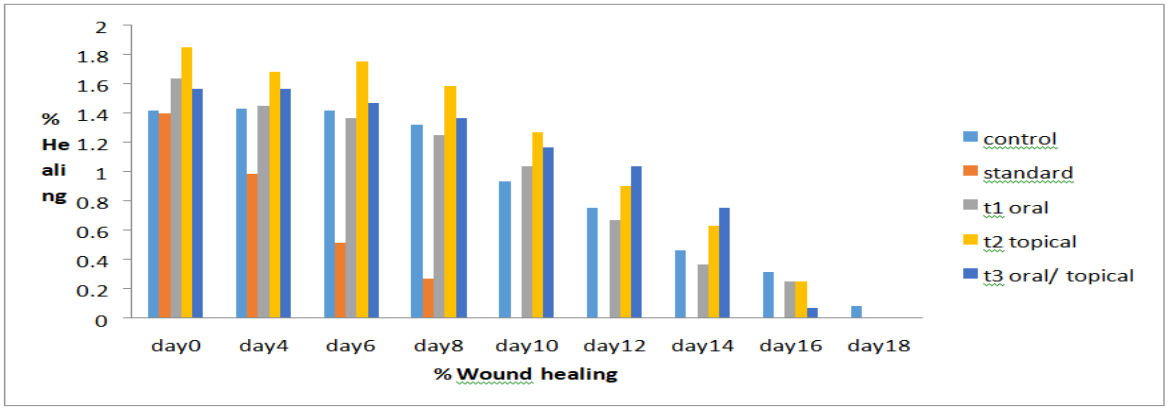


Figure 3.4: Effect of *Bauhinia acuminata* L leaves extract on wound healing

Day	Control	Standard	Oral dose	Topical	Oral / topical
0					
4					
6					
8					
10					
12					
14					
16					
18					
20					

Figure 3.4: Physical estimation of wound healing at various time intervals in control, standard, T1 (Oral), T2 (Topical), T3 (Oral / Topical)

Discussion

Despite the current accessibility of medication regimens for managing antipyretic and wound healing action, they come with specific disadvantages. Consequently, there is a pressing demand for the development of safer and more effective drugs to address these issues. In light of the drawbacks associated with the presently available drug regimens, it becomes imperative to explore and create novel therapeutic options that can enhance the treatment outcomes for antipyretic and wound healing activities. The chemical constituent of flavonoid, tannins, phenol, glycoside, triterpenoid, saponins, carbohydrate, steroids are more responsible for antipyretic and wound healing activity. The use of traditional medicine and medicinal plant in most developing countries, as a normative basis for the maintenance of good health has been widely observed.

The study indicated that Bauhinia acuminata Lleaves extract has both antipyretic as well as wound healing properties. Administration of Brewer's yeast produced an increase in the body temperature of the rats from normal (35.9 ± 0.126ºC) to 38.9 ± 0.0004ºC within 18 hours of yeast injection. In Brewer's yeast induced pyrexia method increase in temperature was 38.9 ± 0.0004ºC. MEBA (250 mg/kg, 500 mg/kg) and paracetamol significantly decrease body temperature to 38.9 ± 0.005ºC, 37.62 ± 0.03ºC and 37.367 ± 0.05ºC, ($p < 0.001$) compared with that of control respectively after 4 h. 250 mg/kg MEBA significantly ($p < 0.001$) and 500 mg/kg ($p < 0.001$) decrease the temperature compared to control. However, the standard drug, paracetamol 150 mg/kg demonstrated the excellent antipyretic activity ($p < 0.001$) compared with that of control. Paracetamol possesses potent antipyretic activities. It may selectively inhibit specific prostaglandin synthesis to achieve its antipyretic effect but does not influence body temperature when it is elevated by factors such as exercise or increase in ambient temperature. Certain phytochemical compounds such as steroids, carbohydrates, tannins, triterpenoids, flavonoid and coumarin glycosides were found to be present in the extract during phytochemical screening. The antipyretic potentials of steroids, tannins, triterpenoids, flavonoid and coumarin glycosides have been reported in various studies. Therefore, the antipyretic activity of MEBA may be due to its contents of steroids, tannins, triterpenoids, flavonoid and coumarin glycosides. Furthermore, indirect evidence seems to support the influence of MEBA on the biosynthesis of prostaglandin (PGE2) which is a regulator of body temperature this may also partly account for its antipyretic activity in yeast-induced pyrexia model. The antipyretic activity of the extract was determined from yeast induce pyrexia method. The leaves extract of Bauhinia acuminata L revealed marked antipyretic activity in brewer's yeast induced pyrexia in rats in the present study the initial rise of temperature after 18 hours of subcutaneous yeast injection was more than 1º C. There was significant difference between the initial mean basal temperature of the different groups and the mean temperature between the groups of pyrexia rats, after 18 hours of yeast injection. Rectal temperature of pyrexia rats was lowered significantly with the test drug and standard drug when compared with control group. Standard drug Paracetamol is more potent than the lower dose of MEBA (250 mg/kg) but higher dose level of MEBA (500mg/kg) is more potent than standard drug. No toxicity was seen in the test drugs treated groups after 24 hours of experiment so the test drug is a safe antipyretic agent.

The present study is based on comparative analysis of the standard povidone iodine ointment and the leave extract ointment of Bauhinia acuminata L in one concentration, for wound healing activity in rats. Administration of 250 mg/kg

MEBA significantly ($p< 0.05$), tropically 5% ointment MEBA significantly ($p<0.001$) and orally/ tropically significantly (0.001) decrease in period of epithelization. However, the standard drug, Povidone iodine 5% ointment demonstrated the excellent wound healing activity ($p < 0.001$) compared with that of control. The wound healing property of methanolic crude extract of Bauhinia acuminata L leaves was may be due to presence of flavonoid, Chrysophanol in leaves extract of Bauhinia acuminata L. The parameters studied included rate of wound concentration and the period of epithelialization in excision wound model.The effect was evident by the decrease in period of epithelialization, increase in the rate of wound concentration, skin breaking strength, granulation tissue dry weight content and breaking strength of granulation tissue. Histopathological study of thegranulation tissue showed increased collagenation when compared to control group of animals. Wound healing method was performed by wistar rats. Wound was created on the back of rat skin with the help of biopsy punch. The standard drug group was treated with standard povidone iodine ointment and test drug groups were treated with oral test dose (250 mg/kg) and topical dose (F1 5% ointment). The effect of wound healing activity in this method was evaluated by determining the % wound concentration and epithelization period (mean±SEM). The studies on excision wound healing method stated that all groups showed decreased wound area from day 1 to day 21. The % wound concentration of excision wound treated with (oral and topical) found to be significantly higher as well as promote more epithelization than the standard ointment. Wound treated with (oral, topical) healed faster and statistically significant as compared to standard and F1.

Conclusion

The methanolic extract of Bauhinia acuminata L leaves exhibited significant antipyretic and wound healing activity at different levels in all the methods used in the study and the results support the use of Bauhinia acuminata Lleaves for the various pyrexia conditions and as and wound in human health. However, further studies need to be done to identify the actual mechanism of action for both the activities. And the whole plant could be further researched for determining the other pharmacological activities, which could serve the human health in future.

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