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Topical Anti-Inflammatory and Anti-Bacterial Activity of *Viola Betonicifolia* Cream in Animal Models

Muhammad **Sajid**, Naveed **Muhammad**, Omer **Shehzad**, Haroon **Badshah***

Department of Pharmacy, Abdul Wali Khan University, Mardan, Pakistan

Abstract:

Background: Viola Betonicifolia is a medicinal plant used locally for various ailments, such as inflammatory and painful conditions.

Objectives: Owing to its traditional uses, pharmaceutical dosage form as Viola Betonicifolia creams (VBC) were formulated from its aqueous extract with different concentrations (1, 3 and 10%). Creams were tested for anti-inflammatory and wound healing activity using paw inflammation edema, neck burning and leg surgical blade cut experimental models. Further the VBC was also subjected for in-vitro antibacterial activity.

Methods: Heated 18 mm metallic coin was used for both induction of edema in the paw and burning of neck of the healthy rabbits. Similarly, anesthetized leg was given a measurable cut with surgical blade for wound healing activity. Inflammation, burn and wounds were treated with VBC in the different concentrations. The antibacterial effect against Staphylococcusaureus, Bacillus pumilus, Pseudomonas aeruginosa and Escherichia coliwas determined by Disc diffusion method.

Results: In comparison with negative control (distilled water treatment), VBC demonstrated significant reduction in the inflamed and burned areas of the paw and neck respectively, showing its potential anti-inflammatory effects. In addition, the VBC 3% exhibited a moderate antibacterial effect with average inhibition of the bacterial cultures compared to standard clarithromycin.

Conclusion: Taken together, VBC cream demonstrated considerable anti-inflammatory effect in paw edema and burn, wound healing effects on skin. Formulation of cream was stable on accelerated humidity and temperature. Therefore, it is suggested to use the formulated form of Viola Betonicifolia for treatment of burns, edema and other types of topical inflammatory disorders.

Keywords: : Viola Betonicifolia, Anti-inflammatory, Burns, Wound healing, Antibacterial.

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Introduction

Over the centuries different medicinal plants have been used for treatment of various ailments all over the world.

Human beings depend on the natural products for treatment of their diseases. 80 % people of the developing countries depends on the natural drugs derived from plants and animals as their primary health care [1].

The practice of the herbal medicines are increased because of cultural beliefs and tremendous biomedical benefits and easy availability [2].

*Voila betonicifolia*belongs to the family Violaceace. A number of pharmacologic activities have been determined for this plant and its potential antipyretic, astringent, diaphoretic, anticancer, febrifuge and purgative effects are determined [3].

Corresponding Author: Dr. Haroon Badshah,
Affiliation: Department of Pharmacy, Abdul Wali Khan University, Mardan, Pakistan
Email: haroonbacha87@yahoo.com

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*Voila betonicifolia*is also used as anti-epileptic and sedative in nervous disorders. Other uses are the treatment of sinusitis, skin and blood disorders and pharyngitis. Its roots are used for the treatment of kidney diseases, pneumonia and bronchitis while its flowers are used against lung troubles, cough and colds and the leaves are claimed to be effective against boils [4].Crude methanolic extract of *Voilabetonicifolia*were tested in mice for in-vivo prokinetic and laxative study [5]. The study demonstrated that methanolic extract of *Viola betonicifolia*shown to be effective in indigestion and constipation.

The pharmacologic activities of *Viola* species are due to the presence of very useful constituents i.e. cyclotides, alkaloids, flavonoids, caffeic acid derivatives, salicylic acid derivatives and triterpenoids etc[6]. Saponins, flavonoids, mucilages, salicylic acid derivatives, coumarines and carotenoids active principles are present in *Violatricolor* [6]. Flowers of *Viola biflora*are used as a astringent, antipyretic, febrifuge, anticancer, antiepileptic, and for nervous disorder [7]. Whole plant of *Viola canescens*is used as astringent, demulscent, purgative, diaphoretic, antipyretic, anticancer and febrifuge. Ethanol extract of *V. mandshurica*exerted anti-obesity effects in high fat diet induced obese mice [8]. Transdermal drug delivery system is effective as well as safe with good compliance over systemic treatment, as in later case toxicity or adverse effects are comparative higher in numbers [9]**.** Topical dosage forms are used locally for prompt effect as compared to oral rout which is passed through various portions of gastro intestine like stomach acids, first pass effects which can alter the chemical structure of the drug and ultimately the pharmacological effect of the drug [10]. Furthermore, the topical therapeutic drugs are safer than systemic use because such type of drugs cause gastric bleeding and ulcer. The potential anti-inflammatory and antinociceptive effects of *Voila betonicifolia*and related species [11, 12, 13] encouraged us to formulate the topical dosage form for the treatment of inflammation, burn and wound. As this plant is commonly used in rural areas of Pakistan for the treatment of various painful and inflammatory conditions [12], so the present study was aimed to evaluate the anti-inflammatory effect of the cream formulated of aqueous fraction of *Voila betonicifolia*.

Methodology

Chemicals

Chemicals and bacterial strains used for this study were officially donated by Ferozsons Laboratories Limited, KPK, Pakistan. All the analytical grade chemicals and bacterial strains are listed in the Supplementary Table 1.

Plant collection and preparation of aqueous extract

Viola betonicifolia whole plant was collected from the Swat (Odigram) Pakistan. The plant material (12 Kg) was washed thoroughly with fresh water to remove sand and dust .The cleaned plant was stored in ventilated room up to dryness. The dry whole plant was grinded with grinder and then the course powder was subjected for extraction.

The whole plant material was placed in plastic bucket for seven days. Distilled water was put in that pot. The material was stirred with cleaned glass stirrer twice daily. After seven days that material were filtered through muslin cloth into another cleaned container. The residue left over the muslin cloth were again placed in another pot along with distilled water for ten days then this materials were filtered through muslin cloth. The filtrate obtained was collected and it was placed for dryness. This dried material was the aqueous extract of *Viola betonicifolia*.

Formulation of cream

Cream was prepared in three different concentrations i.e. 1%, 3%, and 10% according to the previously known method with some modifications [44]. The crude extract of the *Viola betonicifolia*whole plant was passed through the mesh size no 60. Fine powder was obtained. Beaker containing purified water was placed on the heating plate. The *V. betonicifolia* crude extract of required quantity was put in the purified water and was dissolved in it. This beaker was placed on the heating plate and the temperature was raised up to 72 ℃ with continuous stirring. Then the cetomacrogole-1000 and cetostearyl alcohol was put in the heated water containing dissolved aqueous crude extract and the stirring was continued. Subsequently temperature of the mixture was cool down up to 50 ℃. In another beaker the propylene glycol was taken and Nipasept was dissolved in it and was poured in the mixture having temperature maintained at 50 ℃ with stirring. At last the paraffin liquid was poured in the mixture with stirring and the temperature was cool down to room

temperature. A smooth homogenous cream with soft consistency was obtained. The color of the cream was depend on the concentration of the cream as the concentration of crude extract was increased the brackishness was also increased. Quantities of ingredients for 1%, 3% and 10% are shown in Supplementary Table 2, 3 & 4, respectively.

Animals grouping and drug treatment

Rabbits were used for this activity. Rabbits of either sex were purchased from local market and brought to the pharmacology Laboratory for conducting experiments. Animals were screened for any type of diseases especially skin disorder. The healthy rabbits were allowed free movement in the laboratory. Fresh food and water were provided during day and night experimental work. A cleaned environment was provided to the experimental animals. A natural 12/12 hour dark and light along with a room temperature was provided. All the experimental procedures were approved by the local animal ethics committee of the Faculty of Life Sciences, Department of Pharmacy, Abdul Wali Khan University, Mardan, Pakistan (Approval number: Awkum/ORIC/05-008).

Animals were categorized for the experimental purpose as follows:

Group 1: This group was treated with distilled water, DW was applied TID.

Group 2: This group was treated with Cream base. Cream base was applied TID.

Group 3: This group was treated with 1% VBC TID.

Group 4: This group was treated with 3 % VBC TID.

Group 5: This group was treated with 10 % VBC TID.

Group 6: This group was treated with 1% silver sulphadiazine cream TID.

Anti-inflammatory activity

The anti-inflammatory effect of our prepared cream was conducted according to reported method with modifications [14,15]. Animals were divided in test groups as above.

Burn healing effects

The burn healing effect of our prepared cream was conducted according to reported method with slight modifications. Hair were removed from the neck with sharp scissor of each animal and very carefully shaved with surgical blade. The shaved area was locally anesthetized with lignocaine gel. Then lignocaine injection was used to anesthetize that area again. The neck of all selected group were burned with the help of heated coin of 18 mm diameter. The burned animals were treated in different groups as mentioned above.

Paw inflammation or edema

Hair were cut from the paw with sharp scissor of each animal and very carefully shaved with surgical blade. The shaved area was locally anesthetized with lignocaine gel. Then lignocaine injection was used to anesthetize that area again. The paw of all selected group were inflamed with the help of heated coin of 18 mm diameter. The inflamed animals were treated as mentioned in above groups.

The inflamed area was regularly measured with digital Vernier calipers. The percent anti-inflammatory effect was calculated with the following formula:

$$Percent\ Inhibition\ = \frac{Negative\ control - VBC}{Negative\ control} \times 100$$

Wound Healing effects

The hair from the leg area of rabbit was shaved with..the help of sharp scissor. Then it was shaved with sharp sterile blade. The shaved area was locally anesthetized with lignocaine gel. Then lignocaine injection was used to anesthetize that area again. A cut was given to that shaved area with surgical blade. Every cut given was approximately 25 mm in length at least. Then all the animals were treated with VBC 3 % BID and results were obtained.

<u>In vitro Study</u>

<u>Anti-microbial study of VBC 3% by Disc diffusion method</u>

Mueller Hinton agar medium is prepared by dissolving 38 g in 1000 ml of distilled water. It was heated gently up to boiling by swirling and dissolving the medium completely. Media was sterilized by autoclaving at 15 psi for 15 minutes. Media was cooled at 45-50°C. Then it was poured in sterile petri dishes 4 mm deep [16]. Microbial suspension/Inoculum is prepared[17]. Sterile swab is dipped in tube containing microbial suspension diluted to match with 0.5

McFarland turbidity standards (roughly equal to 150 million cells per ml). Swab was streaked on required surface of Mueller Hinton agar medium three times to make a complete lawn of bacterial suspension under the laminar flow hood [18]. It was leaven for 5 minutes to adjust inoculum in media at room temperature and lid was closed on it under laminar flow hood. After 5 minutes interval empty discs were placed with the help of sterilized forceps aseptically on media. 3 grams of VBC 3% was taken and dissolved in 10ml of distilled water. This dilution prepared from our 3% VBC was poured on that empty discs with the help of micro pipette in required quantity. Petri dishes were incubated for 24 hours at 37°c overnight. It was taken out from incubator and zones of inhibition made around the discs were measured in terms of diameter (mm) with the help of digital Vernier calipers. Then it was compared with standard.

Statistical analysis:

The data were analyzed, using Graph pad Prism 5. One way ANOVA followed by Dunnet test. It was applied to find the level of significance. P*<0.05 was consider as significant. Other significant values are expressed as p** <0.01 and p***<0.001.

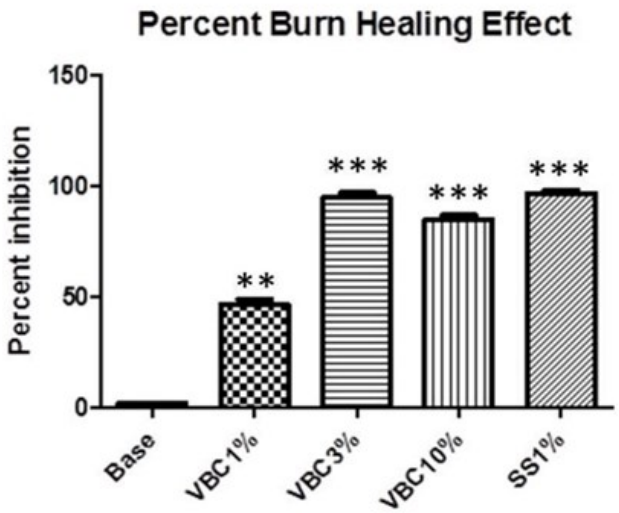
Results:

Anti-inflammatory effect of VBC on burn-induced inflammation

Cream prepared of different concentration i.e. 1%, 3% and 10% was applied on burn area of neck. Burn healing effect for VBC applied on the burn neck area of rabbit in TID dose was recorded for 5 days as shown in the Table 1.

Percent inhibition of inflamed area of neck at day 5 was measured to be 1.87 for base treated control group compared to the distill water treated group. The percent inhibition of inflamed area of neck at final day 5 in comparison with DW treated control group was 46.53, 95.99 and 84.74 for VBC 1%, 3% and 10% respectively. Burn healing effect of SS 1% cream as positive control was also determined with percent inhibition of 97.03. The burn healing effect of VBC 3% and 10% was found to be effective with good significant results (P<0.001). In fact, the strength of VBC 3% was found with more positive results. Graphical representation of percent inhibition of the burn effect of VBC is indicated in Figure 1.

Figure 1: Burn healing effect of base, VBC1%, VBC3%, VBC10% and SS1% compared to the control group following TID application.



Anti-Inflammatory effect of VBC on paw edema

The anti-inflammatory effect of VBC on the inflamed paw following its 1%, 3% and 10%, TID application was recorded for 5 days as shown in the Table 2. The percent anti-inflammatory effect at day 5 of, Base treated group, VBC 1%, VBC 3%, VBC 10% and SS1% was calculated to be 6.14, 93.18, 97.26, 58.82 and 97.95 respectively compared to the DW treatment as negative control group. Anti-inflammatory effect of VBC 1% and 3% for TID application was found more effective on day 4 and 5. Graphical representation of percent anti-inflammatory effect on paw edema of VBC is indicated in Figure 2.

Figure 2: Anti-inflammatory effect of base, VBC1%, VBC3%, VBC10% and SS1% compared to the control group following TID application.

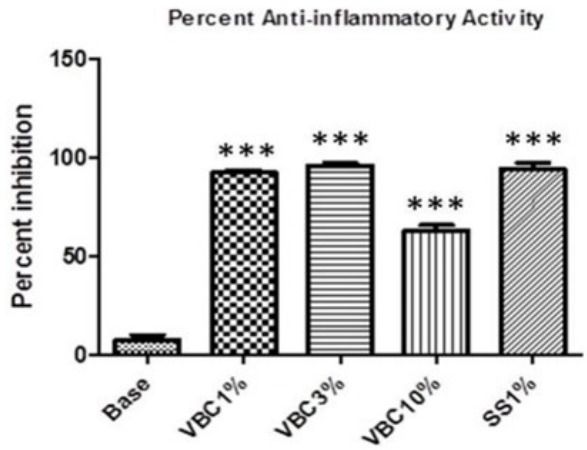


Table 1: Burn healing effect of VBC by TID application

Days	DW	Base	VBC 1%	VBC 3%	VBC 10%	SS1%
1	17.88 ±1.22	17.95 ±2.43	17.14 ±2.31 NS	16.24 ±2.64 NS	17.80± 2.42 NS	18.28 ±2.32 NS
2	17.50 ±1.12	17.45 ±2.63	13.77 ±2.31 NS	11.65± 2.31 NS	14.00±2. 43 NS	6.45 ±2.87***
3	17.30 ±1.24	17.25 ±2.37	11.45 ±2.14 NS	6.23±2. 54**	10.00±2. 52 NS	2.12 ±2.47***
4	17.15 ±1.42	16.89 ±2.56	10.10 ±2.15 NS	2.56±2. 43***	6.23±2.6 4**	0.64 ±2.18***
5	16.85 ±0.23	16.55 ±2.33	9.01 ±2.15 NS	0.67 ±2.87** *	2.35 ±2.21***	0.50 ±1.62***

Table 2: Anti-inflammatory effect of VBC by TID application

Days	DW	Base	VBC 1%	VBC 3%	VBC 10%	SS1%
1	18.14 ±1.25	18.88 ±2.31	16.43 ±2.35 NS	15.23 ±2.43 NS	16.25 ±2.34 NS	11.54 ±2.37***
2	16.27 ±1.37	16.47 ±2.30	12.90 ±2.41 NS	9.64 ±2.39 NS	13.01 ±3.39 NS	3.00 ±2.36***
3	15.33 ±1.20	15.30 ±2.39	7.05 ±2.39*	5.70 ±2.41 **	11.00 ±2.36 NS	1.43 ±2.55***
4	14.93 ±1.12	14.21 ±2.30	3.90 ±3.41**	2.00 ±2.43 ***	8.03 ±3.22 *	0.84 ±1.48***
5	14.67 ±1.30	13.77 ±2.44	1.00 ±2.32** *	0.40 ±2.59 ***	6.04 ±2.31 **	0.30 ±1.02***

Table 3: Wound healing effect of VBC 3% for TID application (Width wise reduction)

Days	DW	Base	VBC 3%
1	8.16±1.7 6	8.72±2.67	10.92±3.76**
2	7.00±1.6 7	8.29±2.54	3.99±3.20***
3	6.85±1.4 5	7.99±2.89	1.79±2.98***
4	6.25±1.8 7	7.10±2.48	0.95±2.19***

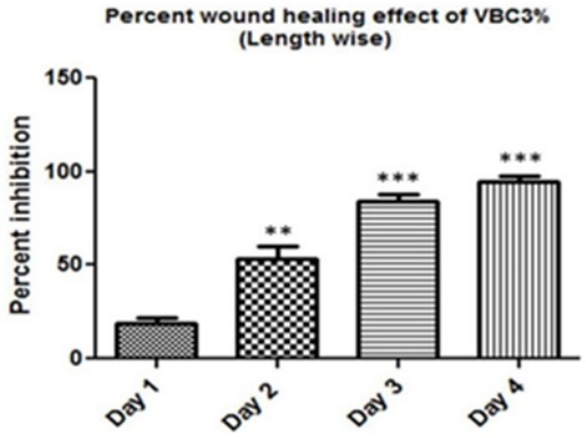
Wound Healing Effect of VBC 3%

Effect of VBC 3% TID (Length wise healing)

The VBC 3% were applied on the wound on the leg of rabbit TID. Length of wound was measured in mm with help of digital Vernier caliper three times a day for 4 days (Table 43). Data was recorded and measured statistically. Decrease of length (wound healing effect) was very significant (P<0.001) in comparison with distilled water and base treatment.

Percent wound healing effect or percent length wise healing of VBC 3% recorded in comparison with distilled water was 1.92, 51.31, 84.59 and 94.02 on day 1, 2, 3 and 4 respectively, graphically represented in Figure 3.

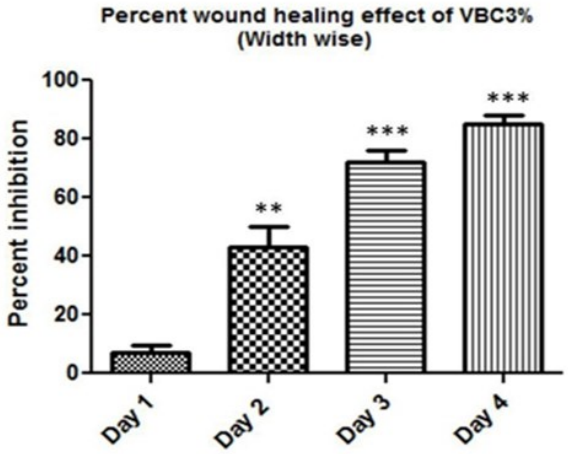
Figure 3: Percent wound healing effect of VBC 3% recorded for TID application (Length wise reduction).



Effect of VBC 3% TID (Width wise healing)

Wound healing effect in terms of width wise reduction was also recorded thrice daily (Table 3). The decrease in width was also with very significant rate (P<0.001). The wound healing effect was calculated in comparison with distilled water and base. Percent wound healing effect width wise at morning time was 2.55, 42.94, 73.22 and 85.05 on 4 consecutive days. It is presented graphically in Figure 4.

Figure 4: Percent wound healing effect of VBC 3% recorded for TID application (Width wise reduction)



Results of antibacterial screening of *Viola betonicifolia* cream

Dilutions of VBC 3% were tested against various gram positive and gram negative bacteria. our results showed that VBC has a significant activity against gram positive bacteria i.e. *Staphylococcus aureus* and *Bacillus Pumilus*. Clarithromycin was used as standard, as shown in the table5.. In addition VBC 3% showed more potent antibacterial effects against *Escherichia coli* compared to that of the standard one. The VBC exhibited a moderate antimicrobial effect and showed an average diameter of inhibitory zone (DIZ) i.e. 17mm in case of *Staphylococcus aureus, Bacillus pumilus* and *Escherichia coli* while in case of *Pseudomonas aeruginosa* the average diameter of inhibitory zone (DIZ) was 8mm.

Table 4: Antimicrobial screening of VBC dilution and standard antibiotic clarithromycin.

Microorganisms	**Diameter of inhibitory zone (DIZ) (mm)**	
	VBC 3% (3mg / 1mL)	**Clarithromycin standard disc**
Staphylococcus aureus	17	34
Bacillus Pumilus	18	31
Pseudomonas aeruginosa	8	10
E. Coli	18	12

Discussion:

Inflammation is protective response which involves host cells, blood vessels and proteins. It eliminates cause of cell injury, remove necrotic cells, tissues and start repairing of damaged tissues.

Inflammation is also initiated because of burn trauma. In severe burns, tissues are damaged seriously and fluids are lost. Skin provides a vital protection to the body. Burns can be caused due to heat, chemicals, electricity, sunlight or nuclear radiations [19]. Due to burns, inflammatory reaction occurs in that area of skin. Edema is formed because of increased microvascular permeability, vasodilation and increased extravascular osmotic activity. Chemical mediators are accumulated in microvasculature of burn area due to direct heat effect. Histamine release cause vasodilation and increased venous permeability [20].

Silver compounds are using worldwide for treating burns, wounds and ulcers but recent studies shows that such compound prolong the wound healing process and also have a cytotoxic effect upon the human cells. in a study performed by ...et al Silver sulphadiazine cream were applied on burns skin of children, and there was small percentage of development of neutropenia and erythematous rashes as a result of hypersensitivity reactions [21]. *Violabetonicifolia* is locally used for treatment of various medical ailment such as analgesic, antipyretic, wound healing [3]. The local population uses the aqueous extract of this plant for the treatment of wounds. Based on its application by local community, we subjected the *Violabetonicifolia* for aqueous extraction. The local application has shown the drug is safe and free of systemic toxicity and as the plants are the potential source of ample of chemical constituents, having different biological actions, therefore the local application is best to avoid the action of all chemicals [22].

For the treatment of inflammatory disorder mostly non -steroidal anti-inflammatory drugs (NSAIDs) are prescribed. These NSAIDs are notorious for the peptic ulcer as their side effect [23]. To avoid such adverse reactions, the topical anti-inflammatory is preferred over the systematic use. In the present study, the VBC 3% cream demonstrated significant anti-inflammatory action. The anti-inflammatory action of this plant is attributed to its chemical constituents. Viola genius is one of the potential sources of anti-inflammatory compounds such as phytosteriods. These phytosteriods having potent anti-inflammatory action [12]. The oral therapy of burn induced wound and inflammation is mostly carried out with sulphonamides which causes many adverse effects such as crystulluria and kernicterus [24]. These problems decrease patient compliance, which leads to failure of therapeutic regimen. To increase patient compliance and success rate of therapy, the topical application will be more fruitful. In present study, the formulated cream demonstrated significant wound healing action. The chemical constituents of this extract having antibacterial moieties. Its anti-inflammatory and burn healing activities are attributed to the chemical constituents of this valuable plant. Cyclotides are main active principles present in plants belong to family Violaceace. The empirical antibacterial activity exhibited by this plant is attributed to the presence of such cyclotides. Cyclotides have the ability to rupture the cell lipid membrane.

Our study proves that VBC 3 % have very significantly reduced in treating burn, inflammation and wounds in TID applications. Hence VBC 3% can be used best for treatment of burns edema and wounds effectively with good consumer compliance. VBC 1% was having significant anti-inflammatory effect in TID application on paw edema. VBC 3% was also checked for wound healing effect in TID application in rabbits. It is very interesting to note that 1% and 10% cream were less potent as compare to 3% formulation. The reason behind this result might be the concentration of anti-inflammatory and antibacterial molecules within extract. As plant's extract having agonist and antagonist constituents at the same time, therefore the action of such extract changing with increasing or decreasing dose or concentration. It means that the antibacterial and anti-inflammatory constitutes are maximum at 3% and that particular concentration is decreasing or antagonizing at other concentrations. The in-vitro antibacterial results further potentiated our study. As the extract diminished the growth of experimental gram positive bacteria therefore the wound or burn induced inflammation was attenuated significantly as compared to negative control.

Other plants belong to this family also gave very useful pharmacological activities. *V. odorata* having significant antibacterial [25, 26], anti-hepatotoxic effects [27] anti-asthmatic [28] and analgesic potentials. Zadeh et al, have formulated syrup from *Viola odorata* flowers. Another specie *Viola tricolor* also demonstrated significant anti-inflammatory, analgesic, anti-bacterial [29] and anti-proliferative activities [30]. Piana et al, 2013 formulated gel from *V. tricolor*. Which was significant anti-inflammatory and anti-nociceptive [31]. More studies are needed to isolate its useful active principles from extract of *Viola betonicifolia* and its topical formulation for treatment of various skin conditions.

Conclusion:

*Viola betonicifolia*is locally used for different inflammatory conditions as well as pathogenic problems. The formulated cream of aqueous extract (VBC 3%) demonstrated significant ($p<0.001$) anti-inflammatory, burn healing, and wound healing effects. Its anti-inflammatory and burn healing activities are attributed to the chemical constituents of this valuable plant. Cyclotides are main active principles present in plants belong to family Violaceace. The empirical antibacterial activity exhibited by this plant is attributed to the presence of such cyclotides. Cyclotides have the ability to rupture the cell lipid membrane. More studies are needed to isolate its useful active principles from extract of *V. betonicifolia* and its topical formulation for treatment of various skin conditions. The present study strongly supports the folklore of this valuable plant to use in various inflammatory conditions including burn inflammation and wound healing.

Ethical approval and consent

The study was approved by the institutional board of studies and informed consent was obtained from each participants included in the study.

Acknowledgment

We thank the study subjects for participating in this study.

Disclosure

The authors report no conflicts of interest.

Author's contributions

MSwas involved in the execution of the project. NM designed, executed the study. OS helped in organization of data. HB supervised the study and wrote the manuscript.All named authors have read and approved the final version of the manuscript.

Data availability

Available from the corresponding author on reasonable request.

References

1. Verma S, Singh SP. Current and future status of herbal medicines. Veterinary world. 2008 Nov 1;1 (11):347.
2. Ahlawat J, Verma N, Sehrawat AR. Globalisation of herbal drugs: A bliss and concern. Int J Sci Res. 2014;3(11):466-74.
3. Ali M, Rauf A, Ben Hadda T, Bawazeer S, Abu-Izneid T, Khan H, Raza M, Ali Khan S, UA Shah S, Pervez S, Patel S. Mechanisms underlying anti-hyperalgesic properties of Kaempferol-3, 7-di-O-α-L -rhamnopyranoside isolated from Dryopteris cycadina. Current topics in medicinal chemistry. 2017 Feb 1;17(4):383-90.
4. Muhammad N, Saeed M, Aleem A, Khan H. Ethnomedicinal, phytochemical and pharmacological profile of genus Viola. Phytopharmacology. 2012;3 (1):214-6.
5. Muhammad N, Khan H, Saeed M, Gilani AH. Prokinetic and laxative effects of the crude methanolic extract of Viola betonicifolia whole plant in rodents. BMC Complementary and Alternative Medicine. 2013 Dec;13(1):1-7.
6. Toiu A, Vlase L, Oniga I, Tamas M. HPLC analysis of salicylic acid derivatives from Viola species. Chemistry of Natural Compounds. 2008 May;44:357-8.
7. Hamayun M, Khan SA, Sohn EY, Lee IJ. Folk medicinal knowledge and conservation status of some economically valued medicinal plants of District Swat, Pakistan. Lyonia. 2006 Dec;11(2):101-13.
8. Sung YY, Kim DS, Kim HK. Viola mandshurica ethanolic extract prevents high-fat-diet-induced obesity in mice by activating AMP-activated protein kinase. Environmental toxicology and pharmacology. 2014 Jul 1;38(1):41-50.
9. Prausnitz MR, Langer R. Transdermal drug delivery. Nature biotechnology. 2008 Nov;26(11):1261-8.
10. Jhawat VC, Saini V, Kamboj S, Maggon N. Transdermal drug delivery systems: approaches and advancements in drug absorption through skin. Int J Pharm Sci Rev Res. 2013 May;20(1):47-56.
11. Muhammad N, Saeed M, Khan H. Antipyretic, analgesic and anti-inflammatory activity of Viola betonicifolia whole plant. BMC complementary and alternative medicine. 2012 Dec;12:1-8.
12. Muhammad N, Saeed M, Aleem A, Khan H. Ethnomedicinal, phytochemical and pharmacological profile of genus Viola. Phytopharmacology. 2012;3 (1):214-6.
13. Antil V, Kumar P, Kannappan N, Diwan A, Saini P, Singh S. Evaluation of the analgesic activity of Viola odorata aerial parts in rats. Journal of Natural Pharmaceuticals. 2011 Jan 1;2(1):24-.
14. Silva MA, Klafke JZ, Rossato MF, Gewehr C, Guerra GP, Rubin MA, Ferreira J. Role of peripheral polyamines in the development of inflammatory pain. Biochemical pharmacology. 2011 Aug 1;82(3):269-77.
15. Theunissen D, Seymour B, Forder M, Cox SG, Rode

H. Measurements in wound healing with observations on the effects of topical agents on full thickness dermal incised wounds. Burns. 2016 May 1;42 (3):556-63.
16. Atlas RM. Handbook of microbiological media. CRC press; 2010 Mar 17.
17. Sykes RB, Bonner DP, Bush K, Georgopapadakou NH. Azthreonam (SQ 26,776), a synthetic monobactam specifically active against aerobic gram-negative bacteria. Antimicrobial Agents and Chemotherapy. 1982 Jan;21(1):85-92.
18. Aaberge IS, Eng J, Lermark G, Løvik M. Virulence of Streptococcus pneumoniae in mice: a standardized method for preparation and frozen storage of the experimental bacterial inoculum. Microbial pathogenesis. 1995 Feb 1;18(2):141-52.
19. Knabl JS, Bauer W, Andel H, Schwendenwein I, Dado PF, Mittlböck M, Römer W, Choi MS, Horvat R, Meissl G, Frey M. Progression of burn wound depth by systemical application of a vasoconstrictor: an experimental study with a new rabbit model. Burns. 1999 Dec 1;25(8):715-21.
20. Çakir B, Yeğen BC. Systemic responses to burn injury. Turkish Journal of Medical Sciences. 2004;34(4):215-26.
21. Atiyeh BS, Costagliola M, Hayek SN, Dibo SA. Effect of silver on burn wound infection control and healing: review of the literature. burns. 2007 Mar 1;33 (2):139-48.
22. Brown MB, Martin GP, Jones SA, Akomeah FK. Dermal and transdermal drug delivery systems: current and future prospects. Drug delivery. 2006 Jan 1;13(3):175-87.
23. Silverstein FE, Faich G, Goldstein JL, Simon LS, Pincus T, Whelton A, Makuch R, Eisen G, Agrawal NM, Stenson WF, Burr AM. Gastrointestinal toxicity with celecoxib vs nonsteroidal anti-inflammatory drugs for osteoarthritis and rheumatoid arthritis: the CLASS study: a randomized controlled trial. Jama. 2000 Sep 13;284(10):1247-55.
24. Cunha BA. Antibiotic side effects. Medical Clinics of North America. 2001 Jan 1;85(1):149-85.
25. Ziad D, Elias A, Roula AM. Antibacterial activity of Rheum rhaponticum, Olea europaea, and Viola odorata on ESBL producing clinical isolates of Escherichia coli and Klebsiella pneumoniae. Int J Pharm Sci Res. 2011 Jul 1;2(7):1669-78.
26. Pränting M, Lööv C, Burman R, Göransson UL, Andersson DI. The cyclotide cycloviolacin O2 from Viola odorata has potent bactericidal activity against Gram-negative bacteria. Journal of antimicrobial chemotherapy. 2010 Sep 1;65(9):1964-71.
27. Qadir MI, Ali M, Saleem M, Hanif M. Hepatoprotective activity of aqueous methanolic extract of Viola odorata against paracetamol-induced liver injury in mice. ||| Bangladesh Journal of Pharmacology|||. 2014 Apr 25;9(2):198-202.
28. Qasemzadeh MJ, Sharifi H, Hamedanian M, Gharehbeglou M, Heydari M, Sardari M, Akhlaghdoust M, Minae MB. The effect of Viola odorata flower syrup on the cough of children with asthma: a double-blind, randomized controlled trial. Journal of evidence-based complementary & alternative medicine. 2015 Oct;20(4):287-91.
29. Witkowska-Banaszczak E, Bylka W, Matławska I, Goślińska O, Muszyński Z. Antimicrobial activity of Viola tricolor herb. Fitoterapia. 2005 Jul 1;76(5):458 -61.
30. Mortazavian SM, Ghorbani A. Antiproliferative effect of viola tricolor on neuroblastoma cells in vitro. Australian Journal of Herbal Medicine. 2012 Jan;24(3):93-6.
31. Piana M, Silva MA, Trevisan G, de Brum TF, Silva CR, Boligon AA, Oliveira SM, Zadra M, Hoffmeister C, Rossato MF, Tonello R. Antiinflammatory effects of Viola tricolor gel in a model of sunburn in rats and the gel stability study. Journal of ethnopharmacology. 2013 Nov 25;150(2):458-65.