RESEARCH ARTICLE

Pharmacological Evaluation of Ayurvedic *Nicotiana tobacum* Formulation for Accelerated Wound-healing: An Experimental Study

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ABSTRACT

Background: The need for pharmacognostic-based compounds with various medicinal actions is increasing to address issues such as drug resistance, new diseases, and the toxicity of already utilized chemical compounds. The plant kingdom provides numerous bioactive molecules that have great promise to help solve these challenges, resulting in the rise of innovative solutions in the pharmaceutical sector. Ayurvedic preparations, including tobacco (*Nicotiana tabacum*) are considered a viable method due to their long history in herbal therapy and several medical advantages.

Methods: The study aims to investigate the wound-healing properties of the tobacco plant. Tobacco leaves were utilized in the production of a traditional ayurveda formulation, which included mashi formulations such as anterdhum padhati mashi, bahirdhum padhati mashi, and muffle furnace mashi, in accordance with traditional ayurvedic methods. Aqueous and alcoholic extracts of tobacco leaves were also produced. The effectiveness of these formulations in wound-healing was evaluated using an Excision model in mice.

Results: The bahirdhum padhati mashi with methanolic discharge showed significantly greater wound-healing capacity compared to a standard medication formulation, as indicated by comparative study.

Conclusion: Ultimately, the bahirdhum padhati mashi made from tobacco shows potential for healing wounds and could be a useful agent for wound-healing. The results of this experimental study suggest that ayurvedic formulations could be effective in treating wound-healing difficulties. Additional research and clinical studies are needed to confirm the safety, effectiveness, and possible use in wound treatment.

Keywords: Ayurvedic formulation, *Nicotiana tabacum*, Wound-healing, Anterdhum padhati mashi, Bahirdhum padhati mashi, Muffle furnace mashi.

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INTRODUCTION

Pharmacognostic-based compounds derived from medicinal plants have garnered considerable interest due to their diverse pharmaceutical applications and potential to address various healthcare challenges, including drug resistance, emerging diseases, and toxicity associated with synthetic chemical compounds. Among the numerous plants with medicinal properties, *Nicotiana tabacum*, commonly known as tobacco and belonging to the Solanaceae family, has been traditionally utilized in different regions for various therapeutic purposes. Its historical use includes the treatment of asthma in India, combating worms in Africa, alleviating infected ulcers in Colombia, and providing relief for dysmenorrhea in Cuba.[1]

Despite the extensive traditional usage of *N. tabacum*, there exists a dearth of systematic studies investigating its potential in wound-healing. Nevertheless, Ayurvedic literature presents a novel approach to harnessing the therapeutic properties of tobacco through the formulation of mashi, an Ayurvedic preparation.[2,3]

Mashi is a black, non-uniform powdered formulation obtained through distinct methodologies, namely *Bahirdhum padhat* (BPM) and *Anterdhum padhati* (APM).[4] The BPM method involves heating and continuously stirring the material in the presence of oxygen at temperatures between 145 to 150°C, while the APM method requires obtaining mashi in the absence of oxygen. In APM, the material is enclosed within

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Sharav samput and multani matti, ignited along with cow dung in *gajaputa* kund, and the remnant is then removed to acquire mashi. Additionally, muffle furnace mashi (MFM) is prepared by subjecting the material to a muffle furnace at 350°C for 45 minutes. These various preparation methods are believed to yield mashi formulations with distinct therapeutic properties.[5]

The present research endeavors to explore and evaluate the wound-healing potential of Ayurvedic mashi formulations derived from tobacco. Both BPM and APM methods, along with MFM, will be subjected to wound-healing assessments. Understanding the wound-healing properties of these mashi formulations may provide valuable insights into the potential integration of traditional Ayurvedic medicine in modern wound management practices.[6]

This study aligns with the growing interest in exploring natural and plant-based remedies for wound-healing, which could lead to novel and pragmatic solutions for addressing wound complications. By shedding light on the wound-healing efficacy of tobacco-derived mashi, this research contributes to the existing knowledge of traditional medicine and may open new avenues for the development of natural and effective wound-healing agents. The investigation of Ayurvedic mashi formulations from *N. tabacum* represents a significant step towards discovering new wound-healing agents with potential implications in modern healthcare practices. The findings from this study may advance the field of wound-healing research and pave the way for further exploration of Ayurvedic formulations as alternative therapeutic approaches.

According to Ayurvedic literature, the method by which mashi is obtained in the presence of oxygen by heating and constantly stirring it at 145 to 150°C is called as BPM and mashi is obtained in the absence of oxygen in which the material is loaded in between *sharavsamput* which is then occluded in multani matti following which it is set on fire along with cow dung in *gajaputa kund.* Subsequently, the remnant is removed and mashi is acquired. This is called APM. Muffle furnace mashi (MFM) is prepared by subjecting the material to muffle furnace at 350°C for 45 minutes. Tobacco was formulated as both BPM and APM along with MF mashi and then the mashi was assessed for its wound-healing ability.[7]

MATERIALS AND METHODS

Raw Materials Collection and Processing

Tobacco leaves have been collected in Aurangabad, India, and then dried in the shade for around 3 to 5 days. The leaves were identified and certified as *N. tabacum* L. of the Solanaceae family by the Department of Botany at Dr. Babasaheb Ambedkar Marathwada University in Aurangabad, India. *N. tabacum* voucher specimen (Accession No. 0694) has been deposited at Dr. Babasaheb Ambedkar Marathwada University's Herbarium in Aurangabad, India.

Figure 1: The mashi formulation

Figure 2: Preparation of anterdhum padhati mashi

Aqueous Extract Preparation Process

Approximately 250 g of tobacco leaves were taken and hydrolyzed with filtered H_2O, then macerated at room temperature for 72 hours. The mixture was subsequently filtered, percolated, and dried.

Development of Alcoholic Extract

An estimated 250 g of tobacco leaves were combined with 95% ethanol and macerated at room temperature for 72 hours. The mixture was subsequently filtered, percolated, and dried. The resulting dehydrated alcohol and water remnants were brown (Figure 1).

Preparation of APM

The dried tobacco leaf powder was placed in two clay pots (Sharavsamput), which were sealed with multani matti. A *gajaputa* (warm cake filled with cow dung cake) was placed on the *gajaputa* kund and left for approximately 50 minutes. When the *gajaputa* cooled down (*swangsheet*), the *sharav* was then removed from the kund, and Mashi was obtained (Figure 2).[4]

Preparing BPM

The dried tobacco leaf powder was transferred to a clay pot, and the burning of tobacco leaves was carried out at a temperature of approximately 140 to 155°C with constant stirring. The process continued until the white smoke disappeared from the leaves, and the carbon particles began to burn. Excessive heat was avoided to prevent the conversion of mashi into white ash (Figure 3).[8]

Preparation of Muffle Furnace Mashi

The estimated amount of dried tobacco leaf powder was transferred to small silica crucibles. The heating of the tobacco leaves was carried out at a temperature of about 285 to 350°C in a furnace until the white smoke disappeared from the leaves and

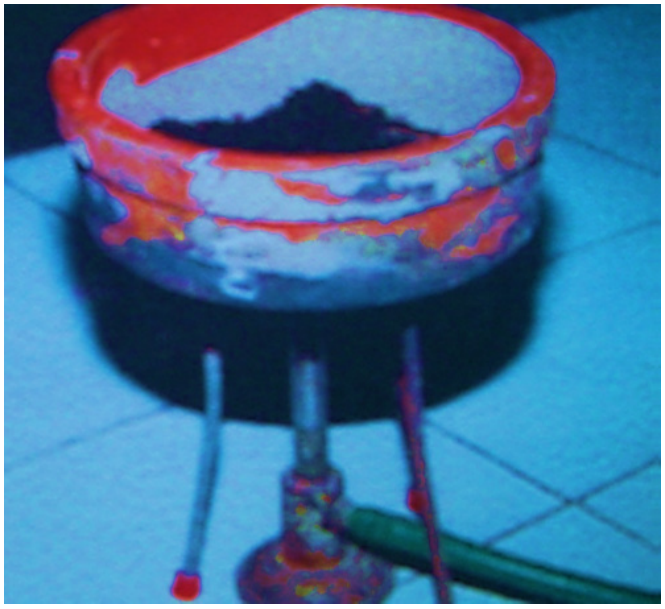

Figure 3: Preparation bahirdhum padhati mashi

the carbon particles began to burn. High heat levels were usually avoided to prevent the conversion of mashi into white ash.

Phytochemical Screening

The extracts and mashi were screened for various phytochemical constituents, including alkaloids, tannins, flavonoids, saponins, sterols, amino acids, reducing sugars, anthraquinone glycosides, cardiac glycosides, and cyanogenetic glycosides.[9]

In experiments with phytochemical medicine, it was found that there were many polyphenolic compounds. At APM and BPM, but the polyphenolic nature was lost at MF mashi. Lack of tannins in this drug was observed under all formations. Where the strong presence of alkaloids has been observed in all formations of tobacco.[10]

Alkaloid detection

A tiny amount of extract or Mashi was treated with diluted HCl acid and filtered. To determine the presence of alkaloids, the filtrate was tested using conventional reagents such as Mayer's reagent, Dragendroff's reagent, Wagner's reagent, and Hager's reagent. The development of a precipitate with any of the reagents showed the presence of alkaloids in the evaluated formulation.[11]

Testing of tannins

A small amount of extract or mashi was combined with a small amount of water and filtered. The filtrate was then treated with 10% solutions of potassium dichromate, sodium chloride, lead acetate, and a half concentration of ferric chloride. The appearance of a precipitate with any of these reagents indicated the presence of tannins.[12]

Testing of flavonoids

In the testing procedure for flavonoids, a measured amount of mashi extract was dissolved in methanol. Subsequently, a concentrated hydrochloric acid layer and magnesium were added to the solution. Upon subjecting the mixture to elevated temperature, a color change to magenta signified the presence of flavonoids.[13]

Testing of saponins

In a graduated cylinder, a little of the mashi formulation was mixed with water and left to incubate for 15 minutes. Due to the presence of a steady foam, saponins were detected.

Testing of sterols

To test for sterols, the mashi formulation underwent extraction with chloroform followed by treatment with acetic anhydride and concentrated H_2SO_4. The emergence of a blue color served as an indicator of the presence of sterols.

Testing of amino acids

The ninhydrin reagent was added to a tiny volume of mashi formulation after it had been mixed with water. The appearance of a purple hue was taken as a good sign that amino acids were present. In addition, a different part of the mashi mixture was mixed with a 10% sodium hydroxide solution, and then a few drops of a 1% copper sulfate solution was added. Further confirmation of the presence of amino acids was provided by the observation of a purple hue in this test.[14]

Reducing sugar test

The extract, also known as the mashi formulation, was filtered after being diluted with water. Adding Benedict's reagent to the filter and heating the mixture for two to three minutes yielded the desired outcome. A reddish hue was seen, signifying less sugars.[15]

Testing of anthraquinone glycosides

A small amount of extract or mashi was broken down by diluted hydrochloric acid in a hot water bath for approximately 10 to 15 minutes. The filtrate was chilled and then combined with a portion of diluted ammonia solution. A pink to cherry red color in the ammonia layer shows the presence of anthraquinone glycosides.[16]

Testing of Cardiac Glycosides

A minute amount of cardiac glycosides was dissolved in pyridine with a small amount of freshly prepared sodium nitroprusside (1–2%) in distilled water and a small amount of freshly prepared sodium hydroxide (10%) in order to analyze cardiac glycosides. The presence of cardiac glycosides was believed to be indicated by the presence of a reddish hue at boundary.[17]

Examination of cyanogenetic glycosides

The investigation of cyanogenetic glycosides involved placing 2 g of the moist sample in a test tube and adding a tiny amount of hydrochloric acid (HCl). After making a sodium picrate solution, the filtration strips were submerged in it. Carefully positioning the strips between two coconut stoves, they were then pushed into the reaction mixture-filled test tube, making sure they did not come into contact with the inside of the tube. For around three hours, the test tube was subjected to temperatures between 30°C. As a result of hydrocyanic acid

synthesis, the presence of cyanogenetic glycosides is confirmed when a red color appears after this period.

Estimation of total phenolic content[18]

Tobacco leaves and mashi extract were subjected to the analysis described by Yang *et al*. to ascertain their total phenolic content. About 1 g of mashi extract was combined with 50 mL of 95% ethanol, and the mixture was left to remain at 30°C for two days before filtering. In a test tube, 1-mL of filtrate, 1-mL of 95% ethanol, and 5 mL of water were mixed. Furthermore, there were 0.5 mL of Folin-Ciocalteu reagent, which is 50% concentration, added. The mixture was then left in darkness for one hour to avoid photodegradation after 1-mL of uniformly added 5% Na_2CO_3 after a brief interval. Next, the absorbance was assessed at a wavelength of 725 nm. By dissolving gallic acid in 95% ethanol, a standard curve was generated. In milligrams per gram of extraction, the total phenolic content was measured.

Toxicological Studies[19,20]

Acute toxicological study

Swiss albino rats of the HA strain, weighing 25 to 30 g and of both sexes, were separated into groups of six animals each. There were five groups created for the study. The "Negative control" group was administered a 4% gum acacia suspension orally using a 20-gauge needle along with normal saline. Four experimental groups were administered aqueous extracts from the standard saline solution orally at doses of 250, 500, 1000, and 2000 mg/kg body weight. Doses over 2000 mg/kg were not given because the substance could not be injected. The test subjects were closely monitored for 24 hours to observe mortality and behavioral changes. Furthermore, the alcohol extract, APM, BPM, and MF were examined for toxicity.

Sub-acute toxicity studies

The dosages in this study were selected arbitrarily and were consistent across all samples. The dosage for level 1 was 200 mg/kg body weight, and for level 2 it was 800 mg/kg body weight. The test subjects in the control group received an equivalent portion of the vehicle. Each animal received the correct dosage daily for twenty-one consecutive days. During the study period, the animals were closely monitored for behavioral changes, qualitative variations, and cases of mortality.[21]

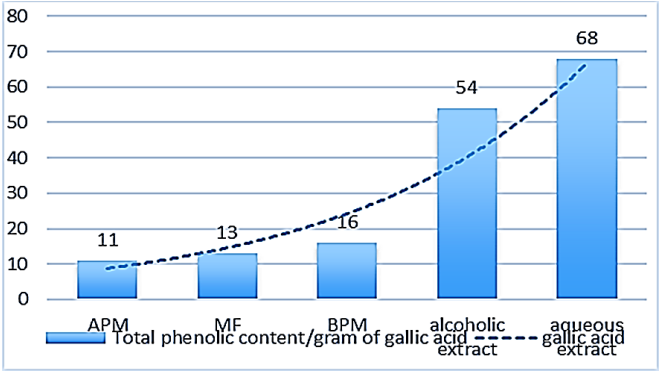

Figure 4: Concentration curve of gallic acid along with Mashi formulation

Evaluation of Wound-healing Function (Excision Model)

The excision model was used to evaluate the wound-healing function in four groups of six mice each. The mice were anesthetized with ether through an open-air mask. The dorsal interscapular area of the mice was used to extract a 500 mm² slice of full-thickness skin. In a public setting, the wounds were disregarded. Two strengths of a methanolic extract of BPM (10 and 15% w/w) and a 0.1% w/w ointment of gentamycin were administered daily to certain groups until complete wound-healing had occurred.[22]

Excellent precision was maintained in measuring the wound area daily, and the healing progress was assessed in square millimeters (mm²). We calculated wound contraction, which is the percentage reduction in the initial wound size. Regular treatments were started right after the procedure and continued until the tenth day of recuperation. At 2, 4, 8, and 10 days post-op, an appropriate scale was used to measure the wound area. A scar's epithelization period is the amount of time it takes for the wound to completely close up and heal.

Analytical Study of Different Forms of Mashi Using Differential Scanning Calorimetry

This study utilized differential scanning calorimetry (DSC) to analyze different types of mashi, including APM, BPM, and muffle furnace mashi (MFM).[23] DSC is a thermal analysis technique that determines a sample's heat flow by measuring temperature changes. By subjecting the mashi samples to controlled heating, we can analyze their thermal properties and transitions, providing valuable insights into their stability and composition. The DSC study aims to elucidate the physical and chemical properties of different mashi formulations to understand their thermal behavior variations, which are important for understanding their medicinal applications and effectiveness.[24]

RESULTS

Phytochemical Screening[18]

Differences in phytochemical makeup were observed across various mashi formulations during screening. Tannins were not found in all formulations save the aqueous extract, although alkaloids were present in all formulations. Sterols were detected in APM, BPM, and MF, but not in the aqueous and alcoholic extracts. The aqueous and alcoholic extracts included saponins, flavonoids, and reducing sugars, but APM, BPM, and MF did not. Anthraquinone glycosides were present in the aqueous and alcoholic extracts but not found in APM, BPM, and MF. Cardiac glycosides were present in all extracts however cyanogenetic alkaloids were not detected in any of the formulations.

Total phenolic content

The total phenolic content was quantified in milligrams per gram (mg/g). Figure 4 displays the phenolic content of the liquid extract (68 mg/g), alcohol extract (54 mg/g), APM (11

mg/g), BPM (16 mg/g), and MF (13 mg/g).

Toxicological Studies

Acute toxicological study

No deaths occurred within 24 hours, and there were no notable alterations in the behavior of the subjects during the study.

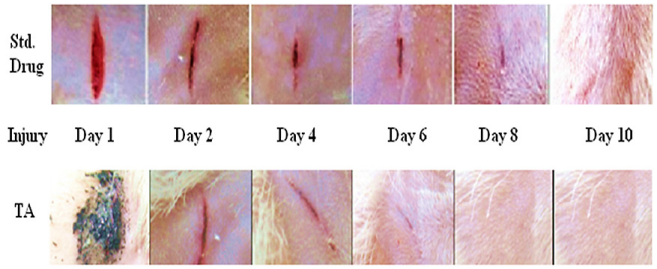

Figure 5: Comparative wound-healing activity of standard drug and BPM formulation on Days 1, 2, 4, 6, 8, and 10

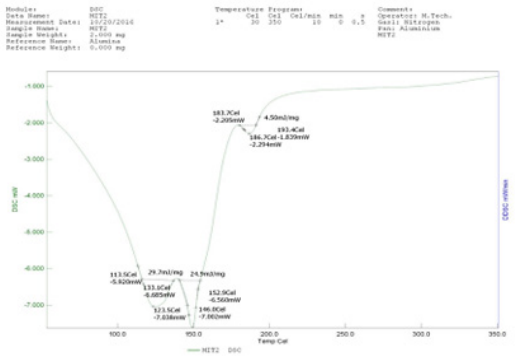

Figure 6: DSC pattern of raw tobacco leaf

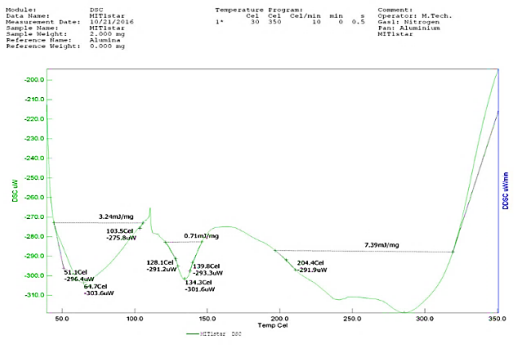

Figure 7: DSC pattern of tobacco formulation APM

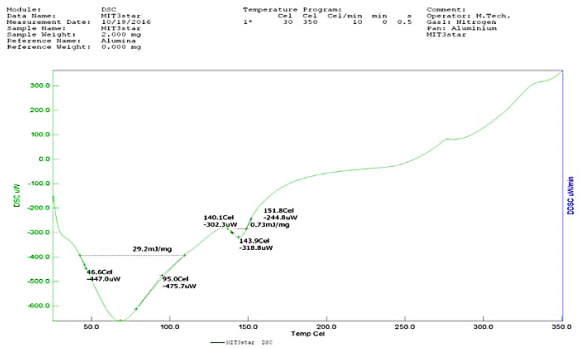

Figure 8: DSC pattern of tobacco formulation BPM

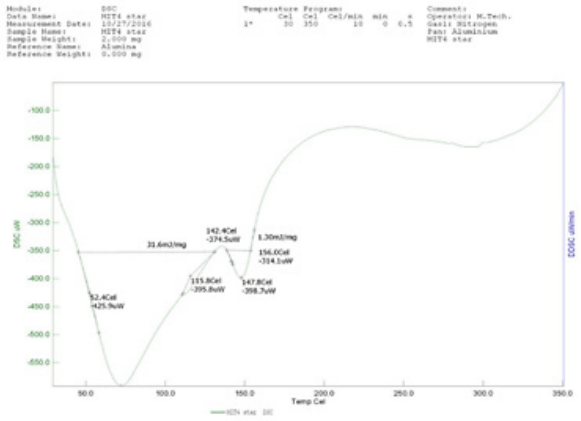

Figure 9: DSC pattern of tobaccoformulation MF

Sub-acute Toxicity Study

The individuals in the sub-acute toxicity research did not experience any significant changes in behavior or death. Up to a dosage of 2000 mg/kg of body weight, all experimental samples were shown to be safe and non-toxic.

Wound-healing Activity[22]

We used the excision model in mice to evaluate the wound-healing efficacy of different formulations. Table 1 shows the wound closure percentage per square millimeter for each group on different days. The control group's wounds took longer to heal, and by day 13, they had completely closed. When the standard antibiotic gentamycin, which is renowned for its quick wound closure, was administered, the wound closed entirely on day 9. Wounds in the group that used mashi's 10%

Table 1: The percentage of wound concentration in the untreated and the test animal was measured

Wound closure (% of original area) per mm² on day (Means ± SEM)						
Group (n = 6)	*Day 2*	*Day 4*	*Day 6*	*Day 8*	*Day 10*	*Complete Closure (days)*
Control	19.3 ± 2.74	63.5 ± 3.04	73.9 ± 0.39	81.4 ± 0.69	88.3 ± 0.18	13.0 ± 0.45
Gentamycin	32.6 ± 3.85*	77.9 ± 1.76**	89.3 ± 1.22**	98.1 ± 0.36**	100.0 ± 0.0***	9.3 ± 0.21***
10% Drug	23.3 ± 1.78	71.6 ± 2.24	85.1 ± 1.36	96.9 ± 0.43	98.3 ± 0.33	10.0 ± 0.36
15% Drug	29.3 ± 4.21	74.8 ± 1.92 *	87.9 ± 1.48*	97.7 ± 0.51*	99.2 ± 0.29*	9.3 ± 0.49*

w/w ointment had completely healed by the tenth day. Table 1 shows that on day 10, the amazing 99.2% closure rate was observed in the 15% w/w ointment group.

Significant differences ($p < 0.001$) were seen between the control group and the gentamycin group in the statistical analysis conducted using student's t-test. Furthermore, $p < 0.05$ and $p < 0.001$, respectively, indicated significant differences between the control group and the groups that were given either 10 or 15% mashi ointment. For instance, the 10% w/w ointment and other mashi formulations were found to hasten wound-healing. Figure 5 shows the wound-healing activity comparison between the 10% BPM formulation and the standard antibiotic Gentamycin. As can be observed in the photographs, the rats' wounds are dressed with the ointment in a progressive fashion from day 1 to 10. The visual data provides additional support for the wound-healing efficacy of the mashi formulation. Based on the findings, the Mashi formulations, particularly the 10% w/w ointment, show promise as a topical wound healer. Clinical trials and additional research are required to validate their safety and therapeutic effectiveness.

Analytical Study of Different Forms of Mashi Using Differential Scanning Calorimetry

In order to investigate the thermal characteristics and transitions of several Mashi forms, such as APM, BPM, and MF, DSC analysis was carried out. The results of DSC analysis as shown in Figures 6-9 for Raw tobacco, APM, BPM and MF formulations respectively show greatly enhance insights into the formulations' physical and chemical properties.

CONCLUSION

The phytochemical screening revealed variations in the composition of different mashi formulations, indicating the presence of various bioactive compounds. The total phenolic content assessment showed varying antioxidant activity among the formulations. The toxicity studies confirmed the safety of the formulations at tested doses. The wound-healing activity demonstrated the potential of BPM methanolic extract in promoting wound closure. DSC analysis provided additional information on the thermal behavior of different mashi formulations. These findings contribute to the understanding of Ayurvedic formulations and their potential applications in wound-healing and pharmaceutical industries. Further research is warranted to explore the mechanisms underlying the observed effects and to optimize the formulation for potential therapeutic use.

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AUTHOR CONTRIBUTIONS

The author's contributions to this research study are as follows: Dr. Aarti Shastri conceptualized and designed the study, conducted an investigation, and provided project administration and supervision. Dr. Arti Swami contributed for analysis of the formulation Dr. Satish Polshittwar contributed for formulation development and Dr Sachin Bhusari performed validation, formal analysis, and data visualization.

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