

## Formulation and Evaluation of Alugbati-Based Nasal Spray: A Preliminary Study for the Adjunct Management of Allergic Rhinitis

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### ABSTRACT

Allergic rhinitis is an inflammation in the nasal passages caused by an allergic response to allergens that affects a large portion of the Asian population, particularly in tropical countries like the Philippines. The Philippine Bureau of Plant Industry promotes *Basella alba*, locally named “Alugbati” which has been reported to have cytotoxic and anti-inflammatory properties, as well as nephroprotective, and wound-healing properties. The purpose of this study is to develop and evaluate the anti-inflammatory effect of *B. alba* Leaf extract as a nasal spray for allergic rhinitis in Sprague Dawley rats. A total of 27 rats were divided into three equal groups for the treatment distribution: the negative control group, the positive control group, and the treatment group which received the formulated *B. alba* nasal spray. White blood cells (WBC) were studied to identify the anti-inflammatory effect in each group. Histological examination graded and observed the following: ciliary loss, a decrease of goblet cells, degree of eosinophil infiltration, and vascular congestion. The Formulation group, on average, had no significant difference in all histological scores when compared to the positive control group but showed clinical significance as observed in the samples microscopically, while on observing WBC count there is a significant difference between the Formulation group and the Positive control group which post-hoc analysis revealed that the Formulation group was more effective in normalizing WBC count 24 hours post provocation/administration. An acute inhalation toxicity test also revealed that the extract is classified as “practically non-toxic”. Therefore, the formulated Alugbati nasal spray is a potential alternative and is effective to be used in an animal model of allergic rhinitis. Further tests such as ELISA are recommended to specifically determine the mechanism of action of the formulation.

**Keywords:** *Allergic rhinitis; Alugbati; Basella alba; Formulation; Nasal spray*

## INTRODUCTION

Allergic rhinitis is an inflammation of the nasal passages caused by an allergic reaction to airborne substances. This disease is the most common atopic disease globally, affecting 10-20% of the population, and the disease prevalence has shown to increase. Severe allergic rhinitis is associated with severe impairments in quality of life, sleep, and employment performance (Dykewicz et al., 2010; Abong et al., 2012). Allergic rhinitis is a prevalent yet underdiagnosed atopic disease that commonly presents at least one of the following clinical symptoms: persistent nasal obstruction, mucous discharge, sneezing, itching and may co-exist with asthma (Chong et al., 2018; Small et al., 2007; Bourdin et al., 2009). The most common therapy for allergic rhinitis is intranasal corticosteroids. They work by reducing inflammation in the nasal mucosa by limiting the influx of inflammatory cells and suppressing the release of cytokines. The onset of therapeutic action for inhaled corticosteroids is 30 minutes due to its broad surface for absorption, but the peak effect might take several hours to days. (Sur et al., 2010; Kimbell et al., 2007). the costs of these medicines risen dramatically, making them unaffordable for anyone who requires and needed them; thus, nasal spray becomes more and more neglected for its high price and this is why people prompts to seek out alternatives and make effective use of herbs orally which is not the fastest choice to treat allergic rhinitis (Luqman et al., 2014). The *Basella alba* plant, locally named 'Alugbati,' is one of the indigenous vegetables promoted by the Philippine Bureau of Plant Industry for consumption. The plant has enormous potential for androgenic activity, antiulcer activity, antioxidant; cytotoxic activity and antibacterial activity; anti-inflammatory activity; depressant activity of the central nervous system; nephroprotective, wound healing, and many more (Kumar et al., 2013; Duke et al., 1985; Grubben et al., 2004; Khare 2007). Reverse Phase-High Performance Liquid Chromatography (RP-HPLC) revealed the presence of secondary metabolites such as naringin, apigenin, ascorbic acid,  $\alpha$ -tocopherol, and Luteolin (Lut), a kind of flavonoid, possesses anti-oxidative, anti-tumor, and anti-inflammatory properties (Gunasekaran, 2015;

Kachchhava, 2006; Krishna, 2012; Rodda et al., 2012).

## Objectives of the Study

The study aims to develop and evaluate Alugbati (*Basella alba* L.) based nasal spray for allergic rhinitis. To the best of the researchers' knowledge, this is the first reported study using *B. alba* for allergic rhinitis by means of nasal spray.

## MATERIALS AND METHODS

### Source of Plant Sample

Leaves of *B. alba* were collected from Bitag Grande, Baggao, Cagayan. Samples were rinsed before disinfecting it with Zonrox (1ml:1L), air-dried at room temperature, and pulverized using a kitchen blender.

### Aqueous Extraction

A 25 gram grinded air-dried *B. alba* leaves (samples) were homogenized with 100 mL distilled water (solvent) in a kitchen blender (1:4 sample to solvent ratio). The prepared solution was kept in a beaker and sonicated for 45 minutes at room temperature ( $25 \pm 1^\circ\text{C}$ ). The ultrasonic treatment was performed using an ultrasonic processor. To maintain a constant room temperature ( $25 \pm 1^\circ\text{C}$ ), an oven thermometer was used, and any slight changes that was observed, freshwater was circulated. Moreover, all sonication was made in the dark to avoid exposure to light which can alter the process. After extraction, the sample residues were dissolved in a quantified solvent level (50 mL distilled water) and was extracted repeatedly until the extracts became clear. The gathered extracts were filtered using a Whatman No. 1 filter paper and will be stored at  $4^\circ\text{C}$  in an airtight container (screw type) until further analysis.

### Maintenance of Sprague Dawley rats

This study was carried out using 25 healthy male Sprague Dawley rats with an average weight of 250–300g, obtained from the authorized seller of laboratory rats located in

Santiago City. The study was started after obtaining animal permit and research clearance from Department of Agriculture, Bureau of Animal Industry (Reference no. AR-2021-140 dated 12/7/2021).

The rats were maintained in cages of no more than five animals each at an ambient temperature of  $26\pm 1^{\circ}\text{C}$ , and they were fed the usual laboratory dieting, each under a 12-hour-light-12-hour-dark cycle for seven consecutive days. During the trial, animals may drink water and consume food ad libitum.

The animals were divided into three groups of nine rats each:

1. Negative control group (ovalbumin [OVA] sensitization and provocation + intranasal PNSS provocation). In this group of rats, sensitization and provocation were made with OVA; PNSS was given as intranasal therapy.
2. Positive control group (ovalbumin [OVA] sensitization and provocation + intranasal Fluticasone Propionate). In this group of rats, sensitization and provocation were made with OVA, and Fluticasone Propionate was given as intranasal treatment.
3. Formulated group (ovalbumin [OVA] sensitization and provocation + intranasal formulated aqueous *B. alba* extract). In this group of rats, sensitization and provocation were made with OVA, and a formulated aqueous *B. alba* extract was given as intranasal treatment

### Sensitization

The initial step of our investigations on animal immunization was conducted following acclimatization to laboratory settings. All animals have been sensitized as an antigen, with the use of OVA (0.3 mg i.p., Sigma, St. Louis, Mo., USA) and aluminum hydroxide (30 mg) in saline (1 ml i.p.) 3 every other day over 14 days (Wen, 2007; Senturk et al., 2017).

### Provocation

Daily actuations using spray bottle with 30 ml 10% OVA solution dissolved in 1 ml PS into both nostrils was used to induce allergic rhinitis in rats sensitized with IP OVA for 14 consecutive days. Allergic rhinitis was observed regardless of severity of typical clinical symptoms that included nasal irritation, sneezing, and nasal secretion. On day 15, which was the first day of provocation, nasal scratching, sneezing, and nasal flow were observed. The AR model was considered successful if symptoms recurred every after provocation (Wen, 2007; Senturk et al., 2017).

### Drug treatment

All groups underwent sensitization with IP OVA for two weeks. After sensitization, AR was provoked by inducing intranasal OVA 1 hour before treatment of the following: Normal Physiological saline (Negative Control group), Fluticasone Propionate (Positive Control), and the Formulated Alugbati Nasal Spray for a total of 3 treatments. The animals' respiration was monitored closely to ensure that they will not stop breathing. The animals was sacrificed after 24 hours following the last medication application. A blood sample was collected through the tail with a process of tail snip technique for complete blood count to detect the anti-inflammatory response of the extract. The nasal cavity, nasal septum, paranasal sinus, and conchae of the animals was removed in one piece. An incision will be made starting just in front of the incisors and stretching all the way to the end of the hard palate for this reason. Tissue samples from the mucosa of the nasal septum was collected for histological analysis.

### Acute Inhalation Toxicity Test

Six (6) healthy young adult male Sprague dawley rats 8 to 12 weeks of age, with body weights ranging from 250-300 will be divided and nebulized individually with the extracts in increasing dose levels of 1.2.3 via nose only nebulization. The toxicity was observed continuously for multiple durations (15, 30, 60,

120, 240 minutes). It was carried out following the OECD TG 433 (OECD, 2018) for Acute Inhalation Toxicity.

### Histological Assessment

The nasal cavity, nasal septum, paranasal sinus, and conchae, of the test animals were removed en bloc. For this purpose, an incision was performed beginning at the level right in front of the incisors and stretching to the end of the hard palate, then tissue samples were taken from the mucosa of the nasal septum. The septal mucosa samples that were taken from the sacrificed animals were fixed in 10% neutral buffered formalin for 72 hours. The nasal mucosa underwent decalcification for 24 hours using nitric formol before cutting it into sections. Tissue samples were taken from the nasal mucosa of the nasal septum specifically at level 2 naso and maxillo turbinates. The tissues were dehydrated in an alcohol series of 70, 80, 90, 96, and 100 percent ethyl alcohol after fixation. The samples were softened in xylene for 3 hours before being molded in paraffin at 60 degrees Celsius. Microtome pieces of approximately 5 µm thickness were sliced after molding. Hematoxylin and eosin were used to stain the sections. An optical microscope was used to analyze stained materials. Ciliary loss, an increase in goblet cells, vascular congestion, and the degree of eosinophil infiltration were assessed under the microscope (Senturk et al, 2017). According to Ercan et al. (2006), the scoring was done semi- quantitatively, with each parameter receiving a score. "0" points in the absence of any modification, "1" for a slight change, "2" for noticeable change, and "3" points for severe change.

### Complete Blood Count (CBC) Testing

All blood samples were collected in EDTA-tube microvettes which were purchased among the pharmacies and boutiques located within the vicinity of Tuguegarao City. Blood samples were collected by cardiac perfusion prior to sacrificing the test subjects. The Complete Blood Count (CBC) testing, including white

blood cell (leukocyte) differential count was determined with the assistance of Department of Agriculture (Carig Norte, Tuguegarao City) research specialist.

### Statistical Analysis

The mean and standard deviation was utilized to determine the anti-inflammatory effects of Alugbati leaf extract nasal spray in terms of decrease in white blood cells, decrease ciliary loss, decrease of eosinophil infiltration, decrease goblet cells, and decrease vascular congestion in the nasal mucosa of the test animals. Furthermore, the One-Way ANOVA (Analysis of Variance) is used to determine whether there are any statistically significant differences among the formulation, positive control, and negative control. Least Significant Difference (LSD) was also used to evaluate the significant differences among various treatments. All statistical runs were performed using SPSS version 26.

## RESULTS AND DISCUSSION

### Phytochemical Analysis of Alugbati aqueous extract in terms of:

**Table 1.** Qualitative screening of ethanol and aqueous extracts of *Basella alba* leaves (Tongco et al., 2015)

Phytochemical Test	Aqueous Extract
Alkaloids	+
Carbohydrates	-
Cardiac Glycosides	-
Anthranol Glycosides	-
Cyanogenic Glycosides	-
Saponins	-
Diterpenes	+
Triterpenes	-
Phenols	+
Tannins	+
Flavonoids	+

Color changes are used in phytochemical screening to determine if particular phytochemicals are present. The presence of phytochemicals such as alkaloids, diterpenes,

phenols, tannins, and flavonoids in aqueous extract was determined in a previous work by Tongco et al. (2015) using qualitative phytochemical screening of extracts from Alugbati leaves. The anticancer, antioxidant, and anti-inflammatory activities of *B. alba* are attributed to phytochemicals found in the aqueous extracts according to the literature. According to Gunasekaran (2015), GS-MS/MS analysis detected 25 compounds, while Reverse Phase-High Performance Liquid Chromatography (RP-HPLC) revealed the presence of or naringin, apigenin, ascorbic acid, and -tocopherol, and luteolin, a flavonoid with anti-oxidative, anti-tumor, and anti-inflammatory properties.

### Formulated Alugbati- based Nasal Spray



**Figure 1.** Nasal Spray with Alugbati Extract

For the formulation of Alugbati-based nasal spray, a 3 ml of alugbati aqueous extract (obtained from Bitag Grande, Baggao, Cagayan) was added to 297 ml of plain normal saline solution (0.9% sodium chloride obtained from a local drugstore). The resulting formulation has 1% alugbati aqueous extract and 99% of plain normal saline solution which was placed into a 30 ml bottle spray. As mentioned in the FDA Guidance of Industry for Nasal spray solution (2002) Inhalation solution and suspension drug products are typically aqueous-based formulations that contain therapeutically active ingredients and can also

contain additional excipients, since the aqueous Alugbati extract has impurities and excipients it came to a decision wherein 1% of the extract will be used for the formulation which also does not exceed the recommended limit concentration based on the GHS classification system when producing Aerosols. Based on the study of Papsin & McTavish (2003), it was imperative that plain normal saline solution (isotonic) must be used in the formulation because using isotonic saline had significant reductions in nasal secretions. Furthermore, in a review of Hermelingmeier et al. (2012) on allergic rhinitis cases treated with isotonic saline nasal irrigation from 1994 to 2010, all patients' symptoms improved, and nasal irrigation was able to reduce the dosage and side effects of glucocorticoids, accelerate nasal mucociliary clearance, and improve quality of life, so isotonic saline irrigation was recommended as a complementary therapy of AR.

### Evaluation of the *Basella alba* L. based nasal spray to Ovalbumin-provoked Sprague Dawley rats in terms of:

#### Organoleptic Properties

**Table 2.** Organoleptic properties of the Alugbati-base Nasal Spray

Organoleptic property	Observation
Color	Brownish-yellow
Odor	Odorless
Texture	Watery
Opacity	Transparent

Table 2 shows the organoleptic test results wherein it was stated in the study of Fitriani et al., (2020) that the senses which are used to define the qualities of crude pharmaceuticals based on form, smell, color, and taste, are employed to specify organoleptic criteria. According to the results, the formulated Alugbati-based nasal spray exhibited no significant smell or odorless with a brownish-yellow color. On the other hand, the formulated nasal spray has a watery texture with a transparent opacity.



Solution (negative control) with a mean of 14.72. This means that the formulated nasal

### Acute Inhalation Toxicity Test

**Table 3.** Mortality rate during nasal toxicity test

Alugbati concentration (mL/L)	Mortality per Time interval				
	After 15 mins	After 30 mins	After 60mins	After 120 mins	After 240 mins
5 mL/L	0/2	0/2	0/2	0/2	0/2
3 mL/L	0/2	0/2	0/2	0/2	0/2
1 mL/L	0/2	0/2	0/2	0/2	0/2

Table 3 shows the mortality rate of sprague dawley rats when exposed to different concentrations of alugbati extract. After 15, 30, 60, 120, and 240 minutes of administration via nose only nebulization. There was no recorded mortality. Therefore, according to the 2018 OECD guidelines for acute inhalation toxicity the extract is classified as “practically non-toxic”.

### Quantity of White Blood Cells

spray has a potential in normalizing white blood cells 24 hours post provocation/treatment.

Table 4.1.b shows the one-way Analysis of Variance (ANOVA) on the white blood cell counts of the test animals when they are grouped by treatments. This demonstrates that there is a significant difference in decreasing white blood cells among the treatments in the

**Table 4.1.a** Quantity of white blood cells 24 hours post provocation/administration of treatment

Variable	Treatments	Mean	Standard Deviation
Decrease in white blood cells	Formulated (Alugbati leaf aqueous extract)	8.22	4.746
	Fluticasone propionate (Positive Control)	13.58	3.358
	Normal Saline Solution (Negative Control)	14.72	2.801

**Table 4.1.b** ANOVA on the difference of the quantity of WBC count when grouped according treatment

Variable	F-value	p-value	Decision
Decreased WBC	6.496	0.006	Reject null hypothesis

Table 4.1.a presents the mean and standard deviation of different treatments in decreasing white blood cells. The formulated Alugbati extract has a mean of 8.22 which has a significant difference with the positive control Fluticasone propionate with a mean of 13.58. But there was no significant difference between the positive control and Plain Normal Saline

overall comparison of the treatments in normalizing white blood cell levels 24 hours post administration.

**Table 4.1.c** Post-Hoc Analysis on the difference of the quantity of WBC count when grouped according treatment

Treatment	Mean	Mean Difference		
		Formulated	Positive Control	Negative Control
Formulated	8.22	0.00	-5.783*	-6.633*
Positive Control	13.58	5.783*	0.00	-0.850
Negative Control	14.72	6.663*	0.850	0.00

\*Significant at 0.05

Table 4.1.c displays the post-hoc analysis, LSD test, on the amounts of white blood cells in Sprague-Dawley rats when they are grouped by treatment. The table reveals that there is a significant difference in normalizing white blood cells 24 hours post administration of treatment between the formulated Alugbati Nasal Spray and the positive control Fluticasone Propionate. According to Bamidele et al. (2020), there were significant decrease ( $p < 0.05$ ) in blood glucose and white blood cell count in *Basella alba* extract treated rats when compared to the two groups of his study. This means that the Alugbati Nasal Spray formulation has a faster response on normalizing WBC, indicating a decrease in inflammation. It was stated in the study of Kumar et al. (2011), by preventing hypotonicity-induced lysis of the erythrocyte membrane, *Basella alba* leaf extracts demonstrated membrane stability. Because erythrocyte membranes are analogous of lysosomal membranes, the extract may also stabilize lysosomal membranes. The stabilization of the lysosomal membrane is critical in limiting the inflammatory response by avoiding the release of activated neutrophil lysosomal contents such as bacterial enzymes and proteases, which induce additional tissue inflammation and damage. The data also demonstrates that there is no statistically significant difference between Fluticasone Propionate (positive control) and Plain Normal Saline Solution (negative control). This means that both have a comparable response when it

**Table 4.1.c** Table 4.1.d. White blood cell count 24 hours post-provocation-administration

Lab No.	Replicates	WBC
		4.0-19.0 $\times 10^9/L$
RADDLD-215182	F1S1	4
RADDLD-215182	F1S2	7.6
RADDLD-215182	F1S3	2.5
RADDLD-215182	F2S1	52.6
RADDLD-215182	F3S1	14.8
RADDLD-215182	F3S2	7.9
RADDLD-215182	F3S3	12.5
RADDLD-215182	P1S1	17.1
RADDLD-215182	P1S2	19
RADDLD-215182	P1S3	10.2
RADDLD-215182	P2S1	14.7
RADDLD-215182	P2S2	10.3
RADDLD-215182	P2S3	13.2
RADDLD-215182	P3S1	15.3
RADDLD-215182	P3S2	13.5
RADDLD-215182	P3S3	8.9
RADDLD-215182	N1S1	15.9
RADDLD-215182	N1S2	13.9
RADDLD-215182	N1S3	16.2
RADDLD-215182	N2S1	19.4
RADDLD-215182	N2S2	11.9
RADDLD-215182	N2S3	12.1
RADDLD-215182	N3S1	13
RADDLD-215182	N3S2	18.1
RADDLD-215182	N3S3	12

comes to normalizing WBC 24 hours post administration. Furthermore, there is a significant difference in formulation between alugbati nasal spray and the negative control.

Table 4.1.d showed the levels of white blood cell count after 24 hours of the last treatment administration. From the different replicates it presents the majority of WBC count within normal values however, the result 52.6, 13.5, and 19.4 were found in the formulated, positive and negative control, respectively, which indicates an above normal result. Moreover, it can be seen that the one replicate from the formulated group yielded 2.5 which indicates a below normal count of WBC. According to Sur & Scandale (2010) the onset of therapeutic action for inhaled corticosteroids is 30 minutes, but the peak effect might take several hours to days. The decrease in WBC counts in the present work is in contrast to the work done by Bamidele et al. (2010) in animal exposed aqueous extract of *B. alba* in which WBC was increased.

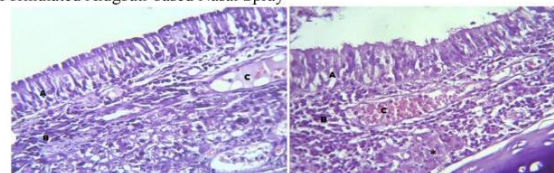
### Quantity of Ciliary loss

**Table 4.2** T-test on the difference on the decrease ciliary loss in the nasal mucosa when grouped according treatment

Variable	t-value	p-value	Decision
Quantity of ciliary loss in the nasal mucosa	0.707	0.496	Accept null hypothesis

Table 4.2 represents the test of difference in terms of decreased ciliary loss in nasal mucosa once grouped by treatment. Because  $p\text{-value}=0.496 > 0.05$  alpha level, there is no significant difference between the formulated alugbati nasal spray and the positive control Fluticasone propionate. This means that the formulated nasal spray is comparable to that of the positive control in terms of the quantity of ciliary loss.

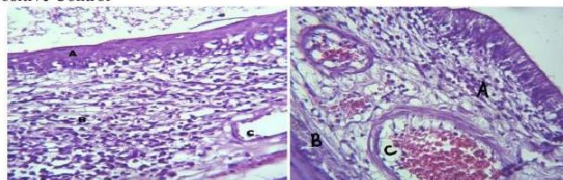
Formulated Alugbati-based Nasal Spray



Goblet cells in the epithelial layer (A). Basement membrane (B). Olfactory Gland (C).

Goblet cells in the epithelial layer (A). Basement membrane (B). Olfactory Gland (C).

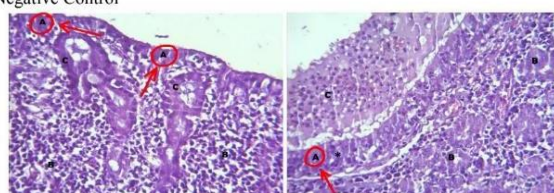
Positive Control



Goblet cells in the epithelial layer (A). Basement membrane (B). Blood vessels (C).

Goblet cells in the epithelial layer (A). Basement membrane (B). Blood vessels (C).

Negative Control



Nasal epithelium (A) with marked ciliary loss and hyperplasia of goblet cell (\*). Marked thickening of the basement membrane, olfactory gland hyperplasia (B) and inflammatory cell infiltration. Inflammatory fluid containing predominantly eosinophils were present in the lumen (C).

Nasal epithelium (A) with marked ciliary loss and hyperplasia of goblet cell (\*). Marked thickening of the basement membrane, olfactory gland hyperplasia (B) and inflammatory cell infiltration. Inflammatory fluid containing predominantly eosinophils were present in the lumen (C).

**Figure 2.1** Histopathological examination of different samples among treatment when ciliary loss is observed.

Figure 2.1 showed no visible ciliary loss on both the Formulated nasal spray and the Positive control compared to the negative control where in evident ciliary loss is present. Which means that there is a clinical significance present in terms of histopathological observations.

### Quantity of Eosinophil infiltration

**Table 4.3** T-test on the difference on the quantity of eosinophils infiltration in the nasal mucosa when grouped according treatment

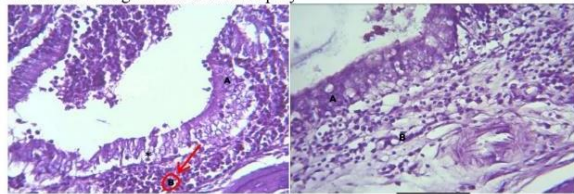
Variable	t-value	p-value	Decision
Quantity of eosinophil infiltration in the nasal mucosa	-0.349	0.734	Accept null hypothesis

Table 4.3 shows the test of difference in terms of the decrease of eosinophils infiltration in the nasal mucosa when grouped according treatment. Since  $p\text{-value}=0.734$  is greater than the alpha level of 0.05 it shows that there is no



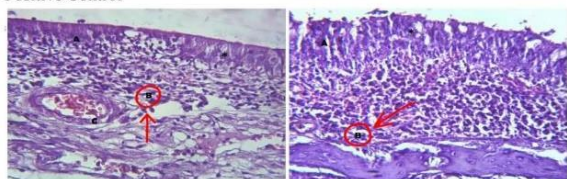
significant difference between the positive control and the formulated nasal spray. This implies that the formulated nasal spray is comparable to the positive control in terms of the quantity eosinophil infiltration in the nasal mucosa.

Formulated Alugbati-based Nasal Spray



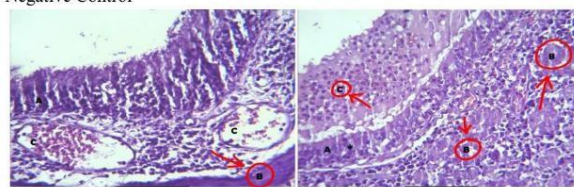
Goblet cells (\*). Epithelial layer (A). Thickened basement membrane with inflammatory cells infiltration (B).

Positive Control



Epithelial layer (A) and goblet cells hyperplasia (\*) Thickened basement membrane with inflammatory cell infiltration (B) Blood vessels (C).

Negative Control



**Figure 2.2** Histopathological examination of different samples among treatment when eosinophil infiltration is observed.

Figure 2.2 showed no visible Eosinophil Infiltration on both the Formulated nasal spray and the Positive control compared to the negative control where in evident Eosinophil migration from the thickened basement membrane is present. Which means that there is a clinical significance present in terms of histopathological observations.

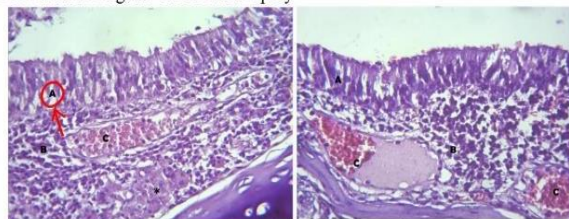
### Quantity of Goblet Cells

**Table 4.4** T-test on the difference on the quantity of Goblet Cells when grouped according treatment

Variable	t-value	p-value	Decision
Quantity of goblet cells	-0.415	0.687	Accept null hypothesis

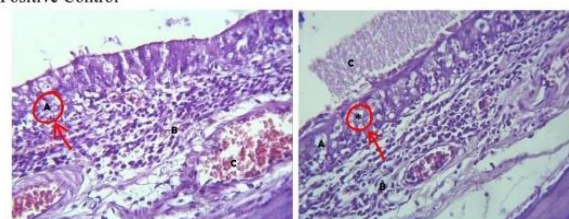
Table 4.4 demonstrates the test of difference in terms of decrease in Goblet cells in the nasal mucosa when grouped by treatment. Since the p-value of 0.687 is more than the alpha level of 0.05, it indicates that there is no significant difference between the positive control and the formulated treatment. This indicates that the prepared nasal spray is comparable to the positive control in terms of the quantity of Goblet cells in the nasal mucosa.

Formulated Alugbati-based Nasal Spray



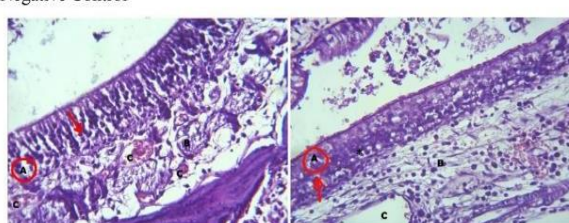
Disorganization and hyperplasia of the epithelial cells and goblet cells (A). Thickened basement membrane (B) and olfactory gland hyperplasia (\*). Blood vessels (C).

Positive Control



Disorganization and hyperplasia of epithelial layer and goblet cells (A). Thickened basement membrane (B) Blood vessels (C).

Negative Control



Hyperplasia of the epithelial layer (A) and goblet cells with numerous intraepithelial inflammatory cells (arrow). Severe swelling of the olfactory glands (B) and congestion of the blood vessels. Hyperplasia of blood vessels was observed (C).

**Figure 2.3** Histopathological examination of different samples among treatment when

Figure 2.3 showed some visible presence and alteration of goblet cells on both the Formulated nasal spray and the Positive control compared to the negative control where in a massive presentation and alteration of goblet cells. Which means that there is a clinical significance present in terms of histopathological observations.

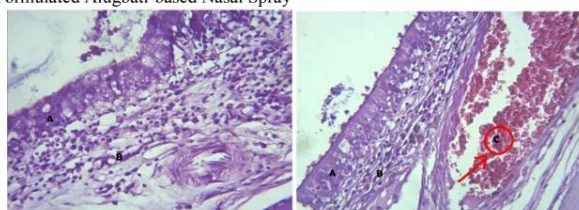
### Quantity of Vascular Congestion

**Table 4.5** T-test on the difference on the quantity of vascular congestion when grouped according treatment

Variable	t-value	p-value	Decision
Quantity of vascular congestion	1.265	0.235	Accept null hypothesis

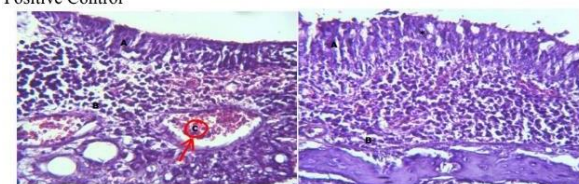
Table 4.5 presents the test of difference in terms of the decrease in vascular congestion in nasal mucosa when grouped according to treatment. Since  $p\text{-value}=0.235 > \alpha=0.05$  it shows that there is no significant difference between the formulated Alugbati nasal spray and positive control Fluticasone propionate. This implies that the formulated nasal spray is comparable with the positive control in terms of the quantity of vascular congestion in the nasal mucosa.

Formulated Alugbati-based Nasal Spray



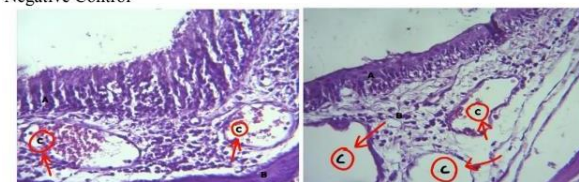
Disorganization of goblet cells (A). Thickened basement membrane (B). Goblet cells in the nasal epithelium (A). Increased cellularity of the basement membrane (B). Congestion of blood vessels was observed (C).

Positive Control



Hyperplasia of the epithelial cells (A). Thickened basement membrane (B). Marked congestion of the blood vessels (C). Disorganization and hyperplasia of the epithelial layer and goblet cells (\*) with ciliary loss (A). Thickened basement membrane (B).

Negative Control



Hyperplastic epithelial layer (A) with numerous intraepithelial inflammatory cells predominantly eosinophils migrating from the thickened basement membrane (B) with inflammatory cell infiltrates. Marked dilation (C) of blood vessels due to congestion. Squamous metaplasia of the nasal epithelium (A) and thickened basement membrane due to edema (B) with marked dilatation of the blood vessels (C).

**Figure 2.4** Histopathological examination of different samples among treatment when vascular congestion is observed.

Figure 2.4 showed visible vascular congestion on all the treatments. Additionally, the negative control had dilation of blood vessels aside from vascular congestion. Which means that there is a clinical significance present in terms of histopathological observations.

### CONCLUSIONS

Based on the findings of this study, it can be concluded that the formulated nasal spray with Alugbati (*Basella alba* L.) aqueous extract is a potential treatment when used in an animal model of experimental allergic rhinitis. It has the ability to normalize the amount of white blood cells within 24 hours after treatment that indicates an increase response on decreasing inflammation in the nasal passages caused by Allergic rhinitis in Sprague Dawley rats. In the histological parameters, the formulated Alugbati nasal spray has an increased effect in the decrease of eosinophils infiltration, decrease ciliary loss in the nasal mucosa, decrease of goblet cells, and decrease in vascular congestion when compared to the positive control statistically. On a clinical perspective it is evident when on observing histopathological samples microscopically there is clinical significance when comparing between treatments. Therefore, formulated Alugbati nasal spray is a potential alternative on treating Allergic Rhinitis.

### RECOMMENDATIONS

The researchers offer several valuable recommendations to enhance the scope and efficacy of their study. Firstly, they suggest replicating the research using different parts of the plant, a strategy that holds the potential to diversify treatment formulations and yield more promising outcomes. Secondly, the researchers strongly advocate the use of ELISA testing for the detection of antigens in biological samples, which could significantly improve the accuracy of their findings.



Additionally, future researchers could explore the isolation of the specific metabolite luteolin, a known anti-inflammatory compound found in the plant extract. This focused approach may shed light on the compound's therapeutic potential. Furthermore, the researchers propose experimenting with alternative organic solvents like ethanol and methanol, varying their concentrations during the extraction process. This approach might optimize the extraction process and potentially yield different bioactive compounds.

To expand the understanding of their formulated nasal spray, the researchers recommend investigating its systemic effects on the research subjects. This broader perspective could provide valuable insights into the treatment's overall impact. Lastly, the researchers suggest conducting a soil analysis to uncover the constituents necessary for the plant's optimal growth and yield potential. This information can be crucial for further cultivation and research efforts. Collectively, these recommendations pave the way for more comprehensive and insightful research in this field.

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