



## FORMULATION AND EVALUATION OF HERBAL GEL FROM ETHANOLIC LEAF EXTRACT OF *SESBANIA GRANDIFLORA* FOR SYMPTOMATIC RELIEF OF MUMPS (PAROTITIS)

### Pharmaceuticals

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### ABSTRACT

Mumps is a common childhood infection caused by the mumps virus. The hallmark of infection is swelling of the parotid gland. Aseptic meningitis and encephalitis are common complications of mumps. Mumps is vaccine-preventable, and however, there have been outbreaks of disease in vaccinated populations. In 2005, a large epidemic peaked in the UK, and in 2006 the American Midwest had several outbreaks. From ancient times the leaf extract of *Sesbania Grandiflora* is applied to the inflamed parotid gland for the symptomatic relief of mumps as a home remedy. But now a days (In 21st century) due to urbanization/ Industrialization the plant are not available commonly. In this context we are aimed to formulate and evaluate herbal gel from leaf extract of *Sesbania Grandiflora* for ready made available to the people to get the relief from mumps. In present study herbal gel is prepared by using Carbopol-934 as a Gelling agent, Methyl paraben and Propyl paraben as Preservatives, Propylene glycol as Permeation enhancer, Triethanolamine as pH adjuster, Ethanol as co-solvent and Water as Solvent. The prepared herbal gel was evaluated for Spreadability, Extrudability, Drug content, Skin Irritation and Ex-vivo permeation studies. In this study anti inflammatory activity was also done on albino rats by taking Diclofenac gel as a standard, the results indicated that the formulated herbal gel is stable, safe, effective and it shows significant anti inflammatory activity when compared with Diclofenac gel.

### KEYWORDS

Mumps; Parotitis; *Sesbania Grandiflora*; Herbal gel; Carbopol; Anti-inflammatory activity.

### INTRODUCTION

Mumps is an acute infectious disease caused by a paramyxovirus. Although the disease is usually mild, up to 10% of patients can develop aseptic meningitis; a less common but more serious complication is encephalitis, which can result in death or disability. Permanent deafness, orchitis, and pancreatitis are other untoward effects of mumps. Classic mumps is characterized by enlargement of the parotid and other salivary glands [1]. Acute Viral Parotitis is nothing but Mumps. Parotitis is an inflammation of one or both parotid glands, the major salivary glands located on either side of the face, in humans. Mumps is a viral infection, so antibiotics aren't effective. Take over-the-counter pain relievers such as acetaminophen or a nonsteroidal anti-inflammatory drug such as ibuprofen to ease symptoms [2].

Topical delivery is an attractive route for local and systemic treatment. Topical application has many advantages over the conventional dosage forms. In general, they are deemed more effective less toxic than conventional formulations. Topical preparation avoids the GI-irritation, prevent the metabolism of drug in the liver and increase the bioavailability of the drug. Topical preparations give its action directly at the site of action [3].

*Sesbania Grandiflora* (Avisa/ Agathi) belongs to the family fabaceae is well know plant commonly known as Vegetable Humming bird. Almost every single part of *S. grandiflora* is used as traditional medicine to treat an array of diseases such as dysentery, stomatitis, fever, small pox, sore throat, headache etc. This plant is also used in Indian traditional system of medicine, Sidha and Ayurveda, for the treatment of various acute and chronic disorders. The dried leaves are often used to make tea and are considered to have good antibacterial, anti helminthic, antitumor and contraceptive properties [4, 5].

### MATERIALS AND METHODS

#### Materials

Carbopol 934 (S.d.fine-chem Ltd), Propylene Glycol (Kemphasol, Mumbai), Methyl paraben (Burgoyne, India), Propyl paraben (S.d.fine-chemLtd,Mumbai), Triethanolamine(Loba Chemie Pvt Ltd), Ethanol(Thermofisher scientific India pvt Ltd), carragennan ( sigma Aldrich) were purchased from the market.

#### Selection and Procurement of plant material

Leaves of *Sesbania Grandiflora* used in present study were selected on the basis of literature survey. *Sesbania Grandiflora* leaves known as "Avisaku" in Andhra Pradesh were collected from local area of Addanki, Guntur and authenticated by Dr. M. Ramaiah, Dept of Pharmacognosy, Hindu college of pharmacy, Guntur.

#### Experimental Animal

Adult wistar albino rats, weighing (150-200g) were procured from Mahaveera enterprises (Regd. No. 1656/PO/Re/S/12/CPCSEA), Hyderabad, Telengana. The animals were feed a normal laboratory pellet diet and water *ad libitum*. They were housed in colony cages under standard laboratory conditions (12:12h light and day cycle, temperature at 25±2°C and relative humidity at 55±10%). The ethical clearance was obtained from Institutional Animal Ethics Committee (HCOP/IAEC/PR-12/2019) at Hindu college of pharmacy, Amaravathi road, Guntur, India for using animal in the present study.

#### Preparation of leaves Extract

The freshly collected leaves of *Sesbania Grandiflora* were dried under shade at room temperature. The dried leaves were coarsely powdered and stored in an airtight container until use. The 50g of crushed plant material is extracted with 250 ml ethanol in soxhlet apparatus at 50-60°C. The process was continued for a total of 16 hours. The collected extract was concentrated on rotary evaporator and concentrated extract was kept in dessicator until used [6].

#### Phytochemical screening

Twenty milligrams of each extract was dissolved in 20 ml of aqueous ethanol solution until the solution was clear. The redissolved extracts were evaluated to determine the presence of alkaloids, carbohydrates, flavonoids, tannins, steroids, and terpenoids according to standard methods [5].

#### Detection of alkaloids

One milliliter each of Wagner, Mayer, and Dragendorff reagents were separately added into 2 ml of the extract solution. A reddish brown precipitate (Wagner), cream colored precipitate (Mayer), and orange/reddish-brown precipitate (Dragendorff) indicated the presence of alkaloids.

#### Detection of carbohydrates

The identification of carbohydrates was performed using a Molisch's test. Three drops of  $\alpha$ -naphthol were placed into 2 ml of the extract solution and 1.5 ml of concentrated sulfuric acid was then steadily added from the sides of a test tube. A violet ring at the interface between the acid and extract layers indicated the presence of carbohydrates.

#### Detection of flavonoids

**Ferric chloride test** One milliliter of 5% (w/v) ferric chloride solution was added to 2 ml of the extract solution. Formation of a bluish color revealed the existence of the phe-nolic nuclei.

**Sodium hydroxide test** One milliliter of 10% (w/v) sodium hydroxide solution was added to 2 ml of extract solu- tion. The intense yellow

color disappeared after adding 1 ml of diluted hydrochloric acid indicated the presence of xanthone groups related to flavonoids.

**Shinoda test** Some magnesium ribbons and concentrated hydrochloric acid were placed into 2 ml of the extract solution. The appearance of orange to red color indicated the existence of flavonoids.

#### Detection of tannins

**Ferric chloride test** One milliliter of 5% (w/v) ferric chloride solution was added to 2 ml of the extract solution. Formation of a blackish blue color indicated the existence of tannins.

**Gelatin test** One milliliter of 1% (w/v) gelatin solution containing sodium chloride (10%, w/v) was added to 2 ml of the extract solution. The white cloudy precipitates which formed revealed the presence of tannins.

#### Detection of terpenoids and steroids

The Liebermann Burchard test was used for the detection of steroids and terpenoids. Ten milligrams of the extract was dissolved in 5 drops of acetic anhydride and then 5 drops of concentrated sulfuric acid were carefully added. After mixing and waiting 5 min the reddish-brown color and green color indicated the presence of terpenoids and steroids respectively.

#### Preformulation Studies

##### Drug-excipient compatibility study by FT-IR spectroscopy

Preformulation testing is the first step in development of dosage forms. preformulation study is the process of optimizing the delivery of the drug through determination of physico-chemical properties of the new compound that could affect the drug performance and development of an efficacious, stable and safe dosage forms. FT-IR spectroscopy was carried out to check the compatibility between drug and polymer. The IR spectra of *Sesbania grandiflora* and physical mixture of extracts and polymer were carried out by using FT-IR (Bruker).

##### Construction of calibration curve

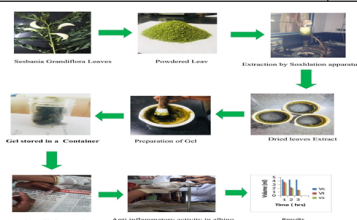
Accurately weighed 1 g of the dried extract was taken in a 100 ml volumetric flask and add sufficient quantity of phosphate buffer (pH 7.4), shaken well to dissolve the active constituents and made up to volume with phosphate buffer (pH 7.4). Aliquots of different concentration were prepared by suitable dilutions after filtering the stock solution and absorbance was measured at 273nm and calibration curve was constructed.

##### Formulation of gel using *Sesbania Grandiflora* leaves extract

The gel was prepared using the dried ethanolic extract of *Sesbania Grandiflora* leaves, carbapol-934 (1%), propylene glycol, ethanol, methyl and propyl parabene, EDTA, tri-ethanolamine and distilled water in a quantity sufficient to prepare 100g of gel. Water required for the formulation is divided in to two parts. In one part the exact amount of extract was dissolved and to this calculated quantity of propylene glycol, ethanol were added and in other part, carbapol-934 was dissolved and to this solution methyl parabene, propyl parabene, EDTA was added. Both of these solutions were mixed in a beaker and tri-ethanolamine was added to the mixture dropwise to obtain the gel consistency [6]. Composition of gel formulation was given in Table 1.

**Table 1 Formulation Of Herbalgel From Ethanolic Leaf Extract Of *S. Grandiflora*.**

Ingredients	Quantity
<i>Sesbania grandiflora</i> extract	1gm
Carbopol	1gm
Propylene glycol	15ml
Methyl paraben	0.50gm
Propyl paraben	0.50gm
Triethanolamine	q.s
Ethanol	10ml
Water	q.s



#### Evaluation of prepared herbal gel

##### Homogeneity

After the gels have been set in the container, developed gel was tested for homogeneity and physical appearance by visual inspection [6].

##### Grittiness

All the formulations were evaluated microscopically for the presence of any appreciable particulate matter which was seen under light microscope. Hence obviously the gel preparation fulfils the requirement of freedom from particular matter and from grittiness as desired for any total preparation [7].

##### Measurement of pH

pH measurement of the gel was carried out using a digital pH meter by dipping the glass electrode completely into the gel system to cover the electrode. The measurement was carried out in triplicate and the average of the three readings was recorded [8].

##### Viscosity study

Viscosity of gel was determined using Brookfield viscometer (S-62, model LVDV-E) at 25°C with a spindle speed of the viscometer rotated at 12 rpm [9].

##### Drug content

Prepared gel formulation (1 g) was taken in a 50 mL volumetric flask and made up to volume with phosphate buffer (pH 7.4) and shaken well to dissolve the active constituents. The solution was filtered through Whatman filter paper and 0.1 mL of the filtrate was pipetted out and diluted to 10 mL with phosphate buffer (pH 7.4). The content of active constituents was estimated spectrophotometrically by using standard curve plotted at 273 nm ( $\lambda_{max}$  of active constituents in the extracts) [9].

##### Spreadability

Two sets of glass slides of standard dimensions were taken. The herbal gel formulation was placed over one of the slides. The other slide was placed on the top of the gel, such that the gel was sandwiched between the two slides in an area occupied by a distance of 7.5 cm along the slides. Hundred g weight of gel was placed on the upper slides so that the gel was between the two slides was pressed uniformly to form a thin layer. The weight was removed and the excess of gel adhering to the slides was scrapped off. The two slides in position were fixed to a stand without slightest disturbance and in such a way that only upper slides to slip off freely by the force of weight tied on it. A 20 g weight was tied to the upper slide carefully. The time taken for the upper slide to travel the distance of 7.5 cm and separated away from the lower slide under the influence of the weight was noted. The experiment was repeated for three times and the mean time was taken for calculation [9].

Spreadability was calculated by using the following formula:

$$S = m \times l/t$$

where, S= spreadability, m-weight tied to upper slides (20 g), l- length of the glass slide (7.5 cm), t- time taken in sec

##### Extrudability Study

A closed collapsible tube containing about 20 g of gel was pressed firmly at the crimped end and a clamp was applied to prevent any roll back. The cap was removed and the gel was extruded. The amount of the extruded gel was collected and weighed. The percentage of the extruded gel was calculated.

##### Skin Irritation Study

The wister albino rats of either sex weighing 150-200g were used for this test. The intact skin was used. The hairs were removed from the rat 3 days before the experiment. The gel containing extract were used on test animals. Gel base was applied on the back of animal taken as control. The animals were treated daily up to seven days and finally the treated skin was examined visually for erythema and edema [6].

##### In-vitro Diffusion studies

*In vitro* diffusion studies were carried out using Franz diffusion cell. An exact amount of formulations (1.0 g) was spread out on membrane positioned between the donor and receptor chambers with an available diffusion area. The receptor compartment was filled with phosphate buffer pH 6.8 and continuously stirred with a small magnetic bar at a speed of 50 rpm during the experiments to ensure homogeneity and maintained at 37±0.5°C. An aliquot of 1mL was withdrawn at specific time intervals up to 3hrs, and was estimated spectrophotometrically at

273 nm. After each withdrawal, the diffusion medium was replaced with an equal volume of fresh diffusion medium. The cumulative percent release was calculated for each time (in hr) interval [7, 9].

**Ex-vivo Permeation studies**

Ex-vivo permeation studies were carried out using goat abdominal skin. The skin was tied to the diffusion cell (donor cell) such that the stratum corneum side of the skin was in intimate contact with the release surface of the formulation in the donor cell as shown in Fig. 1.



**Fig.1. Ex-vivo Permeation Studies Using Goat Abdominal Skin**

Isonic phosphate buffer solution, pH 7.4 (100 mL) was added to a donor compartment prior to be mounted on the diffusion cell. A weighed quantity of formulation equivalent to 1 g of gel was taken on to the skin and was immersed slightly in 100 mL of receptor medium, which was continuously stirred. The entire system was maintained at 37±1 °C. An aliquot of 1 mL was withdrawn at specific time intervals up to 3hrs, and was estimated spectrophotometrically at 273 nm. After each withdrawal, the diffusion medium was replaced with an equal volume of fresh diffusion medium. The cumulative percent release was calculated for each time (in h) interval [9, 10].

**Evaluation Of Anti-inflammatory Activity**

The anti-inflammatory activity of herbal gel formulation was evaluated by the carrageenan-induced rat hind paw edema method. Anti-inflammatory activity of the formulated herbal gel was compared to the marketed gel of diclofenac (Voveran gel). The experimental protocol was designed and approval of Institutional Animal Ethics Committee (IAEC) (Reg. No. 1656/PO/R/S/12/CPCSEA) was obtained. Healthy Wistar albino rats of either sex weighing between 150n200 g were obtained from the disease free small animal house of Hindu college. All rats were fasted for 24hrs before the experiment with water ad libitum. Rats were divided into 3 groups (standard, test, control). 60 min before 0.1 mL 1% carrageenan in isotonic saline was injected sub-plantar into the left hind paw. 0.2g of herbal gel containing 1% sesbania grandiflora leaf extract was applied to the plantar surface of the hind paw by gentle rubbing 50times with the index finger. Rats of the control groups received the plain gel base and 0.2gm 1% diclofenac gel applied in the same way was used as a standard. paw volume was measured immediately after albumin injection and at 1, 2& 3hrs interval after the administration by using a plethysmometer [10, 11]. The percentage inhibition of inflammation was calculated by the following formula:

$$\% \text{ inhibition of test} = \frac{V_c - V_t}{V_c} \times 100$$

Where  $V_c$  volume of the control  
 $V_t$  volume of the test

**Stability Studies**

The main objective of the stability testing is to provide evidence on how the quality of the drug product varies with time under the influence of temperature and humidity. The stability study for the topical herbal gel formulation was done as per ICH guidelines in a stability chamber for a period of 6 months. The formulated gel were filled in the collapsible tubes and stored at different temperatures and humidity conditions of 25°C ± 2°C/60% RH ± 5% RH, 32°C ± 2°C/60% RH ± 5% RH and 40°C ± 2°C/75% RH ± 5% RH. Samples were withdrawn at an initial, first, second, third and sixth months and evaluated for change in color, odor, homogeneity, pH, viscosity, net content [9].

**RESULTS AND DISCUSSION**  
**Phytochemical screening**

The plant extract was subjected for several tests like carbohydrates, reducing sugar, cardiac glycosides, anthraquinone glycosides, amino acids and proteins, starch and triterpenoids, Tannins and phenolic compounds, alkaloids and flavanoids and results were shown in Table2. From the results it was concluded that Carbohydrates, Cardiac glycosides, Anthraquinone glycosides, Triterpenoids, Tannins, Phenolic compounds Alkaloids, Flavanoides were present in the Sesbania grandiflora Leaves extract.

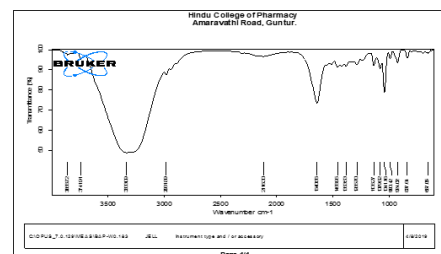
**Table 2 Results Of Phytochemical Screening.**

S.No	Test	Results
1	Carbohydrates	+
2	Reducing sugar	+
3	Cardiac glycosides	+
4	Anthraquinone glycosides	-
5	Amino acids and proteins	+
6	Starch and triterpenoids	+
7	Tannins and phenolic compounds	+
8	Alkaloids	+
9	Flavanoids	+

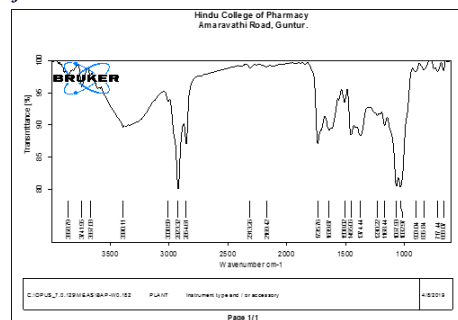
+ sign indicates: Presence of compounds  
 - sign indicates: Absence of compound

**Pre-formulation Studies**

From the FT-IR spectra of leaves extract and physical mixture of the extract, Polymer and other Ingredients, it was observed that the Peaks of major functional groups of Sesbania Grandiflora leaves extract were also observed in spectrum of physical mixture and results showed in Fig2, 3.



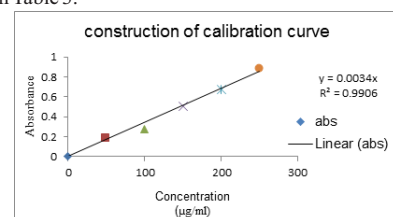
**Fig. 2. FT-IR Spectrum Of Ethanolic Extract Of Sesbania Grandiflora Leaves.**



**Fig. 3. FT-IR Spectrum Of Physical Mixture Of Leaves Extract And Polymer Corbopol-934.**

**Construction of calibration curve**

Standard plot of sesbania grandiflora was plotted as per the procedure in experimental methods. The standard graph of S. Grandiflora (Fig.4) showed good linearity with R<sup>2</sup> value of 0.990, which it indicates that it obeys Beer's- lambert's law in the concentration range of 0-250µg/ml as shown in Table 3.



**Fig. 4. Calibration Curve For Ethanolic Extract Of Sesbania Grandiflora Leaves.**

**Table 3 Data For Construction Of Calibration Curve.**

Concentration( $\mu\text{g/ml}$ )	Absorbance
0	0
50	0.182
100	0.276
150	0.511
200	0.681
250	0.88

**Evaluation of Prepared Herbal gel**

The prepared herbal gel was evaluated for Homogeneity, Grittiness, pH, Viscosity, Spreadability, Extrudability, Drug content, Skin Irritation and Results were shown in Table 4.

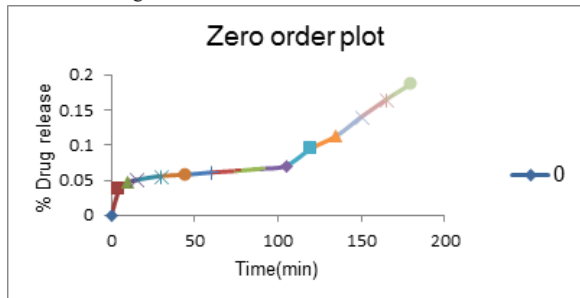
**Table 4 Results Of Various Evaluation Parameters.**

S.No	Evaluation Parameters	Observation
1.	Homogeneity	clear
2.	Grittiness	No grittiness
3.	pH	5.4 - 5.9
4.	Viscosity	0.18cps
5.	Spreadability	7.2cm/sec
6.	Extrudability	91.73%
7.	Skin irritation	No evidence of skin irritation
8.	Drug content	3.5mg

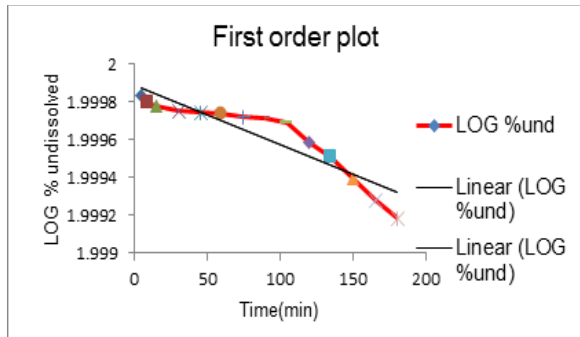
From the results it was clearly evident that the gel formulation showed good gelling property and homogeneity and non greasy characteristics. The pH of the formulation was in the range of 5.4-5.9 which lies in the normal pH range of skin, which indicates that skin compatibility and the herbal gel can be applied to the skin without any irritation. The drug content of formulation was found to be 3.5 $\mu\text{g/gm}$ . The Rheological behavior of gel formulation was studied with Brookfield viscometer the results indicate that formulation followed shear thinning effect with thixotropic property. In case of Spreadability lesser time is taken for the separation 2 slides which indicate better Spreadability. The percentage of Extrudability of formulated gel was found to be more than 90% which indicates the excellent extrudability. There is no evidence of Skin Irritation even at the end of 7<sup>th</sup> day of application. The results of Stability studies reveal that there is no significant change in pH, viscosity, spreadability and drug content.

**In-vitro diffusion studies**

The formulated herbal gel was characterized for drug diffusion study using franz diffusion cell through dialysis membrane according to the procedure and the result were shown in Fig. 5, 6. The results indicate that 99.81% drug was released at the end of three hours.

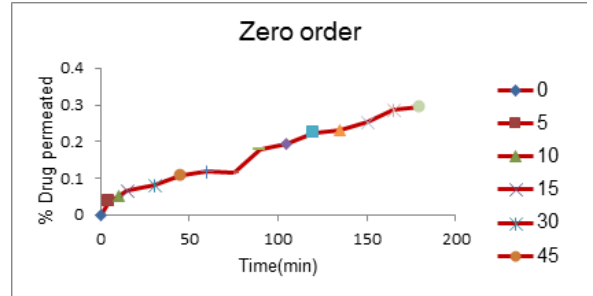


**Fig. 5. Zero Order Plots For In-vitro Diffusion Studies.**

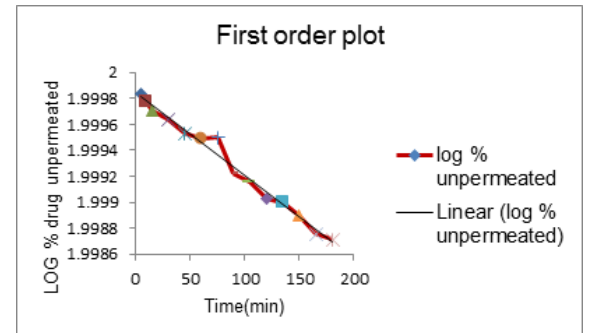


**Fig. 6. First order plots for In-vitro diffusion studies. Ex-vivo permeation studies**

The prepared herbal gel was evaluated for skin permeation studies by using goat skin according to procedure and graphs were plotted. The results were shown in Fig. 7, 8. The results indicate that 97.87% drug was permeated at the end of three hours.



**Fig. 7. Zero order plots for Ex-vivo permeation studies.**



**Fig. 8. First order plots for Ex-vivo permeation studies.**

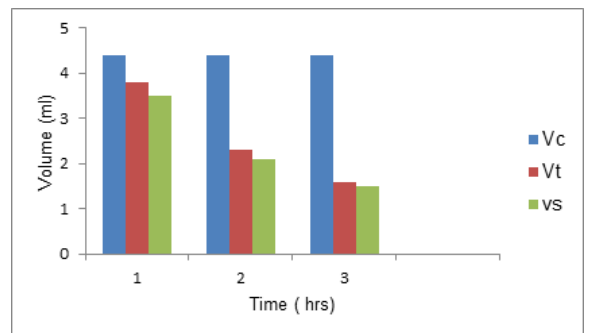
**Evaluation of anti inflammatory property**

The prepared herbal gel was evaluated for the anti-inflammatory activity in albino rats according to the procedure and results were shown in the Table 5 & Fig. 9. From the Results the percent inhibition of inflammation for plant extract was found to be 63%, it was comparable with standard Diclofenac gel (65%).

**Table 5 Results From In-vivo Anti-inflammatory Studies.**

Time (hrs)	$V_{c(ml)}$	$V_{t(ml)}$	$V_{s(ml)}$
1	4.4	3.8	3.5
2	4.4	2.3	2.1
3	4.4	1.6	1.5

$V_c$  - Volume of control  
 $V_t$  - Volume of test  
 $V_s$  - Volume of standard



**Fig. 9. Anti-Inflammatory studies.**

**CONCLUSION**

Mumps is a common childhood infection caused by the mumps virus. Mumps is vaccine-preventable, and one dose of mumps vaccine is about 80% effective against the disease. However, there have been outbreaks of disease in vaccinated populations. In 2005, a large epidemic peaked in the UK, and in 2006 the American midwest had several outbreaks. Complications from mumps are rare, but can be serious if left untreated. Mumps mostly affects the parotid glands. However, it can also cause inflammation in other areas of the body, including the brain and reproductive organs. Orchitis is inflammation of the testicles that may be due to mumps. Because mumps is viral,

antibiotics cannot be used to treat it, and at present, there are no anti-viral medications that can treat mumps. Current treatment can only help relieve the symptoms until the infection has run its course and the body has built up an immunity, much like a cold. From ancient time the leave extract of *Sesbania grandiflora* is applied to the inflamed parotid gland for the symptomatic relief of mumps as a home remedy. But now a day (In 21<sup>st</sup> century) due to Urbanization/ Industrialization the plant are not available commonly. In this context we are aimed to formulate and evaluate herbal gel from leave extract of *Sesbania grandiflora* for ready made available to the people to get the relief from mumps.

The ethonolic leaves extract of *Sesbania grandiflora* was successfully formulated into gel and evaluated for anti inflammatory activity. The evaluation studies revealed that the formulated herbal gel is stable, safe and effective and the anti inflammatory activity is as comparable with diclofenac gel to get the symptomatic relief from mumps.

#### Declaration Of Interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

#### Acknowledgements

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