FORMULATION AND EVALUATION OF HERBAL ANTI-INFLAMMATORY GEL CONTAINING DAucus CARota LINN EXTRACT

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ABSTRACT
The aim of the present work was to formulate and evaluate herbal anti-inflammatory gel containing Anthocyanin extract. Anthocyanin was extracted from the Daucus carota l. using 1% HCl (v/v) in methanol as a solvent. Anthocyanin gels were prepared using various polymers like Xanthan gum, Carbopol 934, HPMC K₁₀₀ M and other additives. All the Anthocyanin gel formulations were evaluated for FTIR, pH, viscosity, spreadability, extrudability, drug content, in-vitro drug release. And results showed that the FTIR spectral analysis suggested that there were no interaction between the drug and formulation additives. pH of formulations were fairly constant about 6.1-6.7 within the range of skin pH. Gels prepared using xanthan gum were less viscous, more spreadability, excellent extrudability and showed better % drug content compared to gels prepared with Carbopol-934 and HPMC K₁₀₀ M. Best formulations in terms of cumulative % drug release were formulations F₁ and F₂ with 90.3% and 87.7% respectively at the end of 6 hours. Hence F₁ and F₂ gels were further studied for In-vivo anti-inflammatory activity by rat paw edema method, gel F₁ proved the best results in inhibiting the edema when compared to diclofenac gel.

KEYWORDS: Anthocyanin, Anti-inflammatory, Herbal gel.

1. INTRODUCTION
Herbal medicines is important, herbs can treat a variety of different ailments and herbs are universal, exists in every human culture on earth. The WHO estimates that 80 percent of the
population of Asian and African countries use herbal medicine for aspect of primarily health care. The scope of herbal medicine for therapeutic purpose is now well-established, safe and effective. Therapeutic agents like anti-inflammatory, anti-cancer, anti-diabetic, anti-microbial, anti-oxidant, anti-depressant and Alzheimer’s disease.$^1$

Gels are semisolid dispersion systems which may contain suspension of either small or large molecules dispersed in a suitable liquid. As per the definition of I.P. “gels are homogeneous, semisolid preparation usually consisting of solutions or dispersions of one or more medicaments in suitable hydrophilic or hydrophobic base”.

Inflammation is a protective immunovascular response that involves immune cells, blood vessels, and molecular mediators. Inflammation is a host response to foreign pathogens, tissue or injury, and it finally leads to the restoration of normal tissue structure and function. It is also defined as the local response of living mammalian tissues to injury from any agent. It is a body defence reaction in order to eliminate or limit the spread of injurious agent, followed by removal of the necrosed cells and tissues.$^2$ Signs of inflammation such as:

1. **Rubor (redness)** – Due to vasodilation of capillaries to increase blood flow.
2. **Tumor (swelling)** – Due to increased vascular permeability and influx of plasma proteins and phagocytic cells into the tissue spaces.
3. **Calor (heat)** – Due to effect on thermoregulatory site of the brain.
4. **Dolor (pain)** – Due to hyperalgesia, sensitization of nociceptors, local release of pain inducers and increased tissue pressure.

For the inflammation studies, topical application is easy and effective as compared to other routes of administration. Such as self-medication is possible, can be applied easily at the site of inflammation. Herbal medicines are safe and there are no side effects as compared to synthetics drugs.

The present study is to investigate and approach of herbal gel formulation with different concentration of polymers. And also comparison of natural, synthetic and semi-synthetic gelling agents for anti-inflammatory studies for the prepared herbal gel containing Anthocyanin extract.
2. MATERIAL AND METHODS

Materials
Black carrot gift sample from horticulture college, Bagalkot. Carbopol-934 (Himedia Mumbai), HPMC K100 M (Himedia Mumbai), Xanthan gum (Lobo chemiePvt.Ltd. Mumbai), propylene glycol (SDFCL Mumbai), triethanolamine (Pioneer chemical co. Delhi), propyl paraben (Genuine chemical co.Mumbai), methyl paraben (NR CHEM Mumbai), ethanol (SDFCL Mumbai), distil water and Carrageenan.

Drug profile
Black carrot\[3\]

Latin name: Daucus carota linn.
Synonyms: Wild carrot, bird’s nest, bishop’s lace, and Queen Anne’s lace.
Biological source: It is a root vegetable of daucus carota sub sp. Sativus belonging to family Apiaceae

Geographical source: Daucus carota is a plant that is native to Europe and Southwestern Asia. The plant probably originated in Persia, carrot seeds have been found in Switzerland and Southern Germany, carrot is a cool-weather plant, but also cultivated in tropical and subtropical regions.

Life cycle: Annual, Biennial.

Plant botanical description
Black carrot (Daucus carota l.) is a root vegetable, usually purple in colour or dark-purple-black, covered in many fine root hairs and ridges depending upon the growing conditions. The root diameter can range from 1cm to as much as 10cm at the widest part. The root length ranges from 5cm to 25cm. The plant growth 30-60cm tall, which can have different forms: spindle, cylindrical or conical, even globular. Flowers development begins when the flat meristem changes from producing leaves to an uplifted, conical meristem capable of producing stem elongation and a cluster of flowers. A large, primary umbel can contain up to 50 umbellets, each of which may have as many as 50 flowers.

Chemical constitutions: Carotenes, α and β-carotenes, thiamine, dietary fiber, vitamin A and C, polyacetylenes. Red carrots also contains lycopene.
Therapeutic uses: Used as lower bad cholesterol (LDL), treat cancer, treat rheumatoid arthritis, breast cancer, diarrhoea, and diabetes.

![Image of daucus carota](A) Flowers, (B) Leaves, (C) Roots, (D) Black carrots, (E) Root vegetable and (F) Seeds.

Fig. 1 Morphology of *daucus carota*linn (a) Flowers, (b) Leaves, (c) Roots, (d) Black carrots, (e) Root vegetable and (f) Seeds.

Methods

Procedure for anthocyanin extraction[^4]

Prepare 1% v/v solution of conc. Hcl to methanol. Chop some fresh black carrots into tiny pieces and place them in above solution. (Like one and half time their volume) cover the beaker and put in a cool, dark place. Let it stand for 24 hours. Later, strain it through filter paper and save it. Discard the sample and extract was evaporated to dryness under at 40° C. Then the Anthocyanin extract obtained was collected and stored in 4° C.

Identification tests for anthocyanin

IR Spectroscopy: The FT-IR spectrum of the obtained sample of the drug was compared with the standard FT-IR spectra of the pure drug.
Solubility analysis: Preformulation solubility analysis was done, which included the selection of suitable solvent to dissolve the drug and also to test its solubility in the dissolution medium, which was to be used.

Phytochemical tests for extract\(^5\)

Test for carbohydrates

Molisch’s test: 2-3ml of methanolic extract was taken in test tube then few drops of α-naphthol solution in alcohol was added and shaken followed by addition of concentrated Sulfuric acid (H\(_2\)SO\(_4\)) from sides of the test tube.

Fehling’s test: 1ml Fehling’s A and Fehling’s B solution, boiled for 1min add test methanolic extract was heated in boiling water bath for 10min.

Test for alkaloids

Dragendorff’s test: To 2-3ml filtrate, add few drops of dragendorff’s reagent.

Test for amino acids

Ninhydrin test: Heat 3ml of methanolic extract with 3 drops of 5% ninhydrin solution and boiled in water bath for 10 min and cool.

Test for proteins

Millon’s test: 3ml of methanolic extract was mixed with 5ml of Millon’s reagent.

Test for flavonoids

Ferric chloride test: To the methanolic extract add 2-3 drops of ferric chloride reagent.

Sulphuric acid test: Methanolic extract was taken and concentrated sulphuric acid was added.

Test for phenolic compounds

Lead acetate test: 2-3ml of methanolic extract, followed by few drops of lead acetate solution.

Dilute nitric acid (H\(_{\text{NO}_3}\)) test: 2-3ml of methanolic extract was added to few drops of diluted H\(_{\text{NO}_3}\) solution.
Test for steroids

Liebermann’s test: To 2ml of methanolic extract add 2ml of acetic anhydride, boil it and then add sulphuric acid.

Test for glycosides

Borntragers’s test: 3ml of methanolic extract and dil.\(\text{H}_2\text{SO}_4\) was boiled and filtered. To cold filtrate, equal volume chloroform, was added and shake well. Separate the organic solvent and slowly add ammonia.

Confirmatory test for anthocyanin\[^{[6,7]}\]

1. To the \textit{Daucus carota} l. extract, aluminium chloride addition will give a shift of 12 nm in spectrophotometer which confirms the Anthocyanin.
2. The \textit{Daucus carota} l. extract, was analysed using UV-Visible spectrophotometer, absorbance between 200 – 700 nm indicates the presence of Anthocyanin.
3. The \textit{Daucus carota} l. extract was mixed with 2M HCl and heated for 5min at 100˚C.
4. To the \textit{Daucus carota} l. extract, add 2M NaOH dropwise.

Analytical methods

Development of UV spectroscopic method

Determination of absorption maxima\[^{8}\]

Absorption maxima are the wavelength at which maximum absorption takes place. For accurate analytical work, it is important to determine the absorption maxima of the substance under study.

Procedure

For the preparation of calibration curve, stock solution was prepared by dissolving 100 mg of accurately weighed Anthocyanin in 100 ml of methanol (1mg/ml) at 100 ml volumetric flask and volume make up to the mark with methanol. From this stock solution 1ml is pipetted out into 10 ml volumetric flask after that volume is adjusted made up to the mark with methanol which gives the concentration of 100μg/ml and subject for UV scanning in the range of 400 - 800 nm using double beam UV-VIS spectrophotometer, (Shimadzu -1601, shimadzu, Japan). The absorption maxima was obtained at 536 nm with a characteristic peak.
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Preparation of calibration curve

Using absorption maxima a standard curve was prepared in the concentration range of 100-600 μg/ml. From the stock solution (1 mg/ml), pipette out 1, 2, 3, 4, 5 and 6 ml into a series of 10ml volumetric flask and volume was made up to the mark 10 ml with methanol to get 100, 200, 300, 400, 500, 600 μg/ml of Anthocyanin respectively. The optical density values of resulting solutions were measured at 536 nm.

FTIR studies[9]

The compatibility between pure drug and gelling agents was detected by FTIR spectra. The potassium bromide pellets were prepared on KBr press. To prepare the pellets the solid powder sample were ground together in a mortar with 100 times quantity of KBr. The finely grounded powder was introduced into a stainless steel die. The powder was pressed in the die between polished steel anvils at a pressure of about 10t/in². For liquid samples thin film of sample liquid is made on pellet. The spectra’s were recorded over the wave number of 3900-1 to 450 cm⁻¹

Characterisation of anthocyanin

In-vitro drug release study[12]

The in-vitro release of Anthocyanin were studied using a modified diffusion testing apparatus. The freshly prepared phosphate buffer (pH 6.8) was used as a diffusion medium. Semi permeable membrane, previously soaked in the diffusion medium for overnight, was tied to one end of a specially designed glass cylinder (open at both ends) having inner diameter of 3.4 cm. 10 mg of extract was accurately pipette into the glass cylinder known as donor chamber. The cylinder was suspended in a beaker (Acceptor chamber) containing 100ml of diffusion medium so that the membrane just touches the surface of the medium. Acceptor chamber was maintained at a temperature of 37 ± 2°C with a stirring rate of 50 rpm using magnetic stirrer. About 5 ml of sample was withdrawn at a specified time intervals and diluted suitably then it was replaced with an equal volume of fresh diffusion medium. The aliquots were analysed at 536 nm using UV spectrophotometer.
Experimental methods

Preparation of anthocyanin gel

Table 1: Composition of gel formulations with different polymers such as Carbopol-934, Xanthan gum and HPMC K100M.

| Code | Drug | Xanthan gum | Carbopol-934 | HPMC K100M | Methyl paraben | Propyl paraben | Ethanol | Propylene glycol | Tri-ethanolamine | Water up to 100 |
|------|------|-------------|--------------|------------|----------------|----------------|---------|-----------------|-----------------|----------------|}
| F_1  | 0.5  | 1           | -            | -          | 0.2            | 0.02           | 5       | 5               | q.s             | 100            |
| F_2  | 0.5  | 1.5         | -            | -          | 0.2            | 0.02           | 5       | 5               | q.s             | 100            |
| F_3  | 0.5  | -           | 1            | -          | 0.2            | 0.02           | 5       | 5               | q.s             | 100            |
| F_4  | 0.5  | -           | 1.5          | -          | 0.2            | 0.02           | 5       | 5               | q.s             | 100            |
| F_5  | 0.5  | -           | -            | 1          | 0.2            | 0.02           | 5       | 5               | q.s             | 100            |
| F_6  | 0.5  | -           | -            | 1.5        | 0.2            | 0.02           | 5       | 5               | q.s             | 100            |

Gel preparation methods\(^{10}\)

For different gels, the concentrated *daucus carota l.* extract (Anthocyanin) as the active ingredient and Carbopol-934, Xanthan gum and HPMC K\(_{100}\)M were used as gelling polymers.

Preparation of Carbopol-934 gel /Xanthan gum/Hpmck100m

First methyl paraben and propyl paraben (as preservative) were dissolved in 40°C purified water and then a specific amount of carbopol-934/xanthan gum/ HPMC K\(_{100}\)M was mixed with it till homogenous using a magnetic stirrer with 1200 rpm for 30 minutes. A determined amount of Anthocyanin extract was weighed and mixed well with ethanol and propylene glycol. This mixture was slowly added to the gel and mixed to achieve a uniform gel. While monitoring the pH, tri-ethanolamine was added to adjust pH of the gel.

Characterization of anthocyanin gels

pH determination\(^{11}\)

The pH of gel were measured by digital pH meter right after formulation, in 48 hours, 1 week, 2 weeks, 1 month and 3 months. One gram of gel was dissolved in 100ml distilled water and stored for 2 hours. The pH meter was calibrated with standard buffers before measurement and each time the measuring was repeated 3 times and the mean was calculated.
Homogeneity$^{[12]}$
All developed gels were tested for homogeneity by visual inspection after the gels have been set in the container. Preparations were tested for their appearance and presence of any aggregates.

Centrifuge test
Formulations were separately centrifuged in a test tube of 10 cm long and 1 cm width for 5, 15, 30 and 60 minutes with 2000 rpm and then studied for sedimentation and gel stability.

Temperature change test$^{[11]}$
To control the formulation stability in different seasons and different temperature conditions, tubes containing formulations were kept in temperatures of 2-8°C, 25°C and 40-45°C and then their appearance quality was controlled after 48 hours, 1 week, 2 weeks, 1 month and 3 months.

Evaluation of anthocyanin gels
Viscosity$^{[13]}$
The viscosity of the prepared gel was measured with a Brookfield Viscometer (Brookfield engineering labs INC. USA). The gels were rotated at 50 rpm using spindle no. 64. At each speed, the corresponding dial reading was noted.

Spreadability test$^{[14]}$
Spreadability was determined by using a modified apparatus which consists of a glass plate block, which was provided by a pulley at one end. Two sets of glass slides of standard dimension were taken. A 20gm of weight was tied to the upper slide carefully and provided with the hook. The formulation of gel (about 2 gm) was placed over one of the slide. The other slide was placed on the top of the gel, such that the gel was sandwich between the two slides in an area occupied by a distance of 6.5 cm along the slide. 100gms of weight was placed on the top of the slides for 5 min to expel air and to provide a uniform film of the gel between the slides. Excess of the gel was scrapped off from the edges. The top slide was then subjected to pull of 20gms weight, with the help of string attached to the hook and the time (in seconds) required by the top slide to cover a distance of 6.5 cm be noted. The experiment was repeated by three times and the mean time was taken for the calculation. Lesser the time taken for separation of the two slides, better the spreadability.
Spreadability was calculated using the following formula:

\[ S = \frac{M \times L}{T} \]

Where, 
- \( L \) = length moved by glass slide (6.5),
- \( T \) = time in seconds,
- \( M \) = weight in pan (tied to upper slide),
- \( S \) = spreadability.

**Extrudability test**[13]

A closed collapsible tube containing about 10gm 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 percentage of the extruded gel was calculated. More quantity extruded better was extrudability. (>90% extrudability: excellent, >80% extrudability: good, >70% extrudability: fair.)

**Drug content**[12]

2g of developed gel was taken and dissolved in 100 ml of phosphate buffer pH 6.8. Then the gel solution was shaken for 2 hr in order to achieve complete solubility of drug. This solution was filtered using filter paper. After suitable dilution, drug absorbance was recorded by using UV-visible spectrophotometer (UV–1608, Shimadzu, Japan) at \( \lambda_{max} \) 536 nm using phosphate buffer pH 6.8 as blank.

**In-vitro drug release studies**[12]

The in-vitro release of Anthocyanin from the prepared formulations was studied using a modified diffusion testing apparatus. The freshly prepared phosphate buffer (pH 6.8) was used as a diffusion medium. Semi permeable membrane, previously soaked in the diffusion medium for overnight, was tied to one end of a specially designed glass cylinder (open at both ends) having inner diameter of 3.4 cm. 10 gm of formulation was accurately pipetted into the glass cylinder known as donor chamber. The cylinder was suspended in a beaker (Acceptor chamber) containing 100 ml of diffusion medium so that the membrane just touches the surface of the medium. Acceptor chamber was maintained at a temperature of 37 ± 2°C with a stirring rate of 50 rpm using magnetic stirrer. About 5 ml of sample was withdrawn at a specified time intervals and replaced with an equal volume of fresh diffusion medium. The aliquots were analysed at 536 nm using UV spectrophotometer.
Anti-inflammatory study\textsuperscript{15}

Animals

Wistar albino rats (200-250g) were obtained from the central animal house of H.S.K. College of Pharmacy and Research Centre, Bagalkot, Karnataka. All the animals were kept under standard husbandry conditions (Temp. 22-28° C; Relative Humidity 65±10%) for 12 hr dark and 12 hr light cycle respectively in standard propylene cages. The animals were fed with standard food (Pranav agro industries, sangli, Maharashtra) and water \textit{ad libitum}. All the experiments were conducted in accordance with direction of Institutional Animal Ethics Committee (IAEC/HSKCOP/MAY 2019/PGI)

To determine the anti-inflammatory study, the over-night starved rats were divided into 4 groups, containing 6 animals in each group as follows:

i. Control group applied with plain gel + carrageenan
ii. Effect of diclofenac gel (voveran 1% gel) + carrageenan
iii. Effect of Anthocyanin [0.5%] gel F1 1% of xanthan gum + carrageenan
iv. Effect of Anthocyanin [0.5%] gel F2 1.5% of xanthan gum + carrageenan

The edema was induced by injection 0.1ml of carrageenan solution (1.0% w/v in normal saline) into the sub-plantar region of the left hind paw after 1hr of drug administration. The paw-volume was measured with the help of Digital Plethysmometer (7140 UGO Basile, Italy) at different intervals of time at 0, 0.5, 1, 2, 3, 4 and 5hr. The results are summarised in the table and fig. The percentage inhibition of paw edema volume of each treated groups is calculated by the equation:

\[
\text{Percentage of inhibition, \% = } \frac{\text{Volume of control} - \text{Volume of test} \times 100}{\text{Volume of control}}
\]

3. RESULT AND DISCUSSION

The present work aimed to formulate and evaluate herbal gel formulations. The herbal gels were prepared with different polymers such as xanthan gum, Carbopol-934 and HPMC K 100 M. The extracted drug and prepared formulation were characterised for FTIR, pH, solubility, phytochemical investigation and spreadability, viscosity, extrudability, drug content, \textit{in-vitro} drug release and \textit{in-vivo} anti-inflammatory study.
Identification tests for anthocyanin

Table 2: Preliminary phytochemical investigation.

<table>
<thead>
<tr>
<th>Test</th>
<th>Observation</th>
<th>Inference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test for carbohydrates: Molish’s test;</td>
<td>Violet ring formed at the junction of two liquid.</td>
<td>Absent</td>
</tr>
<tr>
<td>Fehling’s test:</td>
<td>Yellow colour, then brick red precipitation.</td>
<td>Absent</td>
</tr>
<tr>
<td>Test for alkaloids: Dragendorff’s test:</td>
<td>Orange brown precipitate</td>
<td>Absent</td>
</tr>
<tr>
<td>Test for amino acids: Ninhydrin test:</td>
<td>Purple or bluish colour</td>
<td>Absent</td>
</tr>
<tr>
<td>Test for proteins: Millon’s test:</td>
<td>White precipitated, warm precipitated and turns brick red colour</td>
<td>Absent</td>
</tr>
<tr>
<td>Test for flavonoids: Ferric chloride test:</td>
<td>Green to black colour</td>
<td>Present</td>
</tr>
<tr>
<td>Sulphuric acid test:</td>
<td>Orange to red colour</td>
<td>Present</td>
</tr>
<tr>
<td>Test for phenolic compounds: Lead acetate test:</td>
<td>White precipitate</td>
<td>Present</td>
</tr>
<tr>
<td>Dilute nitric acid test:</td>
<td>Reddish to yellow colour</td>
<td>Present</td>
</tr>
<tr>
<td>Test for steroids: Libermann test:</td>
<td>Green colour appears</td>
<td>Absent</td>
</tr>
<tr>
<td>Test for glycosides: Borntrager’s test:</td>
<td>Ammonical layer turns pink or red colour</td>
<td>Present</td>
</tr>
</tbody>
</table>

Confirmatory test for anthocyanin

1. Addition of aluminium chloride to the Daucus carota l. extract gave a shift of 12 nm in spectrophotometer, this confirms the presence of Anthocyanin.
2. The Daucus carota l. extract was analysed using UV-Visible spectrophotometer, absorbance between 200 – 700 nm. It shows the absorbance at 536 nm, this confirms the presence of Anthocyanin.
3. The Daucus carota l. extract mixed with 2M HCl and heated for 5min at 100°C. Extract remain in purple colour, this confirms the presence of Anthocyanin.
4. The Daucus carota l. extract mixed with 2M NaOH, extract appeared green colour, this confirms the presence of Anthocyanin.

Solubility analysis: Anthocyanin is soluble in water and organic solvents.

Analytical methods

Development of UV spectroscopic method: \( \lambda_{\text{max}} \) of Anthocyanin was found to be 536 nm as it showed maximum absorbance in this wavelength. The result of obtained peak is represented in Fig.no.2.
FTIR studies: IR spectra of the pure drug, polymers and combination of them were represented in Fig. no.4-4c.

Compatibility studies were performed using IR spectrophotometer. The IR spectrum of pure drug, pure polymer and physical mixture of drug and polymers were studied. The characteristic absorption peaks of Anthocyanin were given figure 4 to 4c. The characteristic peak found in pure Anthocyanin were also found in physical mixture of drug and polymer which indicates there were no changes in these main peaks in IR spectra of mixture of drug.
and polymers, which shows that there were no physical & chemical interactions. The peaks obtained in the spectra’s of each sample correlates with the peaks of drug spectrum. This indicates that the drug was compatible with the formulation components.

![Fig. 4: FTIR spectra of anthocyanin.](image1)

![Fig. 4a: FTIR spectra of Anthocyanin + Xanthan gum.](image2)
Characterization of anthocyanin gels

**pH determination of Anthocyanin gel:** pH of formulations were fairly constant about 6.1 – 6.7, which lies in the normal pH range of the skin and with time no skin irritation was observed.

**Homogeneity:** Almost all the formulations were found to be homogeneous and there were no aggregate formation.

**Centrifuge test:** There were no observable sediment in centrifuge tests and the gels kept their uniformity.
**Temperature change test:** All the formulations were subjected to different temperature conditions and there were no appearance changes observed.

**Evaluations of anthocyanin gels**

The prepared Anthocyanins gel formulations were subjected for various evaluation parameters studies and the results data is depicted in Table No.3. All the gel formulations showed good viscous and they were capable to remain in the site of application for prolonged time. Among the prepared formulations, Gels prepared with HPMC K\textsubscript{100}M were more viscous compared to gels prepared with Carbopol-934 and Xanthan gum.

The values of spreadability indicate that the gel was easily spreadable by small amount of shear. Because of less viscosity gels prepared using xanthan gum were more spreadable than gels prepared using carbopol-934 and HPMC K\textsubscript{100}M.

The values of extrudability indicates that the gels were showed good extrudability. Among these formulations, gel prepared with xanthan gum showed excellent extrudability than gels prepared with carbopol-934 and HPMC K\textsubscript{100}M.

All gel formulations showed good % drug content from 92 to 97%. Among these formulations, the formulations F\textsubscript{1} & F\textsubscript{2} showed better percentage drug content than other formulations.

**Table 3: Evaluation parameters of gel formulations.**

<table>
<thead>
<tr>
<th>Formulation code</th>
<th>Parameters</th>
<th>Viscosity in cps</th>
<th>Spreadability in gm.cm/sec</th>
<th>Extrudability in %</th>
<th>Drug content in %</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td></td>
<td>7050</td>
<td>28.88</td>
<td>95.53</td>
<td>97.81</td>
</tr>
<tr>
<td>F2</td>
<td></td>
<td>9080</td>
<td>28.26</td>
<td>93.26</td>
<td>96.56</td>
</tr>
<tr>
<td>F3</td>
<td></td>
<td>10630</td>
<td>25.49</td>
<td>91.04</td>
<td>95.93</td>
</tr>
<tr>
<td>F4</td>
<td></td>
<td>10800</td>
<td>26</td>
<td>90.67</td>
<td>95.31</td>
</tr>
<tr>
<td>F5</td>
<td></td>
<td>10910</td>
<td>23.63</td>
<td>90.69</td>
<td>92.18</td>
</tr>
<tr>
<td>F6</td>
<td></td>
<td>11060</td>
<td>23.21</td>
<td>87.81</td>
<td>93.43</td>
</tr>
</tbody>
</table>

**In-vitro drug release studies**

All the prepared Anthocyanins gel formulations were subjected to *in-vitro* drug release studies and data is shown in the table no.4 and diffusion profiles were given in figures 5 to 5b.
All the gel formulations showed sustained released up to certain period of time. The percentage drug release between every time intervals were fairly constant up to 6 hours.

All the gel formulations showed good percentage drug release. Among these formulations, the formulation containing xanthan gum showed better release than formulations containing carbopol-934 and HPMC K_{100}M. Viscosity is an important physical property which affects the rate of drug release. In general increase in viscosity decreases the rate of drug release. In our study formulation with less viscosity such as gel containing Xanthan gum showed better rate of drug release than formulations with more viscosity such as formulations containing carbopol-934 and HPMC K_{100}M.

Table 4: Cumulative percentage drug release v/s time for pure drug and all gel formulations.

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Pure drug</th>
<th>F1</th>
<th>F2</th>
<th>F3</th>
<th>F4</th>
<th>F5</th>
<th>F6</th>
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<tbody>
<tr>
<td>30</td>
<td>37.68</td>
<td>28.06</td>
<td>27.3</td>
<td>26.06</td>
<td>26.92</td>
<td>27.56</td>
<td>26.56</td>
</tr>
<tr>
<td>60</td>
<td>48.06</td>
<td>34.34</td>
<td>33.56</td>
<td>29.82</td>
<td>30.82</td>
<td>34.84</td>
<td>28.32</td>
</tr>
<tr>
<td>120</td>
<td>62.42</td>
<td>51.42</td>
<td>47.9</td>
<td>34.6</td>
<td>41.36</td>
<td>43.68</td>
<td>39.72</td>
</tr>
<tr>
<td>180</td>
<td>75.77</td>
<td>63.3</td>
<td>57.98</td>
<td>48.8</td>
<td>48.54</td>
<td>49.34</td>
<td>48.24</td>
</tr>
<tr>
<td>240</td>
<td>90.4</td>
<td>80.3</td>
<td>66.32</td>
<td>64.04</td>
<td>63</td>
<td>65.04</td>
<td>63.58</td>
</tr>
<tr>
<td>300</td>
<td>-</td>
<td>85.58</td>
<td>75.34</td>
<td>73.68</td>
<td>75.26</td>
<td>75.96</td>
<td>74.08</td>
</tr>
<tr>
<td>360</td>
<td>-</td>
<td>90.3</td>
<td>87.7</td>
<td>83.26</td>
<td>85.5</td>
<td>86.08</td>
<td>82.03</td>
</tr>
</tbody>
</table>

![Fig. 5: Release profile of anthocyanin.](image-url)
Anti-inflammatory study

The control group had shown significant increase in the paw volume after carrageenan induction in the sub plantar region of the right hind paw; this is due to the release of inflammatory mediators like histamine, serotonin, kinins, PGs and others. The marketed Diclofenac Gel (voveran 1% gel) and Anthocyanin gel F1 & F2 showed significant inhibitory activity against carrageenan induced paw edema inflammation.

Data are given in table no.5 and graph represented in fig. no.6, among these groups gel F1 has showed best % inhibitory (59.07%) compared to marketed diclofenac gel (50.72%) and
gel F2 (55.85%). This observed activity may be due to the inhibition of cyclo-oxygenase enzyme and ultimately this leads to the inhibition of PG synthesis and shows significant activity.

**Table 5: Effect of Anthocyanin gel on carrageenan induced paw oedema volume in rats.**

All values are expressed as mean ± SEM, n=6, *p<0.05, **p<0.01, ***p<0.001 as compared to control group. One way Analysis of Variance (ANOVA) followed by multiple comparisons Dunnett’s test.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Paw Volume in ml (% of edema inhibition)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>½ hr</td>
</tr>
<tr>
<td>Control (plain gel)</td>
<td>1.355±0.033</td>
</tr>
<tr>
<td>Diclofenac</td>
<td>1.107±0.019</td>
</tr>
<tr>
<td>Gel (F1)</td>
<td>1.182±0.017</td>
</tr>
<tr>
<td>Gel (F2)</td>
<td>1.252±0.011</td>
</tr>
</tbody>
</table>

**Fig. 6:** Effect of Anthocyanin gel on carrageenan induced paw oedema volume in rats.

All values are expressed as mean ± SEM, n=6, *p<0.05, **p<0.01, ***p<0.001 as compared to control group. One way Analysis of Variance (ANOVA) followed by multiple comparisons Dunnett’s test.

**4. CONCLUSION**

It can be concluded from the present investigation that Anthocyanin gel formulations can be conveniently prepared using different gelling agents such as Carbopol-934, Xanthan gum and HPMC K100 M at different concentrations. The varying concentration of the three polymers
was found to affect the gel parameters like viscosity, spreadability and extrudability. All gel formulations has shown good homogeneity and good stability.

However, xanthan gum gel formulations proved best choice for formula, since it showed the lesser viscosity, higher the spreadability and excellent percentage of extrudability. Formulation F1 with 1% of xanthan gum showed that there is potential and remarkable protective role in the acute inflammatory model than F2 with 1.5% of xanthan gum. Hence, there is no need to use 1.5% polymers for the preparation of medicines for anti-inflammatory action.

5. REFERENCES
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