A novel poly herbal transdermal patches formulation development and evaluation for treatment of osteoarthritis

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ABSTRACT:

Osteoarthritis causes degenerative changes in the joints i.e. “wear and tear” resulting in pain and impaired function. Pathological cartilage loss occurs due to imbalance in the catabolic& anabolic mechanisms of cartilage remodeling. A Transdermal Delivery of Drugs is a unique method of delivering medicinal agents into the bloodstream via the skin’s surface. The primary purpose for developing these is to create a novel medication delivery system that helps to reduce drug retention, metabolism, and fluctuation through the skin. Ethanolic extract of Cissus quadrangularis:Nigella sativa (80:20) is more efficient in treating osteoarthritis hence same combination was used in formulation of transdermal patch. The patches were prepared by solvent evaporation method and by using HPMC (Hydroxypropyl methylcellulose), Ethyl Cellulose (EC), DibutylPhthalate, Ethanol and chloroform. HPMC is freely water soluble, whereas EC is hydrophobic. So the transdermal delivery systems were prepared using HPMC and EC to study the effect of hydrophilic and hydrophobic nature of polymer on release of drug. Developed Patch formulation was evaluated by considering parameters such as organoleptic characteristics, weight uniformity, thickness, drug content, folding endurance, tensile strength, percentage of moisture content & in-vitro permeation study. Formulation TF 2 was found to be most effective as compared to others. Osteoarthritis activity of Transdermal Dermal patch was evaluated by using collagenase type II-induced osteoarthritis (CIOA) rat model. Out of six formulation TF 2 was found to be most effective in osteoarthritis as compared to others.

Keywords: Osteoarthritis, Transdermal Delivery of Drugs, Cissus quadrangularis and Nigella sativa

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Introduction:

Osteoarthritis: The term Arthritis is made up of two Greek word i.e. “arthro“ meaning joint and “itis“ meaning inflammation.1 Osteoarthritis causes degenerative changes in the joints as a result of everyday “wear and tear” resulting in discomfort pain and impaired function. Osteoarthritis (OA) induces pain, stiffness, loss of mobility2 Osteoarthritis is the second most common rheumatological problem and is most frequent joint disease with prevalence of 22% to 39% in India and inflicts about 4-6 crore Indians.3,4 Osteoarthritis is a degenerative joint disease which causes significant cartilage destruction and morphological destruction toward other joint tissues, biochemical changes in the phase of the disease are more subtle. Water is pushed out of normal cartilage by compressive force, whereas water is drawn in through hydrostatic & osmotic pressure collagen fibres give compressive force, whereas Gibbs–Donnan mechanism as well as cartilage proteoglycans produce osmotic pressure, which pulls water in.5,6

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The collagen framework becomes increasingly disorganized as OA advances, as well as the proteoglycan composition of cartilage diminishes. Collagen fibre breakdown results in a net increase in water content. This is because, while there is a reduction of proteoglycans (and consequently a decrease in osmotic pull) this is offset by a loss of collagen. The collagen fibres of cartilage can become prone to disintegration without the protective effects of proteoglycans, accelerating degeneration. Swelling of the synovial membrane and joint capsule enclosing occurs though it is probably small as compared rheumatic arthritis to inflammation is minimal. The menisci might become injured, wear away and the ligaments within the joint can thicken become fibrotic. The menisci could be fully exhausted as time passed because of which a person may needs a joint replacement new bone outward manifestations termed "spurs" or osteophytes may form on the edges of joint due to lack of the menisci most likely in an effort to improve the alignment of the bone and cartilage surfaces. The volume of subchondral bone expands and becomes less calcified (hypomineralization). All of these changes may wreak havoc on your ability to function. Thickened synovium and subchondral bone lesions have been linked to discomfort in osteoarthritic joints.

Transdermal Delivery of Drugs (TDDS): Transdermal Delivery of Drugs (TDDS) is a unique method of delivering medicinal agents into the bloodstream via the skin's surface. The primary purpose for developing this new technique of medication administration is to reduce drug retention and metabolism in the skin, as well as to speed up drug fluctuation through the skin. It also confirms that therapeutic molecules are transported, with preference towards a precise rate into the bloodstream. TDDS has various advantages including avoiding gastrointestinal medication absorption problems avoiding hepatic first pass mechanism and using painless needle forms. If TDDS toxicity develops, the patch can be readily removed. After permeating the skin and entering systemic circulation the drug will only be carried to the specified location. The TDDS can keep the drug level in the blood at a consistent level using penetration enhancers it is feasible to improve drug transdermal permeability. In order to generate therapeutic activity the area of delivery may or not be different.

The steps involved in transdermal penetration of a drug are as follows:
1. Through the stratum corneum, sorption occurs.
2. Drug permeation through a healthy epidermis
3. Drug moiety uptake by the dermal papillary layer's capillary network
4. The drug must possess some physicochemical properties to reach target site via systemically through stratum corneum

Hadjod: It consists of dried or fresh plant of Cissus quadrangularis belonging to family Vitaceae. Phytoconstituents present in Hadjod are α- and β- amyrine, β- sitosterol, vitamin C, quercitin quadrangularins A, B, C etc. Abundant in calcium ions as well as phosphorus is present in stem extract both of which are necessary for bone formation. Hadjod possess antibacterial activity, antioxidant, antiulcer activity, anthelmintic activity, bone healing activity.

Kalonji: Another name of kalonji is Black cumins or Love-in-a-mist. It consists of dried seeds of Nigella sativa belonging to family Raunculaceae. Pharmacologically active constituent of volatile oil are thymoquinone, dithymoquinone, thymol and thymohydroquinone. Dithymoquinone is the dimerised form of Thymoquinone. Pharmacological properties of N. Sativa seed are as antioxidant activity, analgesic and anti-inflammatory activity, antidiabetic activity, anti-cancer activity, wound healing properties, nephroprotective activity, and effect on nervous system.

Extraction of Plants: The powder of Cissus quadrangularis and Nigella sativawas subjected to ethanolic solvent extraction using soxhlet apparatus separately. Then both extract was concentrated. The extract is then used in transdermal formulation in proportion Cissus quadrangularis:Nigella sativa (80: 20)

Development of transdermal patches
Ethanolic extract of *Cissus quadrangularis* and *Nigella sativa* (80:20) was more efficient in treating osteoarthritis. Transdermal patch is prepared evaluated for anti-osteoarthritis activity. The patches were prepared by solvent evaporation method. The studies describing the use of Hydroxypropyl methyl cellulose (HPMC) in transdermal patches and ophthalmic preparations and ethyl cellulose (EC) transdermal delivery systems as well as other dosage forms for controlled release of drugs. HPMC is freely water soluble, whereas EC is hydrophobic. So the transdermal delivery systems were prepared using HPMC and EC to study the effect of hydrophilic and hydrophobic nature of polymer on release of drug. The backing membrane was purchase from the local market. The drug reservoir was prepared by dissolving HPMC or EC in Chloroform: Ethanol (1:1) mixture. Dibutyl phthalate 15 % (w/w of dry polymer composition) was used as a plasticizer. The drug 200 mg (in 5 ml solvent mixture Chloroform: Ethanol) was added into the homogeneous dispersion under slow stirring with a magnetic stirrer. The uniform dispersion was cast on a backing membrane and dried at room temperature. The films were stored between sheets of wax paper in a desiccator.

**Evaluation of transdermal patch formulations:**

1. **Organoleptic characteristics**24: The parameters such as flexibility smoothness and transparency were observed.
2. **Weight uniformity**25: The weight of identified films was weighed very carefully. The average weight of films was calculated.
3. **Thickness**25: Thickness of film was measured by using a screw gauge having least count of 0.02 mm
4. **Drug content**26: Drug content was found out by dissolving four patches each of 2 cm x 2 cm in 10 ml of Ethanol. 0.1ml of this solution was diluted to 10 ml with hydro alcoholic medium. The absorbance of these solutions were found out at 256 nm of *Cissus quadrangularis* and 325nm of *Nigella sativa* for and the drug content determined using the standard calibration curves.
5. **Folding endurance of films**26: The folding acceptance power of prepared film was measured manually. A piece of film was cut with the help of knife. Strip repeatedly folded at the same place till it broke. The number of times the film was folded at the same place without breaking gave the value of the folding endurance.

**6. Tensile strength**27: In order to determine the elongation as a tensile strength, the polymeric patch was pulled by means of a pulley system; weights were gradually added to the pan to increase the pulling force till the patch was broken. The elongation i.e. the distance traveled by the pointer before break of the patch was noted with the help of magnifying glass on the graph paper, the tensile strength was calculated as kg cm

**7. Percentage of moisture content**27: The films were weighed individually and kept in a desiccators containing activated silica at room temperature for 24 hours. Individual films were weighed repeatedly until they showed a constant weight. The percentage of moisture content was calculated as the difference between initial and final weight with respect to final weight.

**8. In-vitro permeation study**28, 29: The in vitro characterization of transdermal formulations was determined using Franz diffusion cell. The receptor compartment of the diffusion cell was filled with 30.0 ml of phosphate buffered saline (pH 7.4), and in vitro drug release studies were carried out using synthetic cellophane membrane. The prepared formulations 2 cm

**Anti osteo arthritis activity of transdermal patches (Collagenase type II-induced osteoarthritis (CIOA) rat model)**30,31

**Experimental group design**

- **Group 1:** Normal saline (50 µl)
- **Group 2:** Collagenase (50 µl)
- **Group 3:** Collagenase + Indomethacin (3 mg/kg)
- **Group 4:** Collagenase + Test formulation 1
- **Group 5:** Collagenase + Test formulation 2
- **Group 6:** Collagenase + Test formulation 3
- **Group 7:** Collagenase + Test formulation 4
Group 8: Collagenase + Test formulation 5  
Group 9: Collagenase + Test formulation 6

**Body weight, Knee diameter and Paw volume measurement:** Changes in body weight and knee diameter were measured on days 0th, 5th, 10th, 15th, 20th, 25th and 30th. Knee diameter was measured using digital vernier caliper. Mean changes in body weight and joint swelling after treatment were calculated. Paw volume was measured once in a week using plethysmometer.

**Glycosaminoglycans (GAG) release:** Blood was taken from rats before and after the treatment through retro-orbital vein puncture and serum was separated. Extracellular matrix of cartilage contains proteoglycans, which consists of a core protein to which glycosaminoglycans (GAGs) chains are covalently attached. The ability of the cartilage to stand compressive forces is aided by the sulfated GAGs such as chondroitin sulfate. GAG release from explants into the surrounding fluid, is a proven marker of cartilage matrix damage. GAG content in serum was measured by 1, 9-dimethyl methylene blue (DMMB) dye binding assay.

**Result:**

**Formulation of Transdermal Patch**

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>TF1</th>
<th>TF2</th>
<th>TF3</th>
<th>TF4</th>
<th>TF5</th>
<th>TF6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethanolic extract of <em>Cissus quadrangularis</em> and <em>Nigella sativa</em> (80:20) (mg)</td>
<td>200</td>
<td>200</td>
<td>200</td>
<td>200</td>
<td>200</td>
<td>200</td>
</tr>
<tr>
<td>HPMC (Hydroxypropyl Methyl Cellulose)</td>
<td>150</td>
<td>200</td>
<td>250</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>EC (Ethyl Cellulose)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>150</td>
<td>200</td>
<td>250</td>
</tr>
<tr>
<td>Dibutyl Phthalate* (mg)</td>
<td>22.5</td>
<td>30</td>
<td>37.5</td>
<td>22.5</td>
<td>30</td>
<td>37.5</td>
</tr>
<tr>
<td>Ethanol (ml)</td>
<td>2.5</td>
<td>2.5</td>
<td>2.5</td>
<td>2.5</td>
<td>2.5</td>
<td>2.5</td>
</tr>
<tr>
<td>Chloroform (ml)</td>
<td>2.5</td>
<td>2.5</td>
<td>2.5</td>
<td>2.5</td>
<td>2.5</td>
<td>2.5</td>
</tr>
</tbody>
</table>

Note: * 15% w/v Of Dibutyl phthalate to the polymer weight, incorporated as plasticizer. The above formula give patch of 20 Sq. cm area

**Evaluation of Transdermal patch formulations**

1) **Organoleptic Characteristics**

<table>
<thead>
<tr>
<th>Test Formulations</th>
<th>Flexibility</th>
<th>Smoothness</th>
<th>Transparency</th>
</tr>
</thead>
<tbody>
<tr>
<td>TF 1</td>
<td>Flexible</td>
<td>Smooth</td>
<td>Translucent</td>
</tr>
<tr>
<td>TF 2</td>
<td>Flexible</td>
<td>Smooth</td>
<td>Translucent</td>
</tr>
<tr>
<td>TF 3</td>
<td>Flexible</td>
<td>Smooth</td>
<td>Translucent</td>
</tr>
<tr>
<td>TF 4</td>
<td>Flexible</td>
<td>Smooth</td>
<td>Translucent</td>
</tr>
<tr>
<td>TF 5</td>
<td>Flexible</td>
<td>Smooth</td>
<td>Translucent</td>
</tr>
<tr>
<td>TF 6</td>
<td>Flexible</td>
<td>Smooth</td>
<td>Translucent</td>
</tr>
</tbody>
</table>

2) **Weight Uniformity and Thickness**

<table>
<thead>
<tr>
<th>Test Formulations</th>
<th>Weight Uniformity (mg)</th>
<th>Thickness (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TF 1</td>
<td>368.56 ± 1.26</td>
<td>117 ± 3.52</td>
</tr>
</tbody>
</table>

Note: * ISSN 1303-5150 www.neuroquantology.com
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### Table No: 4

<table>
<thead>
<tr>
<th>Test Formulations</th>
<th>Weight Uniformity (mg)</th>
<th>Thickness (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TF 2</td>
<td>415.34 ± 1.06</td>
<td>117</td>
</tr>
<tr>
<td>TF 3</td>
<td>467.81 ± 1.24</td>
<td>122</td>
</tr>
<tr>
<td>TF 4</td>
<td>370.18 ± 1.18</td>
<td>138</td>
</tr>
<tr>
<td>TF 5</td>
<td>419.78 ± 1.24</td>
<td>136</td>
</tr>
<tr>
<td>TF 6</td>
<td>469.18 ± 1.38</td>
<td>148</td>
</tr>
</tbody>
</table>

**Figure No 1:** Weight uniformity - Evaluation parameter of Transdermal Patch

**Figure No 2:** Thickness - Evaluation parameter of Transdermal Patch

3) **Drug Content**
### Test Formulations

<table>
<thead>
<tr>
<th>Test Formulations</th>
<th>Percent Drug content (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TF 1</td>
<td>98.28 ± 0.24</td>
</tr>
<tr>
<td>TF 2</td>
<td>98.74 ± 0.18</td>
</tr>
<tr>
<td>TF 3</td>
<td>99.89 ± 0.15</td>
</tr>
<tr>
<td>TF 4</td>
<td>98.17 ± 0.16</td>
</tr>
<tr>
<td>TF 5</td>
<td>98.07 ± 0.19</td>
</tr>
<tr>
<td>TF 6</td>
<td>97.89 ± 0.14</td>
</tr>
</tbody>
</table>

#### Percentage Drug Content (%)

![Bar chart showing drug content of different test formulations](image)

**Figure No 3:** Drug Content - Evaluation parameter of Transdermal Patch

4) **Folding endurance of films**

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Test Formulations</th>
<th>Folding endurance</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>TF 1</td>
<td>206.32 ± 1.24</td>
</tr>
<tr>
<td>2</td>
<td>TF 2</td>
<td>224.14 ± 2.31</td>
</tr>
<tr>
<td>3</td>
<td>TF 3</td>
<td>245.23 ± 3.62</td>
</tr>
<tr>
<td>4</td>
<td>TF 4</td>
<td>212.23 ± 1.75</td>
</tr>
<tr>
<td>5</td>
<td>TF 5</td>
<td>223.23 ± 2.65</td>
</tr>
</tbody>
</table>
Figure No 4: Folding endurance of films - Evaluation parameter of Transdermal Patch

5) Tensile strength

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Test Formulations</th>
<th>Tensile strength (Kg/cm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>TF 1</td>
<td>2.63 ± 0.34</td>
</tr>
<tr>
<td>2</td>
<td>TF 2</td>
<td>2.74 ± 0.37</td>
</tr>
<tr>
<td>3</td>
<td>TF 3</td>
<td>3.12 ± 0.59</td>
</tr>
<tr>
<td>4</td>
<td>TF 4</td>
<td>2.89 ± 0.69</td>
</tr>
<tr>
<td>5</td>
<td>TF 5</td>
<td>2.94 ± 0.74</td>
</tr>
<tr>
<td>6</td>
<td>TF 6</td>
<td>2.98 ± 0.79</td>
</tr>
</tbody>
</table>
6) Percentage of Moisture Content

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Test Formulations</th>
<th>Moisture Content</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>TF 1</td>
<td>2.28 ± 0.48</td>
</tr>
<tr>
<td>2</td>
<td>TF 2</td>
<td>2.84 ± 0.39</td>
</tr>
<tr>
<td>3</td>
<td>TF 3</td>
<td>2.92 ± 0.89</td>
</tr>
<tr>
<td>4</td>
<td>TF 4</td>
<td>2.09 ± 0.56</td>
</tr>
<tr>
<td>5</td>
<td>TF 5</td>
<td>2.27 ± 0.64</td>
</tr>
<tr>
<td>6</td>
<td>TF 6</td>
<td>2.68 ± 0.81</td>
</tr>
</tbody>
</table>

Figure No 5: Tensile Strength - Evaluation parameter of Transdermal Patch
7) In-vitro permeation study

![Figure No 6: Percentage of Moisture Content-Evaluation parameter of Transdermal Patch](image)

### Table No: 8

<table>
<thead>
<tr>
<th>Time (Hour)</th>
<th>Percent Drug Release</th>
</tr>
</thead>
<tbody>
<tr>
<td>TF 1</td>
<td>TF 2</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>2.18</td>
</tr>
<tr>
<td>2</td>
<td>8.24</td>
</tr>
<tr>
<td>3</td>
<td>16.13</td>
</tr>
<tr>
<td>4</td>
<td>24.36</td>
</tr>
<tr>
<td>5</td>
<td>29.25</td>
</tr>
<tr>
<td>6</td>
<td>37.08</td>
</tr>
<tr>
<td>7</td>
<td>43.52</td>
</tr>
</tbody>
</table>
Evaluation of anti-Osteoarthritis activity of transdermal patch formulations (Collagenase type II-induced osteoarthritis (CIOA) rat model)

1. Effect on body weight

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment</th>
<th>Change in body weight (gm.) (Mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>Normal saline (50 µl)</td>
<td>17.83 ± 3.06</td>
</tr>
<tr>
<td>Group 2</td>
<td>Collagenase (50 µl)</td>
<td>10.15 ± 2.42</td>
</tr>
<tr>
<td>Group 3</td>
<td>Collagenase + Indomethacin (3 mg/kg)</td>
<td>8.83 ± 2.22</td>
</tr>
</tbody>
</table>
### Table No: 10

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment</th>
<th>Knee swelling (mm) (Mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>Collagenase (50 µl)</td>
<td>0.78 ± 0.11</td>
</tr>
<tr>
<td>Group 2</td>
<td>Collagenase + Indomethacin (3 mg/kg)</td>
<td>0.28 ± 0.18</td>
</tr>
<tr>
<td>Group 3</td>
<td>Collagenase + Test formulation 1</td>
<td>0.51 ± 0.19</td>
</tr>
<tr>
<td>Group 4</td>
<td>Collagenase + Test formulation 2</td>
<td>0.39 ± 0.15</td>
</tr>
<tr>
<td>Group 5</td>
<td>Collagenase + Test formulation 3</td>
<td>0.34 ± 0.17</td>
</tr>
<tr>
<td>Group 6</td>
<td>Collagenase + Test formulation 4</td>
<td>0.64 ± 0.12</td>
</tr>
<tr>
<td>Group 7</td>
<td>Collagenase + Test formulation 5</td>
<td>0.56 ± 0.19</td>
</tr>
<tr>
<td>Group 8</td>
<td>Collagenase + Test formulation 6</td>
<td>0.48 ± 0.16</td>
</tr>
</tbody>
</table>

2. Effect on knee diameter

![Figure No 8: Effect on body weight on transdermal Patch](image-url)
3. Effect on paw volume

Table No: 11

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Paw oedema volume (ml)</th>
<th>0 Day</th>
<th>7 Day</th>
<th>14 Day</th>
<th>21 Day</th>
<th>28 Day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>Collagenase (50 µl)</td>
<td></td>
<td>2.58 ±</td>
<td>3.16 ±</td>
<td>3.37 ±</td>
<td>3.46 ±</td>
<td>3.66 ±</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.04</td>
<td>0.01</td>
<td>0.10</td>
<td>0.07</td>
<td>0.05</td>
</tr>
<tr>
<td>Group 2</td>
<td>Collagenase + Indomethacin (3 mg/kg)</td>
<td></td>
<td>2.29 ±</td>
<td>3.08 ±</td>
<td>3.10 ±</td>
<td>2.55 ±</td>
<td>2.40 ±</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.07</td>
<td>0.23</td>
<td>0.04</td>
<td>0.06</td>
<td>0.03</td>
</tr>
<tr>
<td>Group 3</td>
<td>Collagenase + Test formulation 1</td>
<td></td>
<td>2.48 ±</td>
<td>3.17 ±</td>
<td>3.36 ±</td>
<td>2.86 ±</td>
<td>2.91 ±</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.09</td>
<td>0.03</td>
<td>0.05</td>
<td>0.05</td>
<td>0.06</td>
</tr>
<tr>
<td>Group 4</td>
<td>Collagenase + Test formulation 2</td>
<td></td>
<td>2.41 ±</td>
<td>3.14 ±</td>
<td>3.21 ±</td>
<td>2.71 ±</td>
<td>2.78 ±</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.07</td>
<td>0.06</td>
<td>0.06</td>
<td>0.04</td>
<td>0.09</td>
</tr>
<tr>
<td>Group 5</td>
<td>Collagenase + Test formulation 3</td>
<td></td>
<td>2.36 ±</td>
<td>3.11 ±</td>
<td>3.17 ±</td>
<td>2.62 ±</td>
<td>2.66 ±</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.06</td>
<td>0.06</td>
<td>0.05</td>
<td>0.02</td>
<td>0.05</td>
</tr>
<tr>
<td>Group 6</td>
<td>Collagenase + Test formulation 4</td>
<td></td>
<td>2.52 ±</td>
<td>3.36 ±</td>
<td>3.45 ±</td>
<td>2.95 ±</td>
<td>2.96 ±</td>
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<td></td>
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<td>0.03</td>
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<td>0.04</td>
<td>0.05</td>
<td>0.03</td>
</tr>
<tr>
<td>Group 7</td>
<td>Collagenase + Test formulation 5</td>
<td></td>
<td>2.48 ±</td>
<td>3.21 ±</td>
<td>3.27 ±</td>
<td>2.86 ±</td>
<td>2.92 ±</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.06</td>
<td>0.08</td>
<td>0.07</td>
<td>0.07</td>
<td>0.05</td>
</tr>
<tr>
<td>Group 8</td>
<td>Collagenase + Test formulation 6</td>
<td></td>
<td>2.41 ±</td>
<td>3.18 ±</td>
<td>3.21 ±</td>
<td>2.76 ±</td>
<td>2.71 ±</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.04</td>
<td>0.07</td>
<td>0.06</td>
<td>0.05</td>
<td>0.06</td>
</tr>
</tbody>
</table>
Figure No 10: Effect on paw volume on transdermal Patch

4. Effect on Glycosaminoglycans (GAG) release

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment</th>
<th>GAG (µg/ml serum) (Mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>Normal saline (50 µl)</td>
<td>117.66 ± 9.43</td>
</tr>
<tr>
<td>Group 2</td>
<td>Collagenase (50 µl)</td>
<td>806.16 ± 13.68</td>
</tr>
<tr>
<td>Group 3</td>
<td>Collagenase + Indomethacin (3 mg/kg)</td>
<td>525.16 ± 12.75</td>
</tr>
<tr>
<td>Group 4</td>
<td>Collagenase + Test formulation 1</td>
<td>702.12 ± 10.76</td>
</tr>
<tr>
<td>Group 5</td>
<td>Collagenase + Test formulation 2</td>
<td>664.42 ± 9.48</td>
</tr>
<tr>
<td>Group 6</td>
<td>Collagenase + Test formulation 3</td>
<td>628.34 ± 8.84</td>
</tr>
<tr>
<td>Group 7</td>
<td>Collagenase + Test formulation 4</td>
<td>728.37 ± 8.39</td>
</tr>
<tr>
<td>Group 8</td>
<td>Collagenase + Test formulation 5</td>
<td>686.21 ± 8.14</td>
</tr>
<tr>
<td>Group 9</td>
<td>Collagenase + Test formulation 6</td>
<td>658.46 ± 7.89</td>
</tr>
</tbody>
</table>
Conclusion and Outlook: *Sandhivata* (Osteoarthritis) is the most common rheumatological problem hampering the quality of life of the patients and the main aim of the therapy should be focused on improving it. The ethanolic extract of *Nigella sativa* acts as a potent anti-inflammatory and analgesic activity. The ethanolic extract of Cissus *quadrangularis* acts by stimulation of metabolism and increased uptake of the minerals calcium, sulphur, and strontium by the osteoblasts in fracture healing. Combination of both Cissus *quadrangularis* and *Nigella sativa* will be effective in osteoarthritis. It can be concluded from this project work that, from this novel approach, herbal drugs in the extract of the product can transformed into Transdermal Patches for the bioactive properties pertaining to a stable formulation in accordance with its appropriate formulations.

Expanding the use of novel permeation enhancement techniques with macromolecules and other conventional molecules for a wider range of indications is highly desirable for the transdermal industry. Physical enhancement methods afford substantial improvement in the rate of delivery of therapeutic agents across skin. Novel prodrugs would not only help to reach the therapeutic levels for some drugs, but may also help alleviate skin irritation. The incidence and significance of skin irritation reactions will decrease with the increasing availability of physical permeation enhancement methods and new breakthroughs in topical drug formulations, such as liposomes, microemulsions, nanoparticles, and evaporating gels. Breakthroughs in chemical permeation enhancer analogs showing significant improvements in limiting cutaneous irritation show promise for the development of safe chemical enhancers and should be further examined in the future.

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