

Exploring the gelling properties of Plantago ovata-based Arabinoxylan: Fabrication and optimization of a topical emulgel using response surface methodology

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Abstract

Prime objective of the current research was to develop a stable nimesulide emulgel with the help of arabinoxylan, a natural gelling agent extracted from *Plantago ovata*. The response surface methodology was used by a Design Expert 10 software to formulate and optimize the emulgel. The experimental design approach evaluated the impact of independent and dependent variables. Independent variables were different concentrations of arabinoxylan, span 80 and tween 20, whereas, dependent variables were viscosity, pH, and content uniformity. FTIR demonstrated the compatibility of nimesulide with the excipients. Stability study indicated no phase separation and no change in pH for formulation F1, F3 and F4. The negative values of zeta potential revealed the excellent stability of emulgel. Viscosity, spreadability and extrudability values were in desired range. Ex-vivo release studies illustrated 86%, 55% and 66% release of the drug over a period of 24 h from the formulations F1, F3 and F4, respectively. Analgesic effect of the optimized emulgel was significantly higher in test group as compared to control and did not produce any sort of irritation. Therefore, it can be concluded that the newly developed emulgel based on arabinoxylan, as gelling agent, appear to be an effective drug delivery system.

1. Introduction

Human skin is one of the unique and largest organs of the human body that provides approximately 10% of the body mass of an average person, and covers an average area of 1.7 m². This easily accessible organ offers an ideal site to administer therapeutic agents for both local and systemic actions [1]. Unfortunately, most drugs that are taken orally for skin diseases are, on one hand, less effective due to poor bioavailability and on the other hand, they are associated with several undesirable side effects. To overcome limitations of such drugs, topical drug delivery systems that offer numerous advantages over oral drug delivery systems are considered [2]. Topical drug delivery systems are diverse and vary in consistency from solids, semisolids liquids, and semiliquid preparations. Among them, the most popular semisolid delivery systems in the recent times are the gels [3].

Gels are a class of dosage form that contains a bigger portion of aqueous components and allows easy release of the drugs through aqueous vehicles [4]. However, due to their high aqueous concentration the major disadvantage of gels includes their inability to deliver hydrophobic drugs, and therefore, only hydrophilic drugs can be entrapped [5]. Same is the case with the hydrogels that face a limitation in transporting lipophilic drugs [6]. In order to incorporate the hydrophobic drugs within gel system a modified version 'emulgel' is taken into consideration [7]. Emulgel is an emerging topical drug delivery system that provides a greaseless, easily spreadable, easily transferrable, soothing, non-staining, safer, bio-friendly, transparent, attractive appearance, better and effective drug delivery system as compared to other topical drug delivery systems [8]. This system consists of the oil phase, gelling agent, aqueous phase, and emulsifying agent and expressively affects the design of drug release from the dosage form [9]. Moreover, emulgel is considered as a promising concept because of its dual character—the presence of an emulsion and a gel base, combined as a single formulation and therefore, emulgel can efficiently deliver both hydrophilic and lipophilic drugs. Furthermore, by controlling the emulsion bases in emulgels, the drug-release pattern can be controlled for prolonged therapeutic effect at the desired site of action. Notably, a drug delivered through emulgel, being closer to the surface of the skin, results in a larger concentration gradient towards the skin due to high solubilizing capacity, and consequently, provides better skin penetration [10]. Recently emulgels have progressively been used for hydrophilic and hydrophobic drugs with numerous pharmacological effects such as non-steroidal anti-inflammatory drugs, anti-emetic, anti-fungal agents, anti-viral, antibacterial, and local anesthetics [11].

Natural polymers and their derivatives are grabbing attention in the area of drug delivery research because they are usually biodegradable, inexpensive, and can be further modified chemically [12–14]. Within this context arabinoxylan (AX), a natural polymer, forms a highly viscous solution and can form a gel at a very low concentration (0.5–1.5%). The major advantage, other than excellent gelling properties, is thixotropic behavior as compared to commercially used gelling agents [15–17].

Therefore, the current research work was focused on exploring the gelling properties of AX in the development of an efficient emulgel for topical drug delivery. Nimesulide, a model drug highly effective for pain associated with osteoarthritis and backache, belonging to BCS class II (low solubility, high permeability) was incorporated into emulgel. Nimesulide emulgel was then examined regarding its pH, viscosity, spreadability, swelling index, content uniformity, in vitro release profile and in vivo skin irritation and analgesic activity were studied.

2. Materials And Methods

Nimesulide was received as a gift sample from Unixo Labs pharmaceuticals. AX was extracted from (*Plantago ovata*) that was purchased from the local market of Sargodha (Sargodha, Pakistan). Acetic acid, span80, tween 20, and sodium dihydrogen phosphate and sodium hydroxide were purchased from Sigma Aldrich, Germany. Propylene glycol was purchased from Anala R. England. Ethanol was purchased from Riedel-de Haen, Germany. Liquid paraffin was purchased from Merck Millipore and methylparaben was purchased from Sigma Aldrich, USA. Distilled water was taken from the Industrial plant of the University of Sargodha (Sargodha, Pakistan). All other chemicals used in this study were of analytical grade.

2.1. Extraction of AX from *Plantago ovata*

Extraction of AX from *Plantago ovata* was done by method already reported by Saghir *et al.*, using alkali extraction [17]. Briefly, 150g *Plantago ovata* seeds were soaked in 500 mL distilled water overnight. The pH was adjusted to 12 with the addition of 2.5% aqueous NaOH and stirred for 5 min, followed by separation with the help of vacuum filtration assembly. Afterwards, dilute acetic acid was added to the filtrate (until pH was in range of 3–4) for coagulation purposes. The so formed gel was washed with the help of distilled water for 4 days and finally, freeze-dried.

2.2. Experimental Design and Statistical Analysis

As illustrated in Table 1, fifteen formulations (F1 to F15) were designed based upon different concentrations of AX, span 80 and tween 20, being considered the independent variables and their concentrations were chosen by a review of the literature [18–20]. The selection of the ratio factors for AX, span 80 and tween 20 were decided using, Design Expert 10, software, Response surface methodology (Stat-Ease, Inc., Minneapolis, Minnesota, USA) to get optimize emulgel formulations.

The concentration of independent variables AX, span 80 and tween 20 were denoted by A, B and C, respectively. The points for independent variables comprised of lower (-1), medium (0), and higher (+1). For AX and span 80, concentration -1 was assigned to designate minor level concentration of 1%, for medium-level 0 was used for 1.5% and maximum concentration +1 was used for 2.0%. Similarly, for the concentration of tween 20, -1 indicates a lower concentration 0.5%, 0 indicates a medium concentration 0.75% and +1 indicates a higher concentration of 1%. Intercept is indicated as follows;

$$A + B + C + AB + AC + BC + A^2 + B^2 + C^2.$$

2.3. Preparation of Emulgel

From the formulation perspective, polymeric gel base dispersion was initially prepared by dispersing AX in distilled water with constant stirring at 510–550 RPM until the gel was formed and the pH was maintained at 6.5 ± 0.2 using 2.5% aqueous NaOH. For the emulsion, oil phase was prepared by dissolving span 80 in liquid paraffin and aqueous phase by dissolving tween 20 in distilled water. Separately, nimesulide was dissolved in ethanol and methylparaben in propylene glycol owed to the solubility of both ingredients in respective solvent. Both solutions of nimesulide and methylparaben were added to the aqueous phase. In the following, the oily and aqueous phases were separately heated in a water bath at 70°C. Upon obtaining uniformity, drug loaded aqueous phase was added to the oil phase with continuous stirring until the temperature reached to room temperature. The drug-loaded emulsion was incorporated into the polymeric gel base dispersion of AX steadily with continuous stirring to obtain homogenized emulgel. [19, 20]. Afterwards, the three dependent variables (responses) viscosity, pH and drug assay were monitored and recorded.

Table 1
Formulation design of Emulgel and dependent variables (Viscosity, pH and drug content).

Formulation code	Arabinosylane (%w/w)	Span 80 (%w/w)	Tween 20 (%w/w)	Viscosity (Cps) mean + SEM	pH mean + SEM	Drug Content (%) mean + SEM
F1	1.5	1.5	0.75	17987 ± 2.08	5.6 ± 0.03	106 ± 0.06
F2	1.5	2	0.75	16548 ± 49.9	5.9 ± 0.03	98 ± 0.31
F3	2	2	0.5	29857 ± 504.79	6.8 ± 0.08	115 ± 0.05
F4	1	1	1	11445 ± 70.40	6.1 ± 0.03	85 ± 0.28
F5	1.5	1.5	0.5	18377 ± 35.37	5.8 ± 0.05	105 ± 0.52
F6	2	1	1	28765 ± 127.69	6.5 ± 0.08	92 ± 0.57
F7	1	1.5	0.75	13654 ± 128.77	6.4 ± 0.03	60 ± 0.88
F8	2	2	1	30676 ± 89.29	6.4 ± 0.03	104 ± 0.80
F9	1	1	0.5	12543 ± 15.16	6.4 ± 0.04	85 ± 0.30
F10	2	1	0.5	26378 ± 90.35	6.1 ± 0.03	89 ± 0.90
F11	1.5	1	0.75	16424 ± 45.33	6.3 ± 0.03	97 ± 0.30
F12	1	2	1	12543 ± 154.70	6.6 ± 0.18	90 ± 0.0
F13	1.5	1.5	1	16980 ± 80.55	6.1 ± 0.03	103 ± 0.6
F14	2	1.5	0.75	28543 ± 273.20	6.6 ± 0.04	68 ± 0.31
F15	1	2	0.5	13658 ± 22.66	6.4 ± 0.03	92 ± 0.31

2.4. Checkpoint Analysis and Optimization Model Validation

Design Expert 10, software (Stat-Ease, Inc., Minneapolis, Minnesota, USA), completed statistical validation of the polynomial equations, which was determined using the software's ANOVA requirement. The models were assessed using R^2 values and statistically significant coefficients. The optimal formulations were chosen based on their attractiveness as determined by Design-Expert software acceptance criteria. The Design-Expert software featured a variety of 3D reaction surface graphs. To validate the experimental model and polynomial equations, three optimal checkpoint formulations were chosen over the experimental region. The response qualities of the improved checkpoint formulation variables were tested. To compute the percentage prediction error, the experimental values of the answers were quantitatively compared to the projected values.

2.5. Characterization of emulgel

2.5.1. Physical Appearance

Visual inspection of formulations was done for their homogeneity, appearance, color, phase separation, and consistency [21].

2.5.2. Viscosity Study and pH determination

A viscosity test of all formulations was performed on a Brookfield viscometer (Brookfield, Massachusetts, USA) at 50 rpm and 100 rpm over a period of 10 min. The pH of all formulations was measured by using a digital pH meter [22].

2.5.3. Drug Content Measurement

The amount of drug entrapped into the emulgel formulations was determined photometrically. Briefly, 1 g of each formulation was mixed with phosphate buffer (pH 7.4) (1:100 w/v) in a volumetric flask and continued stirring for 30 min. The mixture was further diluted with phosphate buffer (1:10 v/v) before measuring the absorbance using a UV spectrophotometer (Shimadzu, UV-2401 PC, Japan) against 392nm. A standard curve of the pure drug within the range of 1–10 µg/mL in phosphate buffer was used for quantification.

2.5.4. Stability Study

The stability studies were performed for all the formulations stored in well-closed glass containers for a period of 90 days at room temperature. Samples were collected at predetermined intervals 0, 30, 60 and 90 days, and their physicochemical parameters were evaluated [23].

2.5.5. Spreadability Measurement

A spreadability test based on 'Slip' and 'drag' characteristics of emulgel was performed for the optimized formulations. For this purpose, a glass slide was fixed on the wooden box and 1 g of each formulation was placed on this slide, separately. A second glass slide was used to cover in such a way that the emulgel was sandwiched between two glass slides. Afterwards, a standard weight of 1 Kg was placed on the sandwiched slides in order to remove any entrapped air and to get a uniform layer emulgel. After an incubation of 5 min, the excess emulgel that drained out of the slides was scrapped off and the weight was uplifted. In the following, the time required for the separation of two slides was noted in seconds [24].

The following formula was used to calculate spreadability,

$$S = \frac{M \times L}{T} \text{Equation 1}$$

Whereas; S = Spreadability, M = Weight Knotted to slide, L = Length of glass slide, T = Time required for the separation of two slides

2.5.6. Extrudability Measurement

As a measure of thickness of the emulgel formulations, extrusion from lacquered aluminum collapsible tube on application of weight (in g) required to extrude at least 0.5 cm ribbon of emulgel in 10 s was measured. The measurement of extrudability (g/cm²) of each formulation was done in triplicates [25].

$$\text{Extrudability} = \frac{\text{Applied weight to extrude emulgel from tube (g)}}{\text{Area in cm}^2} \text{Equation 2}$$

2.5.7. Particle size (PS), polydispersity index (PDI), and zeta potential (ZP) determinations

A Malvern zeta-sizer (Worcestershire, UK) was used to determine the average globule size, polydispersity index, and zeta potentials of optimized emulgel formulations as per the concepts of dynamic light scattering and electrophoretic mobility theories, respectively.

2.5.8. Photomicrography

Under a light microscope, the morphology of the emulsion was examined. A formulation that displayed the best release was examined for shape, globule size, and agglomeration. The formulation was mounted on a glass slide and viewed under a microscope.

2.5.9. Fourier-Transform Infrared Spectroscopy (FTIR)

FTIR of pure Nimesulide, AX, and physical mixture of AX and nimesulide was done by KBr disc method by employing Bruker FTIR (Tensor 27 series, Bruker Corporation, Germany) instrument, using attenuated total reflectance (ATR) technology accompanying

software OPUS data collection. All the spectrum was obtained at $400\text{--}4000\text{cm}^{-1}$ under similar operating conditions [26].

2.5.10. Ex-vivo Release Study

Ex-vivo drug release study was performed for the optimal formulation with the help of Franz diffusion cell (PermeGear, Inc. 1815 Leithsville Road, Hellertown, PA 18055 USA) using rabbit skin as described in literature (28–30). Briefly, rabbit skin was freshly collected from the local animal house of the university and after rinsing with normal saline was clamped between donor and receptor chamber of the Franz cell. One gram of emulgel was applied over the rabbit skin and phosphate buffer (pH 7.4) maintained at $37^{\circ}\text{C} \pm 0.5$ was used as a diffusion medium. Stirring was continued with a magnetic stirrer to ensure the diffusion of the media and meanwhile, 0.1 mL samples were drawn at different time intervals (30 min, 1 h, 2 h, 4 h, 6 h, 12 h, and 24 h), and every time replaced with the same volume of the buffer maintained a $37^{\circ}\text{C} \pm 0.5$. Then the samples were diluted properly to check the absorbance at the UV spectrophotometer. The accumulative quantity of drugs released through the skin was calculated as a function of time [27].

2.5.11. In-vivo Study

All the protocols for the in-vivo study were pre-approved by the Ethical Committee of the Faculty of Pharmacy, University of Sargodha (PREC4532). The in vivo experiment for analgesic activity was conducted on healthy adult Wistar rats weighing 150–200 g, whereas skin irritation was conducted on healthy albino rabbits weighing about 1.5 ± 0.5 kg. Animals were allowed to acclimatize for one week before the experiment. Animals were kept at ambient conditions (room temperature of $25 \pm 2^{\circ}\text{C}$, relative humidity of $65 \pm 5\%$, and light/dark cycle of 12 h) and had free access to standard pellet diet and fresh water [28]. This study was conducted in accordance with ARRIVE guidelines (<https://arriveguidelines.org>).

2.5.11.1. Analgesic Activity

Two groups of rats were made, three rats per group. Group 1 was considered as standard group and group 2 was considered as test group. Group 1 or the standard group was treated with the marketed formulation of nimesulide. Group 2 or the test group was treated with the topical formulation of emulgel. *In-vivo* analgesic activity of nimesulide was examined by the Hot plate method. Each rat was kept on a hot plate (55°C) for 10 sec and the latency period (period at which animals show the reaction to the pain stimulus) or PRT (pain reaction time) was measured. 0.5 g of the respective formulation was applied on the dorsal surface of the right hind paw of rat with gentle rubbing. The time (in seconds), spent licking the paw was considered an indicator of pain response.

2.5.11.2 Skin Irritation Test

The test was conducted to explore the safety and skin compatibility of the optimized formulation. Two groups of rabbits were made, three rabbits each group. Group I was considered as control group, received no treatment while group II was considered as test group, and received topical treatment of emulgel.

Before the study, rabbits were shaved and circumscribed dorsal skin area. A sample (0.5 g) of the formulation was applied once daily for 7 days, and the rabbit's skin was inspected for any signs of irritation, redness, and inflammation. A scoring system was considered and average scores were recorded. The scoring system was designed as follows; 0: no signs of irritation, 1: slight irritation, 2: moderate irritation, and 3: severe irritation [29].

2.5.12. Statistical Analysis

For *In-vivo* study, the statistical analysis of all the result was accomplished utilizing GraphPad Prism 7.0 Graph Pad Software (San Diego, CA, USA). The data were analyzed using student t test. P value of < 0.05 was considered as significant. All the results are depicted as value Mean $\pm$ SEM.

3. Results And Discussion

3.1. Formulation and Optimization Design by RSM

The experimental design was used for the optimization of the formulation variables, as the response surface methodology required 18 experiments. As illustrated in Table 2, dependent (A, B and C indicating Arabinoxylan, Span 80 and Tween 20 respectively also depicted their minimum, medium and maximum levels) and independent variables (R1, R2 and R3 indicating viscosity, pH & drug

contents respectively). Table 3 indicates co-efficient for responses (a second-order polynomial equation defines the experimental link between independent variables and their responses in comparison to actual factors) and Table 4 comprises numerical optimization of formulations.

Optimal conditions for three dependent variables (responses) were predicted by the desirability function and appropriate concentration ranges were chosen. Five options that are used by the program to shape the desirability indices include none, minimum, maximum, within range, and target. Our focus was to make formulations with good viscosity, acceptable pH range, and content uniformity. According to established criteria, the optimum situations for our formulation’s desired response were; the concentration of AX at 1.5%, the concentration of span 80 was 2% and the concentration of tween 20 was 0.75%. Design-Expert software was used to obtain optimized formulations and create the mathematical equations shown in (3), (4), and (5). The responses, viscosity (Y 1) and pH (Y 2), and percent drug diffusion (Y3) were found to be significantly higher (Y 1, 30676) in F8; (Y 2 6.8) and (Y3 115%) in F3 respectively. The response surface models were developed with Design-Expert software by applying coded values of factor levels to estimate the quantitative impacts of various combinations of variables on Viscosity, pH, and percent drug diffusion. The following is a representation of the model described:

Viscosity = 17957 + 8038AX + 772.3span-40tween20 + 397.625AX×span + 676.87AX×tween-197.62span×tween + 3567.11AX²-1045.39span²80 + 147.107tween²Eq. 3

pH = 6.01 + 0.05AX + 0.07span + 0.02tween + 0.0125AX×span + 0.0125AX×tween-0.037span×tween + 0.453AX² + 0.053span²-0.096tween²Eq. 4

DrugAssay = 95.98 + 6.6AX + 5.1span-1.2tween + 3.25AX×span-0.75AX×tween-2span×tween-22.22AX² + 6.27span² + 12.77tween²Eq. 5

Table 2
Selection of ranges for dependent and independent variables.

Dependent								
Factor	Name	Min	Max	Coded Low		Coded High	Mean	Std. Dev.
A	IAX	1. 0000	2. 00	-1 ↔ 1. 00		+ 1 ↔ 2. 00	1. 50	0. 3835
B	Span 80	1. 0000	2. 00	-1 ↔ 1. 00		+ 1 ↔ 2. 00	1. 50	0. 3835
C	Tween 20	0. 5000	1. 0000	-1 ↔ 0. 50		+ 1 ↔ 1. 00	0. 7500	0. 1917
Independent								
Response	Name	Units	Analysis	Min	Max	Mean	Std. Dev.	Model
R1	Viscosity	cps	Polynomial	11445	30676	19440. 00	6518. 31	Quadratic
R2	pH	pH	Polynomial	5. 6	6. 8	6. 24	0. 3071	Quadratic
R3	drug assay	%	Polynomial	60	115	94. 22	12. 41	Quadratic

Table 3
Co-efficient Table for responses.

	Intercept	A	B	C	AB	AC	BC	A ²	B ²	C ²
Viscosity	17957.3	8038	772.3	-40	397.625	676.875	-197.625	3567.11	-1045.39	147.107
p-values		< 0.0001	0.1601	0.938	0.496	0.2594	0.7322	0.0058	0.307	0.8818
pH	6.01071	0.05	0.07	0.02	0.0125	0.0125	-0.0375	0.453571	0.0535714	0.0964286
p-values		0.5978	0.464	0.8316	0.9053	0.9053	0.7221	0.0319	0.7672	0.5964
drug assay	95.9881	6.6	5.1	-1.2	3.25	-0.75	-2	-22.2262	6.27381	12.7738
p-values		0.0152	0.0447	0.5913	0.2125	0.7625	0.4286	0.0007	0.1665	0.0147

Table 4
Numerical optimization of formulations.

Name	Goal	Lower	Upper limit	Lower	Upper	Importance
		limit		weight	weight	
AX	Maximum	1	2	1	1	3
Span 80	Within range	1	2	1	1	3
Tween 20	Within range	0. 5	1	1	1	3
Viscosity	Within range	11445	30676	1	1	3
pH	Within range	5. 6	6. 8	1	1	3
Drug assay	Maximum	60	115	1	1	3

3.2. Fitting of Data to the Model

Formulation F2 showed a significant percent drug diffusion (Y3) and pH (Y2) and Viscosity (Y1) among the formulations and demonstrated good quality with the best spreadability. Using Design Expert 10, software (Stat-Ease, Inc., Minneapolis, Minnesota, USA), the responses observed for 18 different formulations were fit to a design model at the same time as in Fig. 1. A negative number implies that the effect favors the optimization, whereas a positive value suggests that the factor and the response have an inverse connection. The independent variable (gelling agent concentration) was discovered to have a negative influence on the responses: percent drug diffusion (Y3) as well as spreadability. To assess the impact of the independent factors on response and choose the best formulation, three-dimensional response surface plots were created as described in Fig. 2.

3.3. Data Analysis

Multiple regression was used to generate a polynomial equation using the percentage drug diffusion and viscosity acquired at various values of the three independent variables (X1, X2, and X3), the results are displayed in Table 5. The correlation coefficient (R^2) of viscosity and pH was determined to be 0.9535 and 0.9273 respectively, suggesting that the selected variables for each are well matched. Moreover, the "Pred R-Squared" values fairly agree with the "Adj R-Squared" for both of them. The percentage of drug diffusion values tested for various formulations exhibited a broad range of values (i.e. from a minimum of 60.8% to a maximum of 115.5%), and the R^2 was determined to be 0.7951. The "Pred R-Squared" is 0.6459, which is reasonably close to the "Adj R-Squared" of 0.7010. In general, the findings demonstrate that the factors chosen for the investigation have a significant impact on the percentage drug diffusion and when two variables are adjusted at the same time, the interaction terms reveal how the percentage drug diffusion varies. Positive coefficients for interactions between two variables indicate an adverse influence on percent drug diffusion, whereas negative coefficients for all three independent variables suggest a beneficial effect on percent drug diffusion.

Table 5
Summary of results of regression analysis for responses 1, 2 and 3.

Model	R ²	Adjusted R ²	Predicted R ²	SD
Viscosity (Y1)	0.9535	0.9415	0.9160	0.43
pH (Y2)	0.9273	0.9193	0.8351	0.87
Drug content (%) (Y3)	0.7951	0.7010	0.6459	1.06

3.4. Percentage Yield of Arabinoxylan from *Plantago ovata*

AX was extracted from *Plantago ovata* by the alkali extraction method with a percentage yield of 39%. AX extracted via mild alkali treatment forms a strong gel, however, harsh alkali treatment decreases the viscosity and elasticity of gel. The reason behind is decreased FA (ferulic acid) content of AX with harsh alkali treatment and decreased FA content cause a decrease in gelling capacity of AX [30]. The content of FA in AX is an important factor affecting the gel structure. Increases in FA content usually result in smaller mesh sizes and lower molecular weight and higher cross-linking capacity, as a result, gels with more compact structures are formed. [31, 32].

3.5. Viscosity and pH determinations

A viscosity test is recommended for semi-solid dosage forms in order to investigate the flow properties. The viscosity of the different emulgel formulations was in the range of 10000-30000cps, whereas, the ideal range for the viscosity is 12500–21100 cps. The outcomes of the viscosity studies, as illustrated in Table 1, show that formulation containing 1.5% AX had the value of viscosity within the ideal range. On one hand, a lower concentration (1% AX) was either at a lower borderline or below the ideal range of viscosity and on the other hand, a higher concentration (2% AX) exhibited the value of viscosity either at the upper borderline or above the range of viscosity. By increasing the concentration of AX, the absorption capacity of water decreases due to cross-linking and a complex network of polymers [33].

The results of the pH of emulgel formulations are also mentioned in Table 1. The pH of all formulations was is in the range of 5.6 to 6.8, therefore, making sure that there is minimum risk for irritation and is compatible with the skin physiology (the pH of skin is 5.5) [34].

3.6 Stability testing

The emulgel was examined visually for phase separation, pH and content uniformity. Among all formulation, F1, F3 and F4 were found to be incubated under given conditions, whereas all other formulations undergone phase separation. Formulations appear to be creamy with a smooth homogeneous texture and glossy appearance. Table 6 indicates results of stability testing F1, F3 and F4 formulations.

Table 6
Outcomes of the stability studies of optimized formulations.

Formulation												
	F1				F3				F4			
Days	0	30	60	90	0	30	60	90	0	30	60	90
Colour	x	x	x	x	x	x	x	x	x	x	x	x
Appearance	xx	xx	xx	xx	xx	xx	xx	xx	xx	xx	xx	xx
pH	5.6 ± 0.05	5.7 ± 0.03	5.8 ± 0.02	5.0 ± 0.09	6.8 ± 0.05	6.8 ± 0.04	6.5 ± 0.01	6.3 ± 0.06	6.1 ± 0.01	6.0 ± 0.03	5.9 ± 0.02	6.1 ± 0.01
Drug content	106 ± 0.09	105 ± 0.03	105 ± 0.06	102 ± 0.05	117 ± 0.05	115 ± 0.04	116 ± 0.05	115 ± 0.03	85 ± 0.05	84 ± 0.10	84 ± 0.05	82 ± 0.03
Phase separation	No	No	No	No	No	No	No	No	No	No	No	No
x = Creamy												
xx = Homogeneous												

3.7. Spreadability and determination of extrudability

Spreadability is inversely proportional to the viscosity, the less viscous formulation exhibit more spreadability and vice versa [35, 36]. The spreadability of the optimized formulations, as given in Fig. 3, was found to be in the required range for good spreadability (20-29g.cm/sec). Moreover, it was found that extrudability of emulgel was a function of viscosity and concentration of gelling agent AX in formulations. Extrudability decreased with an increase in the viscosity and concentration of AX in these formulations, 1.5%, 2% and 1% for formulations F1, F3 and F4, as depicted in Fig. 3.

3.8. Zeta potential, droplet size distribution and PDI

When formulating a stable emulgel system, zeta potential, droplet size distribution, and PDI are the most important factors to consider [37]. The emulgel stability is determined by the amplitude of the zeta potential. The charge on the surface of dispersed globules in an emulgel formulation is measured by the zeta potential. When all particles of emulgel have large positive or negative zeta potential, the repulsion forces between them increase and dispersion stability is achieved. There is no force to keep the particles away from colliding if their zeta potentials are low, resulting in dispersion instability. The ideal range of zeta potential for emulgel stability is + 50mV to -50mV.

Figure 4 indicates the zeta potential of formulations F1, F3, and F4 was - 46.5, -34.7 and - 46.5 respectively. The negative zeta potential is beneficial for enhanced stability of the formulations. Emulgel formulations with either negative or positive zeta potential, i.e., surface charges, have enhanced stability. This is because of the increased electrostatic repulsion between the droplets or globules of the emulsion system and thus it will prevent droplet coalescence. The value of zeta potential lies in the range which confirms the stability of emulgel formulations. The term PDI represents the system's polydispersity and its values are 0.0 to 1.0. The PDI for nimesulide emulgel formulations ranged from 0.945, 0.975 and 1.00 for F1, F3 and F4, respectively and these values depict the heterogenous dispersion of droplets in the formulation [38].

3.9. Photomicrography

Figure 5 demonstrates the microscopic view of emulgel formulations F1, F3 and F4. The presence of oil droplets of the emulgel can be seen clearly that act as reservoir for lipophilic drugs and the sustained release of the drug is manifested by the complex gel structure making them ideal carriers for drug delivery.

3.10. Ex-vivo Release Study

The release of drug from emulgels depends on multiple factors including gelling agent concentration, spreadability, emulsifying agent, and viscosity. Figure 6 elaborates the ex-vivo release profile of the model drug, nimesulide. The average release of nimesulide from the formulations F1, F3 and F4 was 86%, 55% and 66%, respectively, within 24 h. The overall concentration of AX in these formulations was approximately 1.5%, 2% and 1% respectively. An incomplete release in case of F3 may be associated to the slightly higher concentration of polymer that makes a complex gel network. The viscosity of the newly developed emulgels, affected by swellability of AX, played a significant role in overall release of the drug. The added possible reason might be due to higher cross-linking density which ultimately decreases the elasticity of polymeric structure, therefore restricting the movement of drug solution [39, 40]. Optimized formulation F1 having maximum ex-vivo cumulative drug release was therefore selected for FTIR, in-vivo analgesic activity and skin irritation test.

3.12. FTIR Analysis

FTIR of AX, pure nimesulide, and optimized emulgel formulation F1 is described in Fig. 7. In the FTIR spectrum of AX, the absorption bands at 3595cm⁻¹ indicate OH-stretching, the main band at 2866cm⁻¹ is linked with the aliphatic CH-stretching and the bands from 1750-2000cm⁻¹ illustrate the deformation by water absorption. A further look indicates the presence a peak at 1483cm⁻¹ (conforming to the CH₂ group), 1352cm⁻¹ (CH), 1134 cm⁻¹ (arabinosyl side chain) and the peak at 1010cm⁻¹ showing C-C, C-O, and C-O-H group stretching [41]. FTIR spectrum of pure nimesulide provides the fingerprint region from 2500-4000cm⁻¹. The individual strong peak was observed at 3423 cm⁻¹ that revealed the N-H stretching (amide group). The peak of the drug at 1490 cm⁻¹ confirms the presence of NO₂ group, while the peak at 1315. 2 cm⁻¹ reveals the presence of CH3 group and 1157. 20 cm⁻¹ is for S = O group that confirm the drug purity [42]. FTIR of emulgel containing nimesulide clearly confirms the presence of the drug in the emulgel. The sharp peak of nimesulide at 3423cm⁻¹is clear in the spectrum, revealing the presence of amide group of drugs in emulgel.

3.13. In-vivo analgesic activity

Analgesic effect in treated groups of rats and control was evaluated by observing the response (raising or licking paw, jumping) to pain stimulus and outcomes of the study are described in Fig. 8. The F1 treated group demonstrated an overall hike in the lapse time compared to control group and therefore, hinting a higher analgesic effect. It was observed that the treated group produced a maximum delay in reaction time (analgesic effect) at 1.5 h that was approximately three folds higher than the control group. The possible reason of better analgesic effect in treated group indicates that drug shows more efficacy in emulgel formulation. PEG was used in nimesulide emulgel which act as a permeation enhancer. After application, integrity of stratum corneum got changed, leading to enhanced percutaneous absorption of developed formulation as compared to marketed product [43]. Statistically, the outcomes of the study, analgesic effect, were found significantly different (*p*< 0.05) for the treated group as compared to control group in the student's *t*-test.

3.14. Skin irritation test

For skin irritation test, procedure for the skin irritation grading was conducted every day extended over a period of one week. As depicted in the Fig. 9, the signs of skin irritation such as significant redness or abrupt change in morphology was found absent for both the treatment and control groups. A very mild redness was observed but that is considered a normal skin reaction to the repetitive rub-on of the topical formulation on the same spot. The scoring system from grade 0 to grade 3 was used to estimate skin irritation. In scoring system, score 0 value meant no signs of irritation, score 1 indicated slightly patchy erythema, score 2 patchy erythema and a score of 3 severe erythema with or without edema [44]. The outcomes of the skin irritation via the scoring system are demonstrated in Table 7.

Table 7 Evaluation of optimized Emulgel for skin irritation in control and treated group.							
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Control Group	0	0	0	0	0	0	0
Treated Group	0	0	0	0	0	0	0

4. Conclusion

This study was designed to evaluate natural biomaterial AX as gelling agent in emulgel formulation. Results indicated that AX extracted from *Plantago ovata* is an excellent gelling agent in emulgel formulation. The topical emulgel made from AX possesses good homogeneity, spreadability, stability and gradual drug release characteristics. The program response surface methodology proved to be a significant help in optimizing the formulation and later, the best formulation in term of drug release was picked for numerous studies. The three times higher delay in the lapse times for the treated group (F1 formulation) clearly indicated the higher analgesic activity of developed formulation. Hence, it can be concluded that developed emulgel containing nimesulide was better able to promote the analgesic activity via penetration in to stratum corneum and could be used as carrier for other drugs in future needs.

Declarations

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Author Contributions: Conceptualization, Arshad Mahmood, Alia Erum and Ume Ruqia Tulain.; methodology and investigation Nadia Shamshad Malik, Aneeqa Saleem and Muhammad Zubair Malik.; writing—original draft preparation Alia Erum, Ume Ruqia Tulain and Nadia Shamshad Malik.; writing—review and editing Mohammed S Alqahtani, Mahwish Siddiqui, Asif Safdar and Abdul Malik. All authors have read and agreed to the published version of the manuscript.

Ethics Statements: Guidelines for care and use of laboratory animals of Faculty of Pharmacy, University of Sargodha, Sargodha, Pakistan, were used to perform animal studies, and these studies were duly approved by the animal ethics committee of University of Sargodha.

Conflicts of Interest: The authors declare no conflict of interest.

Data Availability Statement: The authors confirm that the data supporting the findings of this study are available within the article.

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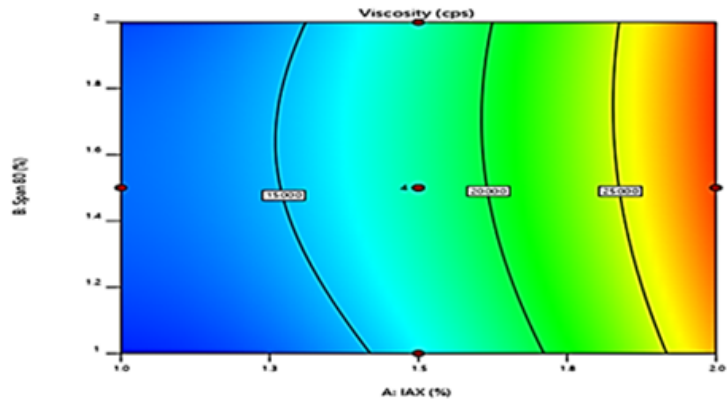
Figures

Factor Coding: Actual

Viscosity (cps)
● Design Points

11445 30676
X1 = A: IAX
X2 = B: Span 80

Actual Factor
C: Tween 20 = 0.75

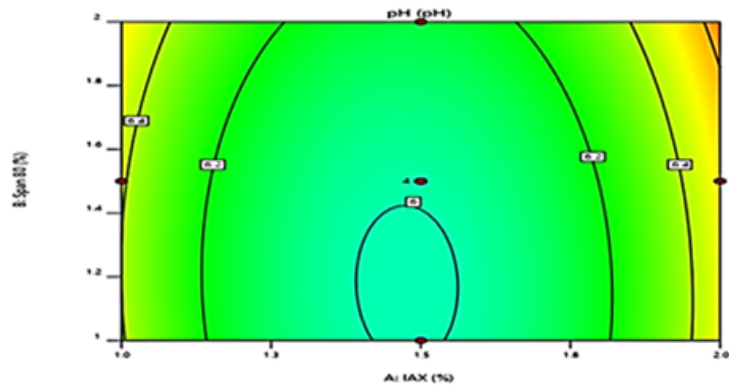


Factor Coding: Actual

pH (pH)
● Design Points

5.6 6.8
X1 = A: IAX
X2 = B: Span 80

Actual Factor
C: Tween 20 = 0.75



Factor Coding: Actual

drug assay (%)
● Design Points

60 115
X1 = A: IAX
X2 = B: Span 80

Actual Factor
C: Tween 20 = 0.75

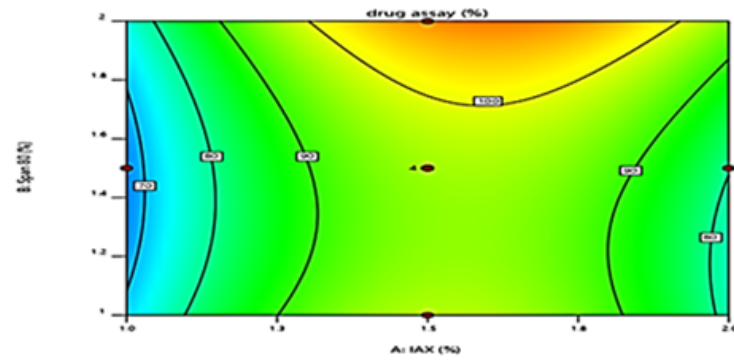


Figure 1

Contour plot of viscosity, pH and drug assay.

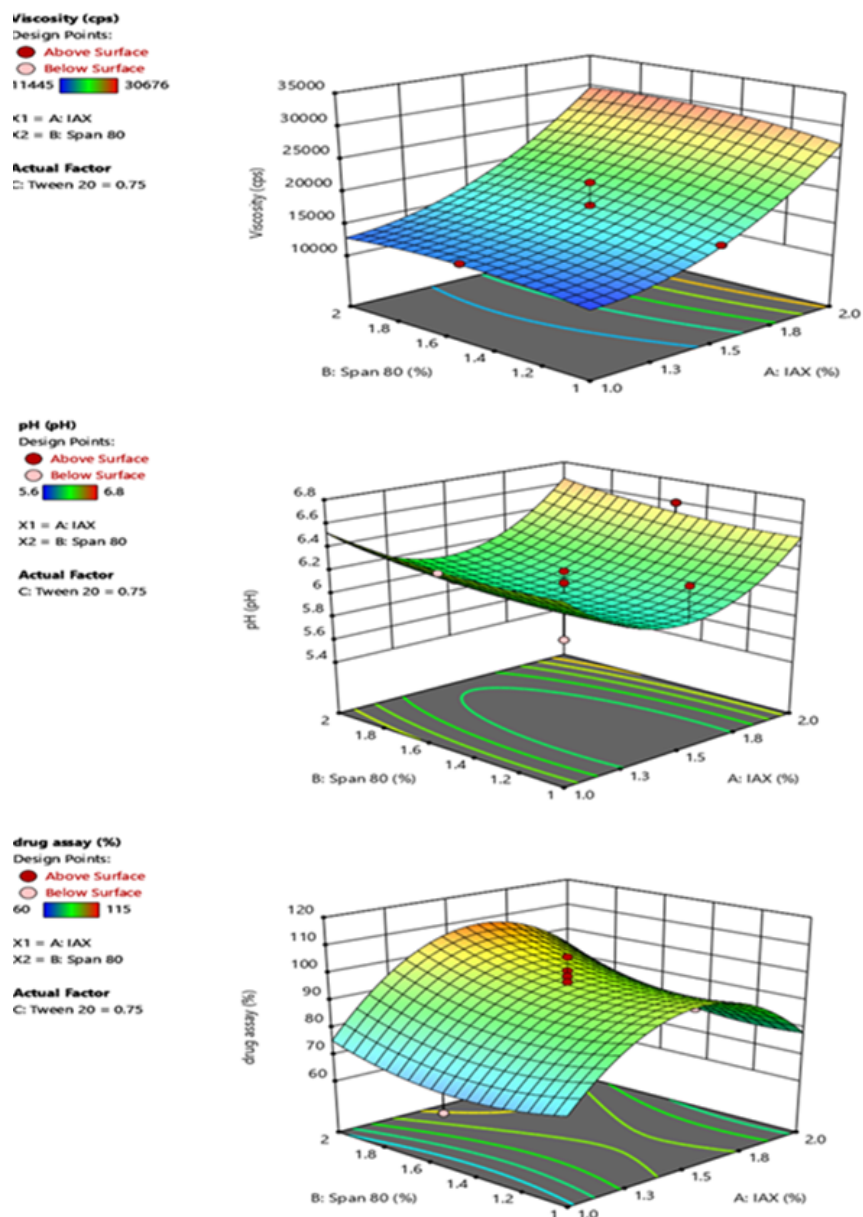


Figure 2

3-D graph of viscosity and drug assay.

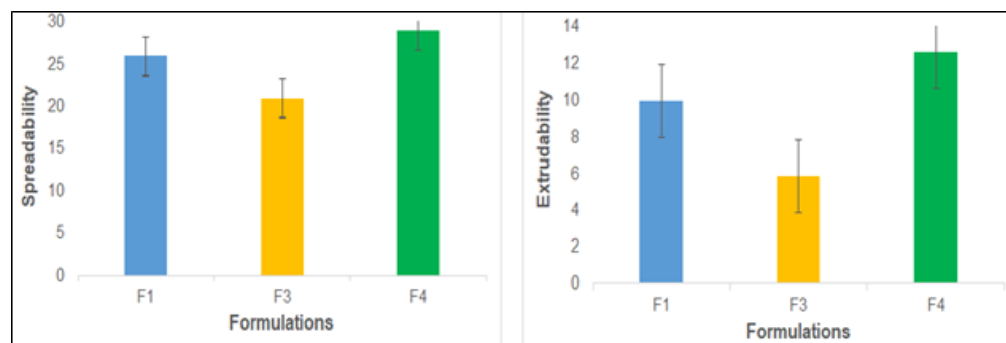


Figure 3

Spreadability and extrudability of the optimized emulgel formulations.

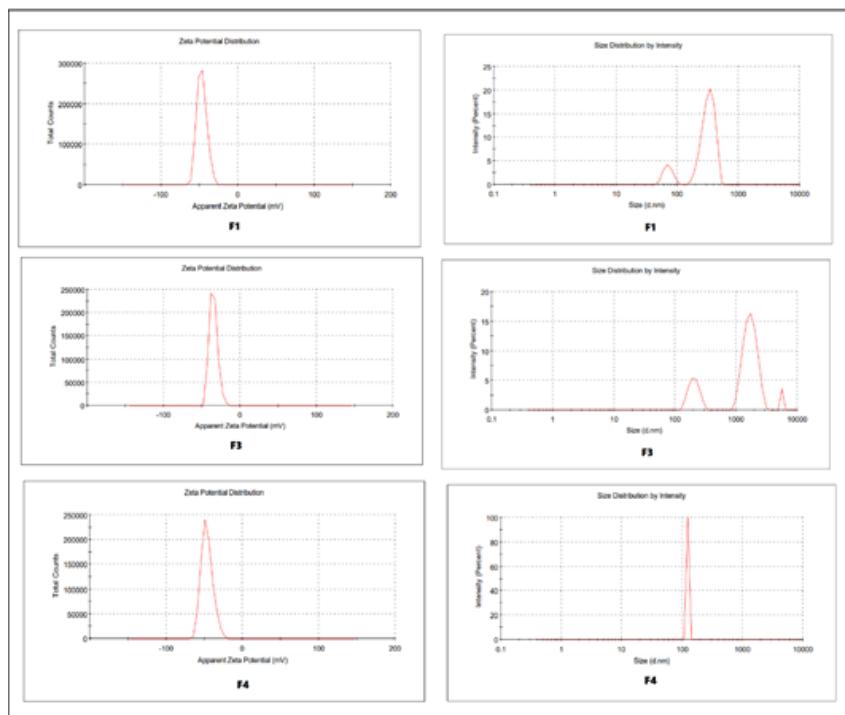


Figure 4

Zeta potential and globule size distribution of Formulation F1, F3, F4.

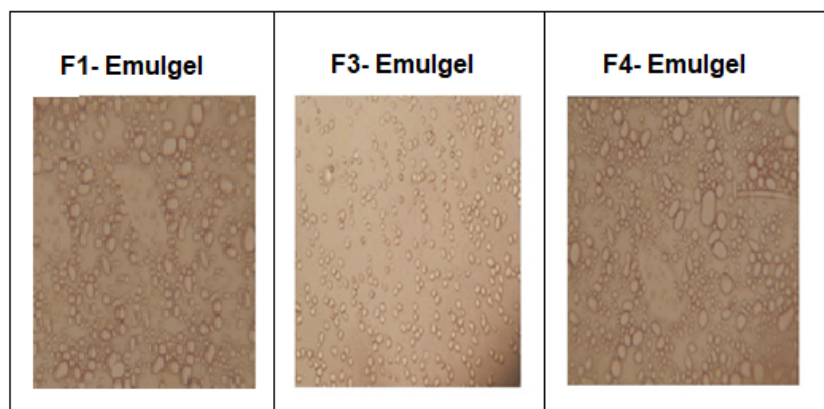


Figure 5

Microscopic view of optimized emulgel formulations F1, F3 and F4

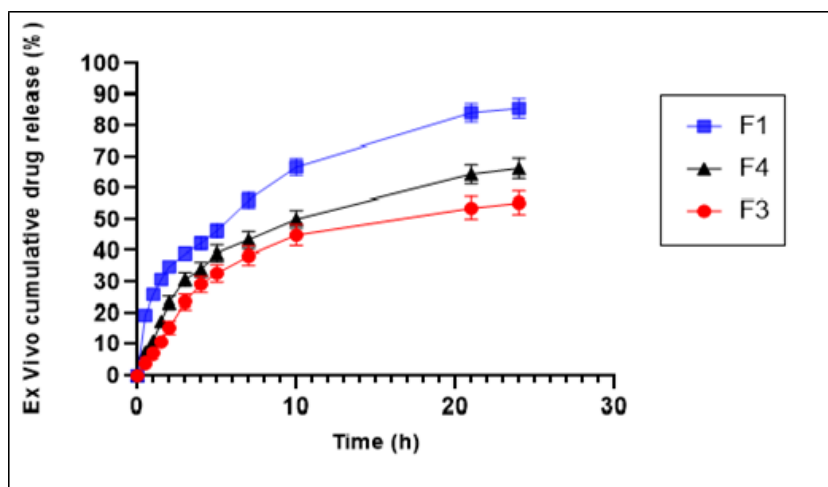


Figure 6

Ex-vivo cumulative % release of nimesulide from formulations F1, F3 and F4

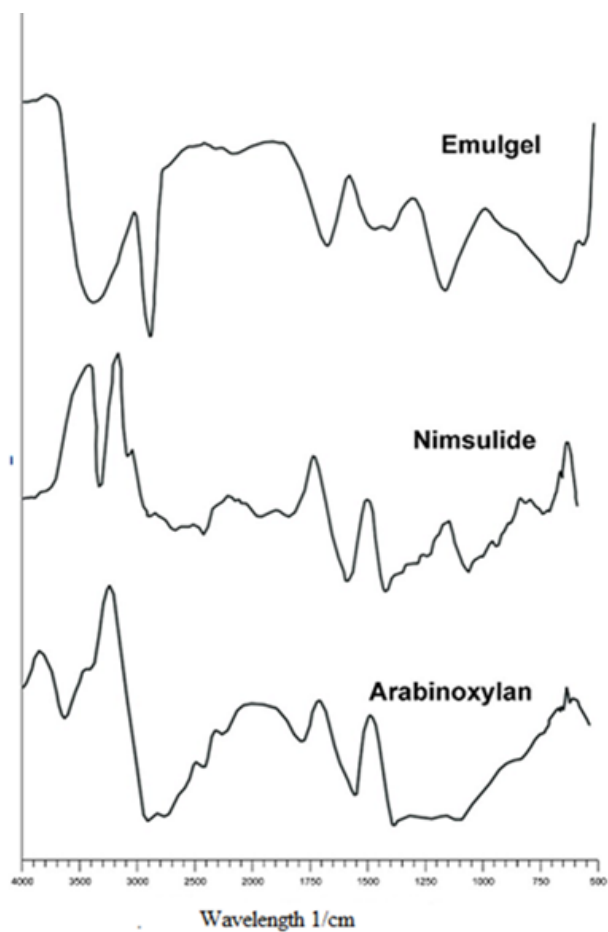


Figure 7

FTIR of AX, pure nimesulide and optimized emulgel formulation F1

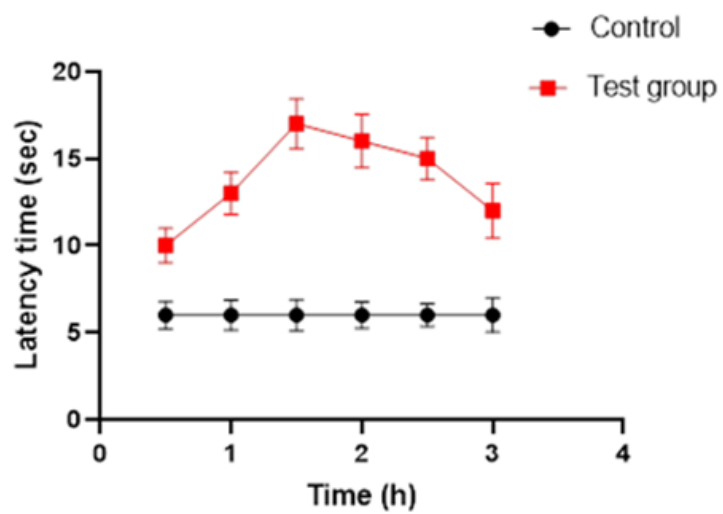


Figure 8

Pain reaction time in test and control group.

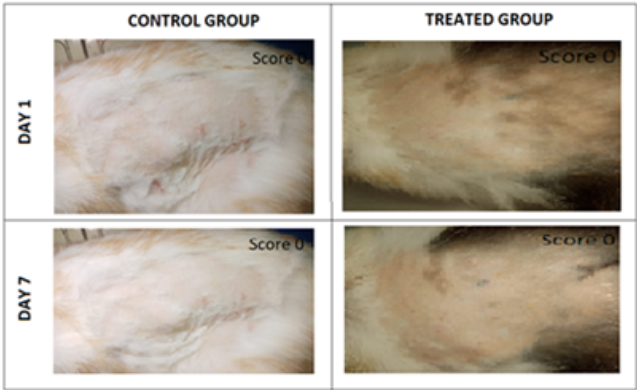


Figure 9

Skin irritation test in control and treated group.