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Development and In-Vivo Validation of a Buxus papillosa–Salvia hispanica Seed Oil-Based Topical Gel for Attenuating Inflammation and Oxidative Stress

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ABSTRACT: Background: Inflammation and oxidative stress are closely associated with the progression of several inflammatory disorders. Plant-derived seed oils may provide a promising approach for developing topical formulations with complementary anti-inflammatory and antioxidant effects. The present study aimed to develop and evaluate a topical gel containing Buxus papillosa and Salvia hispanica seed oils.

Methods: Five Carbopol 940-based gel formulations (F1–F5) were developed and evaluated for physicochemical characteristics, in-vitro release, release kinetics, ex-vivo skin permeation, stability, and skin irritation. The optimized formulation was further evaluated for anti-inflammatory and antioxidant activity using a formalin-induced paw edema model. Biochemical parameters including superoxide dismutase (SOD), glutathione (GSH), total protein, and catalase were assessed.

Results: The combined formulation F5 demonstrated favorable physicochemical characteristics, with 94.03% cumulative in-vitro release and 80.24% ex-vivo skin permeation at 12 h. Release kinetics showed the highest correlation with the Higuchi model ($R^2 = 0.9967$). F5 produced the greatest reduction in formalin-induced paw edema among the experimental formulations, with paw volume reduced to 0.92 ± 0.008 , compared with 1.48 ± 0.014 in the disease-control group and 0.87 ± 0.005 with indomethacin. F5 also markedly restored SOD, GSH, total protein, and catalase levels compared with the disease-control group. No visible skin irritation was observed during the 7-day assessment.

Conclusion: The B. papillosa–S. hispanica seed-oil-loaded topical gel, particularly F5, demonstrated promising anti-inflammatory and antioxidant activity, together with favorable release, permeation, stability, and skin-tolerability characteristics. These findings support further mechanistic and translational investigation of this plant-derived topical formulation.

1. INTRODUCTION

Inflammation is a complex and tightly regulated biological response that protects tissues against injury and harmful stimuli. Although acute inflammation is essential for maintaining tissue homeostasis and initiating repair, its excessive or persistent activation can contribute to tissue damage and the progression of several pathological conditions. Inflammatory responses are accompanied by the release of multiple mediators, including histamine, serotonin, prostaglandins, and cytokines, which collectively promote vascular changes, edema formation, and recruitment of inflammatory cells [1]. Importantly, inflammation

is closely interconnected with oxidative stress, as excessive generation of reactive oxygen species (ROS) can amplify inflammatory signaling and compromise cellular integrity. The formalin-induced inflammatory response used in the present study provides an established experimental model for investigating changes in paw edema and associated biochemical alterations [2].

Oxidative stress represents an important component of inflammatory tissue injury and occurs when the generation of reactive oxygen species exceeds the capacity of endogenous antioxidant defense systems. Enzymatic antioxidants such as superoxide dismutase (SOD) and catalase, together with reduced glutathione (GSH), constitute an important defense network against oxidative damage [3]. In the present thesis, formalin-induced inflammation was associated with marked alterations in these endogenous antioxidant parameters, whereas treatment with the investigated seed-oil formulations partially restored antioxidant defense. In particular, restoration of SOD and catalase activity suggests attenuation of oxidative burden and improved endogenous protection against reactive oxygen species [4].

The search for naturally derived therapeutic agents with both anti-inflammatory and antioxidant properties has consequently received considerable attention. *Buxus papillosa* C.K. Schneid. and *Salvia hispanica* L. represent two botanically distinct plant sources with reported pharmacological potential [5]. The thesis investigation demonstrated the presence of several classes of bioactive phytoconstituents in the seed oils of both plants, including alkaloids, flavonoids, phenolic compounds, tannins, terpenoids, steroids, and quinones. The observed phytochemical profile provides a plausible basis for investigating these oils as sources of bioactive compounds with potential antioxidant and anti-inflammatory effects [6].

Salvia hispanica (chia) has attracted increasing scientific interest because of its biologically active seed constituents and oil. Previous studies cited in the thesis have reported antioxidant, anti-inflammatory and other pharmacological properties of chia-derived preparations. Recent literature has particularly explored the capacity of *S. hispanica* seed oil and extracts to modulate oxidative stress and inflammatory responses, supporting further investigation of chia-derived bioactives in therapeutic applications. In parallel, *Buxus papillosa* has been investigated for its pharmacological properties, with previous studies identifying steroidal alkaloids and other bioactive constituents and reporting antioxidant and diverse pharmacological activities [7].

Despite the pharmacological potential of these individual plant sources, the therapeutic relevance of combining *B. papillosa* and *S. hispanica* seed oils within a topical delivery system remains insufficiently explored. A combination approach may provide complementary biological effects arising from the different phytochemical profiles of the two oils. In the present work, *B. papillosa* oil exhibited comparatively greater representation of terpenoid and steroid constituents, whereas *S. hispanica* oil showed notable phenolic and flavonoid characteristics. Such differences provide a rational basis for investigating whether their incorporation into a single formulation can enhance the overall pharmacological response [8].

Topical drug delivery offers an attractive strategy for the localized management of inflammatory conditions because incorporation of bioactive agents into a semisolid dosage form can facilitate direct application to the affected site [9]. Accordingly, the present study developed oil-loaded topical gels using Carbopol 940 as the gelling agent. Five formulations were prepared to evaluate the individual and combined effects of *B. papillosa* and *S. hispanica* oils, with the F5 formulation containing both oils in a 1:1 combination. The formulations were further evaluated for physicochemical characteristics, in-vitro release, stability, and skin irritation to establish their suitability as topical preparations [10].

The pharmacological evaluation provides an important translational component of the study. In the formalin-induced paw edema model, treatment with the individual oil-loaded gels reduced inflammatory edema, whereas the combination formulation produced the greatest reduction among the test formulations [11]. Paw volume was reduced from 1.48 ± 0.014 in the disease-control group to 0.92 ± 0.008 following treatment with the combination formulation, compared with 0.87 ± 0.005 for the indomethacin-treated standard group [12]. Furthermore, the combination formulation produced a stronger restoration of antioxidant defense, with SOD and catalase levels approaching those observed with the standard treatment [13].

Importantly, the developed formulations did not produce observable dermatological reactions during the seven-day skin irritation assessment, supporting their preliminary topical tolerability under the experimental conditions. These findings, together with the anti-inflammatory and antioxidant responses, provide a basis for further investigation of the combined seed-oil formulation as a potential plant-derived topical intervention [14].

Therefore, the present study was designed to develop and evaluate a topical gel containing *Buxus papillosa* and *Salvia hispanica* seed oils and to investigate its anti-inflammatory efficacy and influence on oxidative stress-associated biochemical parameters in a formalin-induced inflammation model. The study further compared the combined formulation with individual oil formulations and a standard anti-inflammatory treatment to determine whether combination therapy provides an enhanced pharmacological response.

2. MATERIALS AND METHODS

2.1 Formulation of *Buxus papillosa* and *Salvia hispanica* Seed Oil-Loaded Topical Gels

Topical gel formulations containing *Buxus papillosa* and *Salvia hispanica* seed oils were prepared using Carbopol 940 as the gelling polymer. Briefly, Carbopol 940 (1% w/w) was dispersed in distilled water under continuous stirring and allowed to hydrate completely. The respective seed oils were subsequently incorporated into the hydrated polymer dispersion.

Five formulations (F1–F5) were prepared. F1 and F2 contained *B. papillosa* seed oil at concentrations corresponding to 0.5 and 1.0 mL, respectively, whereas F3 and F4 contained *S. hispanica* seed oil at 0.5 and 1.0 mL, respectively. F5 contained a combination of *B. papillosa* and *S. hispanica* seed oils in a 1:1 ratio (1 mL each). Propylene glycol (5% v/v) and glycerin (5% v/v) were incorporated as humectants and penetration-enhancing excipients. Methyl paraben (0.15% w/w) and propyl paraben (0.05% w/w) were used as preservatives. Triethanolamine was added as required for pH adjustment, and distilled water was added to make the final formulation weight up to 100 g.

Table 1. Composition of seed oil-loaded topical gel formulations

Ingredient	F1	F2	F3	F4	F5
Carbopol 940 (% w/w)	1.0	1.0	1.0	1.0	1.0
<i>B. papillosa</i> oil (mL)	0.5	1.0	—	—	1.0
<i>S. hispanica</i> oil (mL)	—	—	0.5	1.0	1.0
Propylene glycol (% v/v)	5.0	5.0	5.0	5.0	5.0
Glycerin (% v/v)	5.0	5.0	5.0	5.0	5.0
Methyl paraben (% w/w)	0.15	0.15	0.15	0.15	0.15
Propyl paraben (% w/w)	0.05	0.05	0.05	0.05	0.05
Triethanolamine	q.s.	q.s.	q.s.	q.s.	q.s.
Distilled water	q.s. to 100 g	q.s. to 100 g	q.s. to 100 g	q.s. to 100 g	q.s. to 100 g

2.2 Physicochemical Evaluation of the Topical Gels

The prepared formulations were evaluated for their physical appearance, clarity, occlusiveness, washability, and organoleptic characteristics by visual examination. The pH of each formulation was measured using a digital pH meter, and measurements were performed in triplicate.

2.2.1 Spreadability

Spreadability was evaluated by placing 1 g of gel between two horizontal glass plates measuring 20 × 20 cm². A standardized 500-g weight was applied to the upper plate, and the diameter of the spread gel was recorded after 5 min. Spreadability was calculated using the standard relationship described in the thesis methodology.

2.2.2 Viscosity and Homogeneity

The viscosity of the formulations was measured using a Brookfield viscometer at 100 rpm using spindle no. 6. Homogeneity was assessed visually for uniform appearance and the absence of aggregates or particulate matter.

2.3 In-Vitro Release Study

The in-vitro release of the prepared formulations (F1–F5) was investigated using a Franz diffusion cell. A cellulose acetate dialysis membrane with a molecular weight cut-off of 12,000–14,000 Da was soaked overnight in phosphate buffer (pH 7.4) before use.

Approximately 1 g of each gel formulation was uniformly applied to the membrane in the donor compartment. The receptor compartment contained 20 mL of phosphate buffer (pH 7.4) containing 20% ethanol to maintain sink conditions. The diffusion medium was maintained at $37 \pm 0.5^\circ\text{C}$ and continuously stirred at 100 rpm.

At predetermined intervals of 0.5, 1, 2, 4, 6, 8, 10, and 12 h, 1 mL samples were withdrawn from the receptor compartment and immediately replaced with an equivalent volume of fresh diffusion medium maintained at the same temperature. Samples were analyzed using UV–Visible spectrophotometry at the predetermined wavelength corresponding to the major phytoconstituent.

2.4 Ex-Vivo Skin Permeation Study

Ex-vivo skin permeation was evaluated using freshly excised goat abdominal skin obtained from a local slaughterhouse. The skin was carefully cleaned to remove adhering adipose and connective tissues and subsequently washed with normal saline. The prepared skin was equilibrated in phosphate buffer (pH 7.4) before being mounted on a Franz diffusion cell with the stratum corneum facing the donor compartment.

Approximately 1 g of gel was uniformly applied to the skin surface. The receptor compartment contained 20 mL phosphate buffer (pH 7.4) containing 20% ethanol and was maintained at $37 \pm 0.5^\circ\text{C}$ with continuous stirring at 100 rpm. Samples of 1 mL were withdrawn at 1, 2, 4, 6, 8, 10, and 12 h and replaced with an equal volume of fresh diffusion medium. The samples were analyzed spectrophotometrically, and cumulative permeation was calculated. Each experiment was performed in triplicate.

2.5 Stability Study

The stability of formulations F1–F5 was evaluated for 3 months under different storage temperatures ($28 \pm 2^\circ\text{C}$, $30 \pm 2^\circ\text{C}$, and $35 \pm 2^\circ\text{C}$). During the study period, the formulations were periodically examined for changes in colour, pH, viscosity, homogeneity, and spreadability. All measurements were performed in triplicate and expressed as mean $\pm$ SD.

2.6 Skin Irritation Study

The preliminary dermal tolerability of the prepared gel formulations was evaluated in Wistar rats of either sex weighing approximately 150–200 g. Hair was removed from the treatment area 3 days before the experiment. The respective gel formulations were applied daily for 7 days, and the treated sites were examined for visible erythema and edema.

2.7 In-Vivo Anti-Inflammatory Evaluation

2.7.1 Experimental Animals and Treatment Groups

Wistar rats were randomly allocated into eight experimental groups, with six animals per group. Group I served as the normal control, whereas Group II served as the disease-control group. Groups III and IV received F1 and F2, respectively; Groups V and VI received F3 and F4, respectively; Group VII received F5 containing the 1:1 combination of *B. papillosa* and *S. hispanica* oils. Group VIII received 1% w/w indomethacin gel as the standard treatment.

2.7.2 Formalin-Induced Paw Edema

Formalin-induced paw edema was used to evaluate the anti-inflammatory activity of the formulations. Paw volume was initially measured using the mercury displacement method. Inflammation was induced using formalin, and changes in paw volume were monitored over the experimental observation period. The degree of edema inhibition was used as an indicator of anti-inflammatory activity.

2.7.3 Formalin-Induced Nociception

The anti-nociceptive activity of the topical formulations was evaluated using the formalin-induced paw nociception model. The study was conducted in a blinded manner, with the observer unaware of treatment allocation. Formulations F1–F5 were

applied topically to the plantar surface of the right hind paw 60 min before administration of 20 μ L of 1% formalin. Indomethacin gel (1% w/w) served as the standard treatment.

Nociceptive behavior was assessed by recording the duration of paw licking and biting. The early neurogenic phase (0–5 min) and late inflammatory phase (15–45 min) were evaluated, and percentage inhibition of nociceptive response was calculated relative to the disease-control group.

2.8 Biochemical Assessment

Following the final treatment schedule, blood and tissue samples were collected for biochemical evaluation. Serum was separated following centrifugation at 3000 rpm for 15 min. Tissue homogenates were prepared using appropriate buffers for the estimation of oxidative stress and antioxidant parameters.

2.8.1 Superoxide Dismutase

SOD activity was determined based on inhibition of pyrogallol auto-oxidation under alkaline conditions. The change in absorbance was monitored at 420 nm using a UV–Visible spectrophotometer. SOD activity was expressed as units per milligram of protein.

2.8.2 Catalase

Catalase activity was determined by monitoring the decomposition of hydrogen peroxide spectrophotometrically at 240 nm. The reaction was monitored for 3 min, and enzyme activity was calculated from the rate of decrease in absorbance and expressed as units per milligram of protein.

2.8.3 Total Protein

Total protein content was determined using the Bradford protein assay, with absorbance measured at 595 nm.

2.8.4 C-Reactive Protein

Serum C-reactive protein (CRP) was determined using a commercially available CRP-latex diagnostic kit according to the manufacturer's instructions.

2.9 Statistical Analysis

Experimental data were expressed as mean $\pm$ SEM. Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test. Differences were considered statistically significant at $p < 0.05$.

3. RESULTS

3.1 Physicochemical Characteristics of Seed Oil-Loaded Gels

All five formulations (F1–F5) exhibited a smooth and homogeneous appearance without visible lumps, grittiness, or phase separation. F1 and F2 showed a light green to pale-brown appearance, F3 and F4 were faint yellowish, while the combined formulation F5 showed a slightly darker appearance. All formulations demonstrated good homogeneity.

The pH of the formulations ranged from 6.18 to 6.78, indicating a relatively skin-compatible pH range. F1 showed the highest pH (6.78), whereas F4 showed the lowest (6.18). Viscosity ranged from 3000 to 4500 cP, with F1 showing the highest viscosity and F2 the lowest. The optimized F5 formulation exhibited a viscosity of 3800 cP. Spreadability ranged from 9.60 ± 0.05 to 11.73 ± 0.01 mm, with F5 showing a spreadability of 10.50 ± 0.02 mm.

Table 1. Physicochemical characteristics of seed oil-loaded topical gels

Formulation	pH	Viscosity (cP)	Homogeneity	Spreadability (mm)
F1	6.78	4500	Good	11.73 ± 0.01
F2	6.25	3000	Good	9.85 ± 0.04
F3	6.62	4200	Good	11.20 ± 0.03

F4	6.18	3100	Good	9.60 ± 0.05
F5	6.40	3800	Good	10.50 ± 0.02

3.2 In-Vitro Release Profile

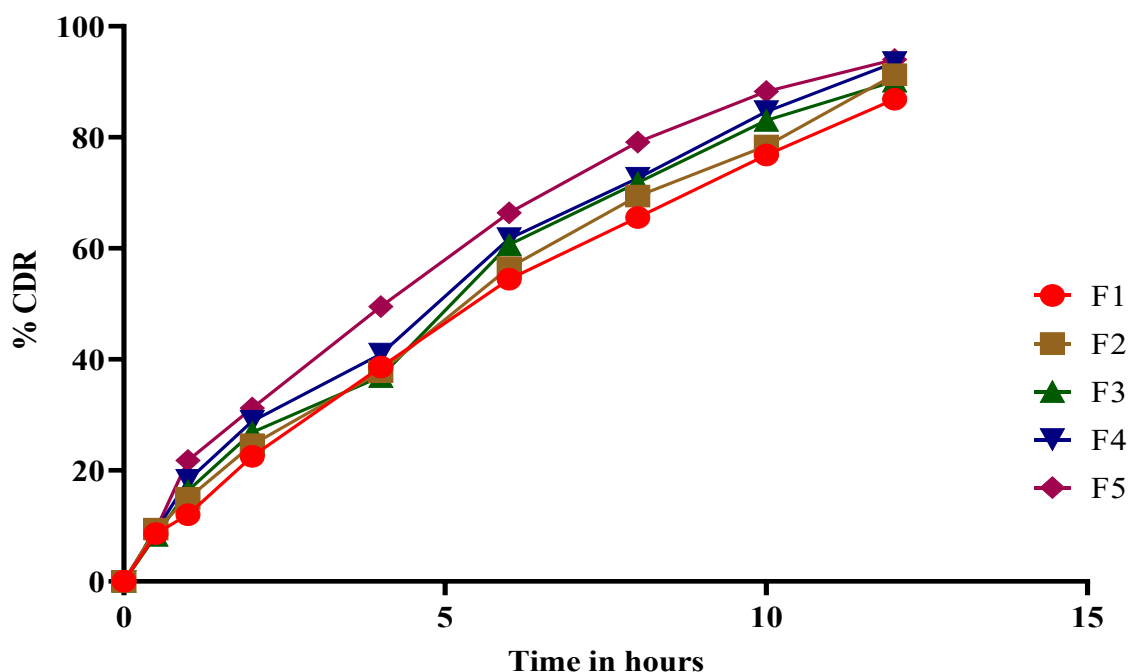
All formulations demonstrated an initial burst release followed by sustained release over the 12-h study period. The cumulative release increased progressively with time.

At 12 h, F5 demonstrated the highest cumulative release (**94.03%**), followed by F4 (**93.48%**), F2 (**91.26%**), F3 (**90.12%**), and F1 (**86.80%**). Based on its comparatively higher release profile, F5 was selected as the optimized formulation for subsequent investigations.

Table 2. Cumulative in-vitro release after 12 h

Formulation	Cumulative release (%)
F1	86.80
F2	91.26
F3	90.12
F4	93.48
F5	94.03

Figure 1. *In-vitro* drug release of seed oil extract gel F1-F5.



3.3 Release Kinetics of Optimized F5 Formulation

The release data of F5 were fitted to zero-order, first-order, Higuchi, and Korsmeyer–Peppas models. The **Higuchi model showed the highest correlation coefficient ($R^2 = 0.9967$)**, followed by the Korsmeyer–Peppas model ($R^2 = 0.9824$) and first-order model ($R^2 = 0.9810$). The zero-order model demonstrated comparatively lower linearity ($R^2 = 0.9537$).

These findings indicate that release from F5 was predominantly governed by a **diffusion-controlled mechanism through the hydrated polymeric matrix**.

Table 3. Mathematical modelling of F5 release kinetics

Release model	R^2
Zero-order	0.9537
First-order	0.9810
Higuchi	0.9967
Korsmeyer–Peppas	0.9824

Figure 2. Zero order plot for release kinetics of seed oil extract gel (F5).

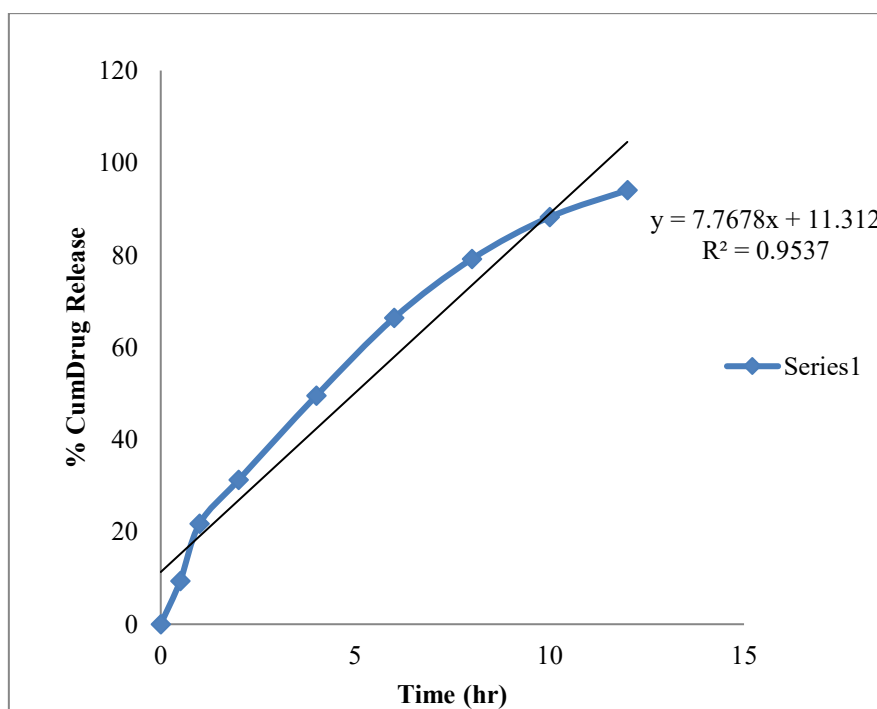


Figure 3. Higuchi plot for release kinetics of seed oil extract gel (F5).

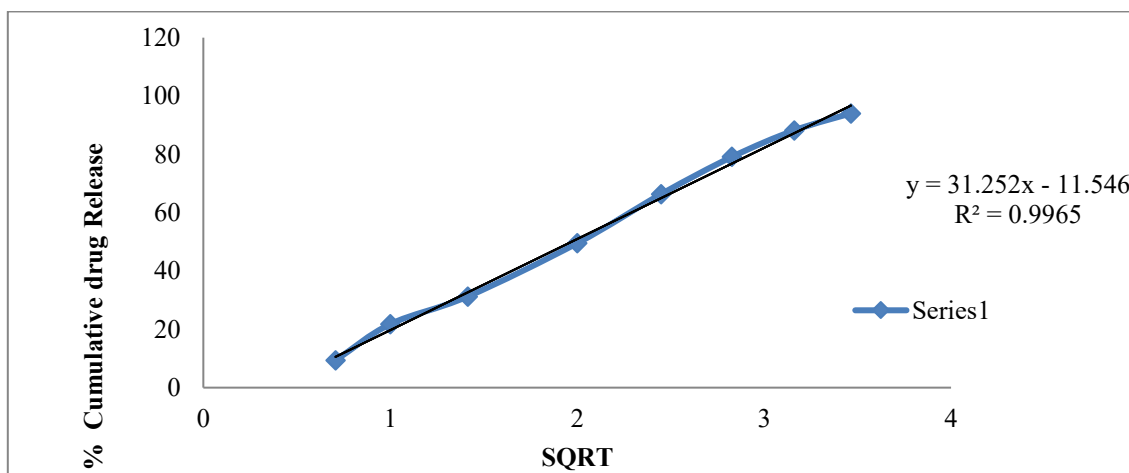


Figure 4. First order plot for release kinetics of seed oil extract gel (F5).

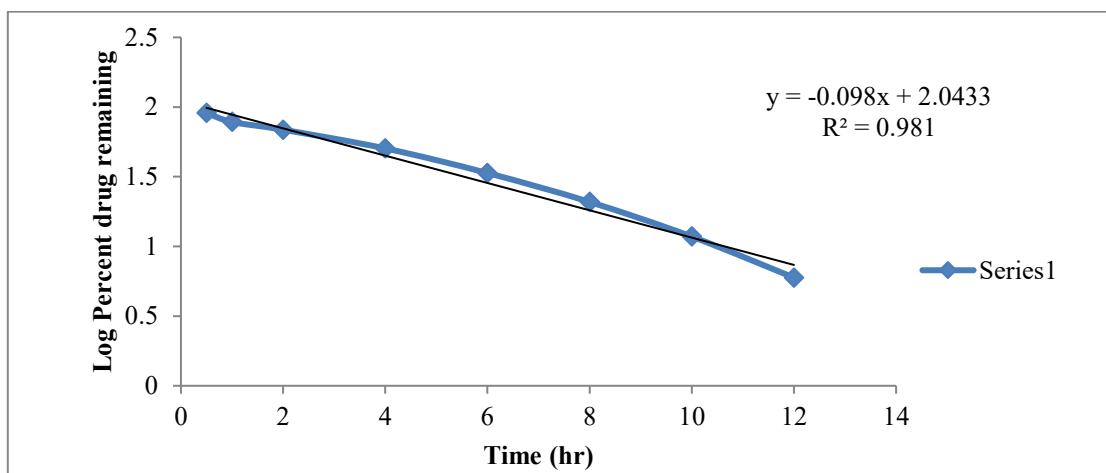
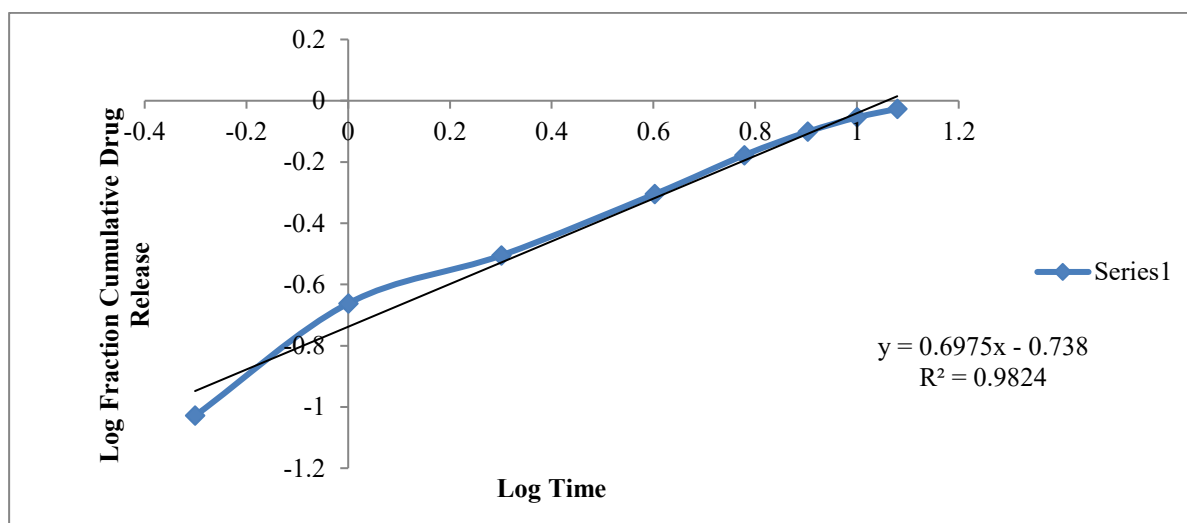


Figure 5. Peppa's plot for release kinetics of seed oil extract gel (F5).



3.4 Ex-Vivo Skin Permeation

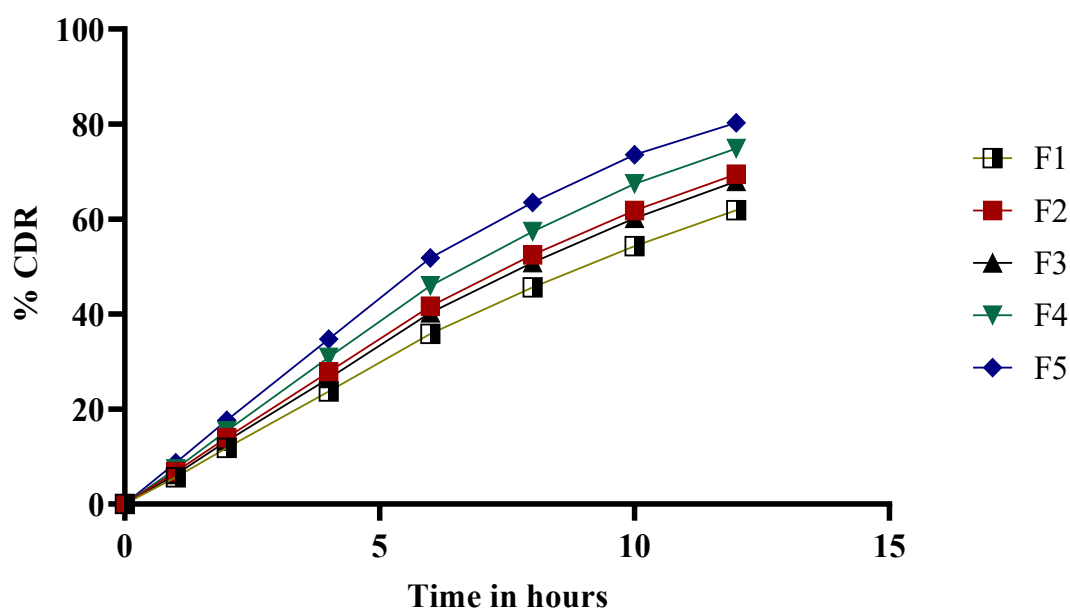
All formulations demonstrated a progressive increase in cumulative permeation during the 12-h study. F5 exhibited the highest cumulative permeation (**80.24%**), followed by F4 (**74.85%**), F3 (**71.26%**), F2 (**69.42%**), and F1 (**61.84%**).

Thus, the combination formulation showed the highest permeation among the tested formulations, supporting its selection for further pharmacological evaluation.

Table 4. Cumulative ex-vivo skin permeation at 12 h

Formulation	Permeation (%)
F1	61.84
F2	69.42
F3	71.26
F4	74.85
F5	80.24

Figure 6. *Ex-vivo* drug release study of seed oil extract gel F1-F5.



3.5 Stability of the Formulations

All formulations remained physically stable during the 3-month stability study. No major changes were observed in colour, homogeneity, pH, viscosity, or spreadability under the tested storage conditions. F5 maintained a pH of **6.38 ± 0.04**, **6.35 ± 0.05**, and **6.32 ± 0.04** during the first, second, and third months, respectively, while its viscosity remained within **3750–3800 cP**.

These observations indicate good short-term physical stability of the Carbopol-based formulations.

3.6 Skin Irritation Study

No visible erythema, edema, redness, or other dermatological reactions were observed following application of F1–F5 for seven consecutive days. All formulations were graded **“A – No reaction”** on each observation day, indicating absence of observable skin irritation under the experimental conditions.

3.7 In-Vivo Anti-Inflammatory Activity

3.7.1 Effect on Formalin-Induced Paw Edema

Formalin administration produced a marked increase in paw volume compared with the normal control (1.48 ± 0.014 vs. 0.82 ± 0.005). Treatment with all seed-oil-loaded formulations reduced paw volume compared with the disease-control group.

Among the individual oil formulations, F4 produced the greatest reduction in paw volume (1.01 ± 0.008), followed by F2 (1.08 ± 0.020), F3 (1.15 ± 0.008), and F1 (1.22 ± 0.03). Notably, the combination formulation F5 produced the greatest reduction among the experimental formulations, with paw volume decreasing to 0.92 ± 0.008 , approaching the value observed with the indomethacin-treated standard (0.87 ± 0.005).

Table 5. Effect of seed oil-loaded gels on formalin-induced paw edema

Group	Paw volume
Normal control	0.82 ± 0.005
Disease control	1.48 ± 0.014
F1	1.22 ± 0.03
F2	1.08 ± 0.020
F3	1.15 ± 0.008
F4	1.01 ± 0.008
F5	0.92 ± 0.008
Indomethacin	0.87 ± 0.005

Key finding: F5 reduced paw volume by approximately 37.8% compared with the disease-control group, based on the reported mean values.

3.8 Effect on Oxidative Stress and Antioxidant Parameters

3.8.1 Superoxide Dismutase

Formalin administration markedly reduced SOD activity from 8.52 ± 0.48 in the normal-control group to 3.12 ± 0.492 in the disease-control group.

Treatment increased SOD levels in all formulation groups. F5 produced the greatest restoration among the test formulations (7.63 ± 0.408), approaching the standard indomethacin group (7.98 ± 0.376).

3.8.2 Glutathione

GSH levels decreased from 7.85 ± 1.02 in the normal-control group to 2.95 ± 0.81 following formalin-induced inflammation. Treatment with the formulations restored GSH levels, with F5 producing the highest response among the test formulations (7.15 ± 1.34), compared with 7.42 ± 1.25 for indomethacin.

3.8.3 Total Protein

Total protein decreased substantially following formalin administration, from 72.40 ± 2.186 in the normal-control group to 45.23 ± 1.39 in the disease-control group.

Treatment with F1–F5 progressively restored total protein levels. F5 produced the highest value among the test formulations (69.23 ± 2.10), approaching the standard group (70.88 ± 2.05).

3.8.4 Catalase

Catalase activity decreased from 88.41 ± 2.32 in the normal-control group to 28.66 ± 1.75 in the disease-control group.

All treatment formulations increased catalase activity, with F5 producing 55.18 ± 2.16 , compared with 56.85 ± 2.04 in the indomethacin group. F5 therefore demonstrated the strongest antioxidant response among the experimental formulations.

Table 6. Summary of antioxidant and biochemical parameters

Group	SOD	GSH	Total protein	Catalase
Normal control	8.52 ± 0.48	7.85 ± 1.02	72.40 ± 2.186	88.41 ± 2.32
Disease control	3.12 ± 0.492	2.95 ± 0.81	45.23 ± 1.39	28.66 ± 1.75
F1	5.14 ± 0.66	4.85 ± 1.34	55.33 ± 2.20	39.86 ± 1.16
F2	6.25 ± 0.586	5.95 ± 1.16	62.65 ± 2.14	46.93 ± 2.06
F3	5.83 ± 0.408	5.40 ± 0.81	58.62 ± 1.96	43.95 ± 1.85
F4	6.92 ± 0.586	6.45 ± 1.25	65.43 ± 2.39	50.66 ± 2.16
F5	7.63 ± 0.408	7.15 ± 1.34	69.23 ± 2.10	55.18 ± 2.16
Indomethacin	7.98 ± 0.376	7.42 ± 1.25	70.88 ± 2.05	56.85 ± 2.04

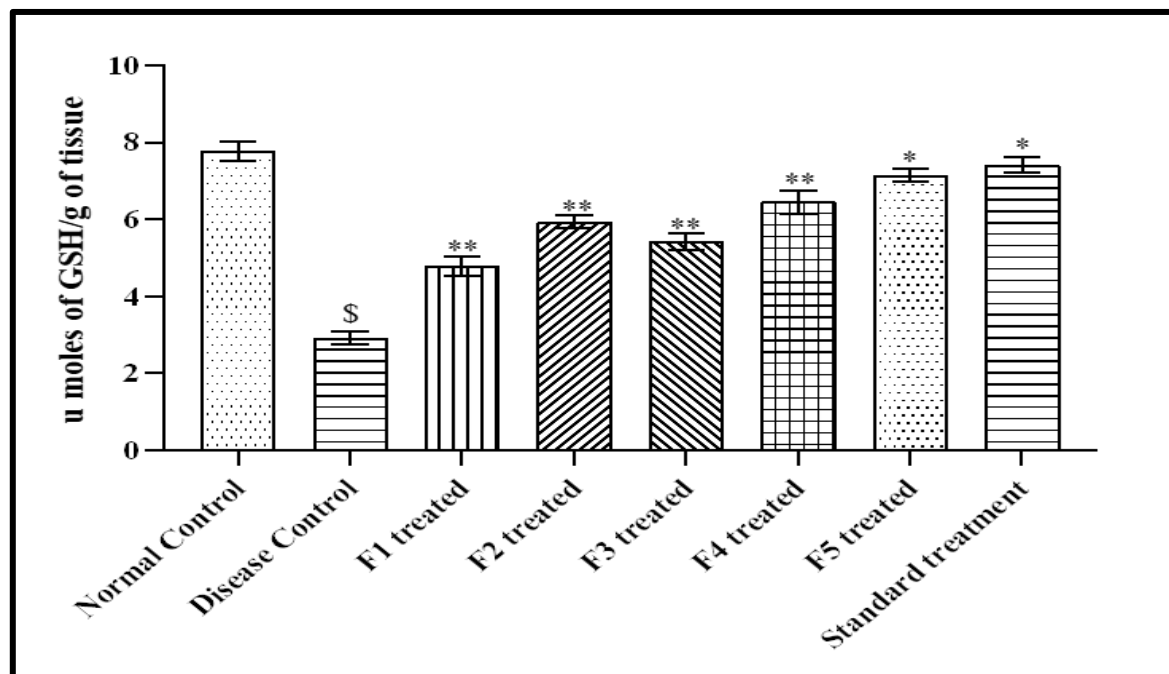
3.8.2 Glutathione

GSH levels decreased from 7.85 ± 1.02 in the normal-control group to 2.95 ± 0.81 in the disease-control group. Treatment with F1–F5 increased GSH levels, with F5 producing the highest response among the experimental formulations (7.15 ± 1.34), compared with 7.42 ± 1.25 in the indomethacin group.

Table 7. Effect of seed oil-loaded gels on GSH levels

Group	GSH level
Normal control	7.85 ± 1.02
Disease control	2.95 ± 0.81
F1	4.85 ± 1.34
F2	5.95 ± 1.16
F3	5.40 ± 0.81
F4	6.45 ± 1.25
F5	7.15 ± 1.34
Indomethacin	7.42 ± 1.25

Figure 7. Graph of the level of Glutathione. Data are shown as mean $\pm$ S.D. One-way ANOVA with Tukey's post-hoc test was used. \$p<0.05 vs. Control group, *p<0.05 vs. Disease Control, **p<0.05 vs. Standard Treatment.



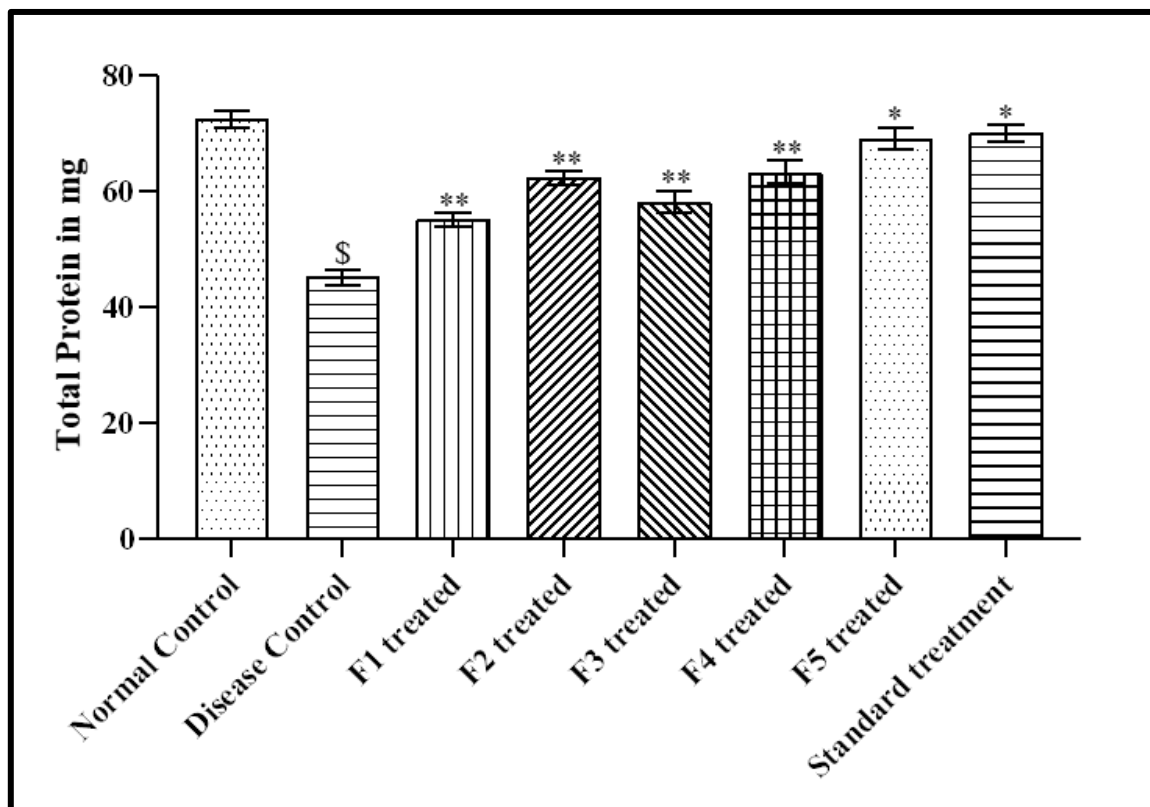
3.8.3 Total Protein

Total protein decreased from 72.40 ± 2.186 in the normal-control group to 45.23 ± 1.39 in the disease-control group. Treatment with the formulations restored total protein levels, with F5 producing 69.23 ± 2.10 , approaching the indomethacin group (70.88 ± 2.05).

Table 8. Effect of seed oil-loaded gels on total protein levels

Group	Total protein
Normal control	72.40 ± 2.186
Disease control	45.23 ± 1.39
F1	55.33 ± 2.20
F2	62.65 ± 2.14
F3	58.62 ± 1.96
F4	65.43 ± 2.39
F5	69.23 ± 2.10
Indomethacin	70.88 ± 2.05

Figure 8. Graph of Total Protein level. Data are shown as mean $\pm$ S.D. One-way ANOVA with Tukey's post-hoc test was used. \$p<0.05 vs. Control group, *p<0.05 vs. Disease Control, **p<0.05 vs. Standard Treatment.



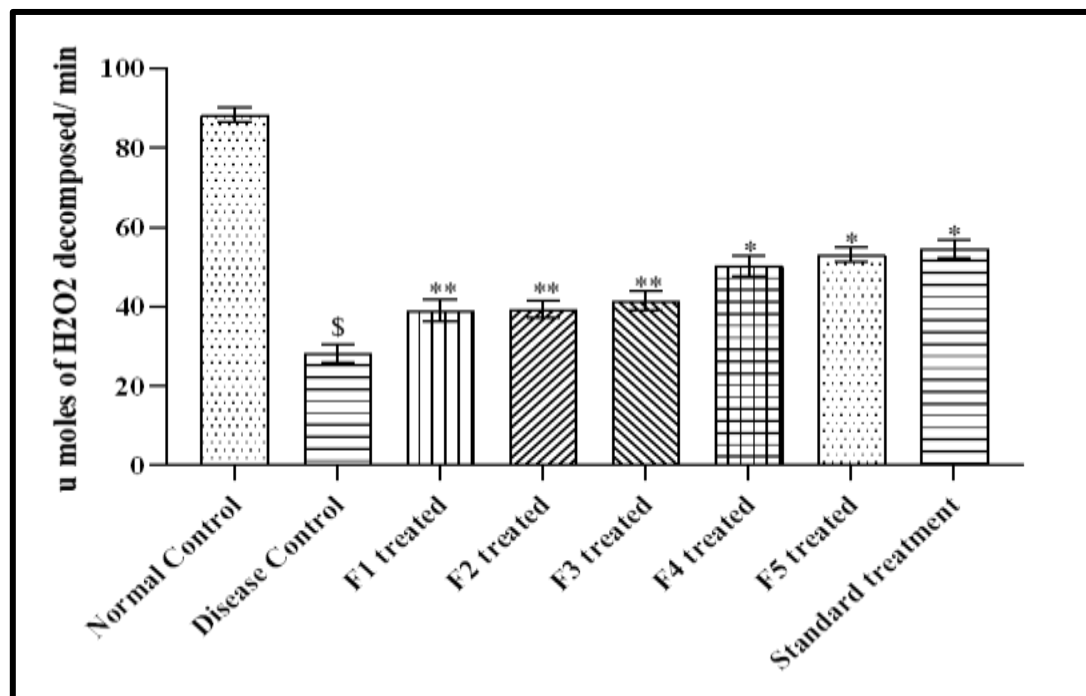
3.8.4 Catalase

Catalase activity decreased from 88.41 ± 2.32 in the normal-control group to 28.66 ± 1.75 in the disease-control group. All treatment formulations increased catalase activity, with F5 showing the highest activity among the experimental formulations (55.18 ± 2.16), approaching the indomethacin group (56.85 ± 2.04).

Table 9. Effect of seed oil-loaded gels on catalase activity

Group	Catalase activity
Normal control	88.41 ± 2.32
Disease control	28.66 ± 1.75
F1	39.86 ± 1.16
F2	46.93 ± 2.06
F3	43.95 ± 1.85
F4	50.66 ± 2.16
F5	55.18 ± 2.16
Indomethacin	56.85 ± 2.04

Figure 9. Graph of Catalase level. Data are shown as mean $\pm$ S.D. One-way ANOVA with Tukey's post-hoc test was used. \$p<0.05 vs. Control group, *p<0.05 vs. Disease Control, **p<0.05 vs. Standard Treatment.



3.9 Overall Findings

Collectively, the combination formulation F5 (1:1 *B. papillosa* + *S. hispanica* seed oils) demonstrated the most favorable overall profile among the experimental formulations. F5 showed the highest in-vitro release (94.03%), highest ex-vivo permeation (80.24%), and the greatest reduction in formalin-induced paw edema (0.92 ± 0.008). It also produced the strongest restoration of SOD, GSH, total protein, and catalase among the test formulations.

4. DISCUSSION

The present study was designed to develop a topical gel incorporating *Buxus papillosa* and *Salvia hispanica* seed oils and to investigate its physicochemical performance, skin permeation characteristics, anti-inflammatory activity, and influence on oxidative stress-associated biochemical parameters. The principal finding of the study was that the combined formulation F5, containing *B. papillosa* and *S. hispanica* seed oils in a 1:1 ratio, consistently demonstrated a more favorable overall pharmacological and biopharmaceutical profile than the formulations containing either oil individually.

The physicochemical characteristics of the prepared formulations indicated that incorporation of the seed oils into the Carbopol 940-based gel system produced physically acceptable topical formulations. The pH values remained within the range of approximately 6.18–6.78, suggesting compatibility with topical application, while F5 showed a pH of 6.40. The relatively uniform appearance and good homogeneity observed across the formulations further indicated adequate dispersion of the oils within the polymeric matrix. The viscosity and spreadability characteristics of F5 were also suitable for topical application. These findings are important because the performance of a topical formulation depends not only on the pharmacological activity of its active constituents but also on its ability to remain physically stable and spread uniformly over the skin [15].

The release and permeation studies provided additional evidence supporting F5 as the optimized formulation. F5 exhibited the highest cumulative release at 12 h (94.03%) among the tested formulations. Furthermore, F5 demonstrated the highest ex-vivo skin permeation (80.24%). The greater release and permeation observed with the combination formulation may facilitate greater availability of the incorporated bioactive constituents at or across the skin interface. Topical plant oils have previously been investigated for their ability to contribute to skin barrier modulation, antioxidant effects, and anti-inflammatory activity, while their physicochemical characteristics can also influence dermal penetration [16].

Importantly, the release kinetics of F5 showed the highest correlation with the Higuchi model ($R^2 = 0.9967$), suggesting predominantly diffusion-controlled release from the hydrated Carbopol matrix. This behavior is desirable for a topical formulation because controlled diffusion may provide sustained availability of the incorporated oil constituents rather than an exclusively rapid release. The combination of high cumulative release and favorable permeation therefore provides a formulation-level explanation for the subsequent biological performance of F5 [17].

The enhanced performance of F5 may also be related to the complementary chemical characteristics of the two plant oils. The thesis phytochemical investigation identified multiple classes of bioactive constituents in both oils, including phenolic compounds, flavonoids, terpenoids, steroids, alkaloids, tannins, and related constituents. The presence of chemically diverse constituents in the two oils provides a plausible basis for complementary biological effects. However, synergy cannot be definitively claimed from the present experimental design alone, because a formal pharmacological synergy analysis was not performed. Therefore, the superior performance of F5 is more appropriately described as an enhanced or complementary effect rather than proven molecular synergy [18].

The anti-inflammatory findings provide strong support for the biological relevance of the formulation. Formalin administration produced a substantial increase in paw volume in the disease-control animals, confirming successful induction of inflammation. Treatment with each seed-oil formulation reduced the inflammatory response, but the combination formulation F5 produced the greatest reduction among the experimental formulations. Paw volume decreased to 0.92 ± 0.008 with F5 compared with 1.48 ± 0.014 in the disease-control group, whereas the indomethacin-treated group showed 0.87 ± 0.005 . Thus, F5 approached the response obtained with the reference anti-inflammatory treatment, although the present findings should be interpreted as preclinical evidence rather than evidence of equivalence to indomethacin [19].

The anti-inflammatory response observed with the seed-oil combination is biologically plausible because plant-derived oils can contain lipid-soluble bioactive molecules capable of influencing inflammatory and oxidative processes. Previous literature has documented anti-inflammatory and antioxidant effects associated with several topical plant oils, supporting the broader rationale for investigating botanical oils as topical therapeutic agents. In addition, *S. hispanica* has been reported to possess biologically active constituents and its seed oil is particularly rich in polyunsaturated fatty acids. Experimental studies have also reported improvements in antioxidant status following chia seed or oil administration.

A particularly important finding of the present study was the effect of F5 on endogenous antioxidant defenses. Formalin-induced inflammation markedly reduced SOD activity from 8.52 ± 0.48 in normal animals to 3.12 ± 0.492 in the disease-control group. Treatment with F5 restored SOD activity to 7.63 ± 0.408 , approaching the value observed with indomethacin (7.98 ± 0.376). This restoration suggests that the anti-inflammatory effect of the formulation may be accompanied by attenuation of inflammation-associated oxidative imbalance.

A similar pattern was observed for GSH and catalase. GSH decreased markedly following formalin administration but increased to 7.15 ± 1.34 following F5 treatment, compared with 2.95 ± 0.81 in the disease-control group and 7.42 ± 1.25 in the indomethacin group. Catalase activity showed a comparable response, increasing from 28.66 ± 1.75 in the disease-control group to 55.18 ± 2.16 following F5 treatment, compared with 56.85 ± 2.04 following indomethacin. These observations are consistent with the concept that inflammatory tissue injury is closely associated with disruption of antioxidant defenses. SOD, catalase, and GSH constitute major components of cellular antioxidant protection, and restoration of these systems may help limit oxidative damage [20].

The response of F5 was also evident in total protein levels. Formalin-induced inflammation reduced total protein from 72.40 ± 2.186 in normal animals to 45.23 ± 1.39 in the disease-control group, whereas F5 restored the value to 69.23 ± 2.10 . Although total protein is a relatively nonspecific biochemical parameter, its recovery alongside SOD, GSH, and catalase suggests an overall improvement in the biochemical disturbances associated with the inflammatory state.

The absence of observable skin irritation during the seven-day assessment further supports the preliminary dermal tolerability of the formulations. All F1–F5 formulations were graded as showing no visible reaction during the observation period. This finding is particularly relevant for a topical preparation because efficacy must be considered alongside local tolerability. Nevertheless, the short duration and limited experimental design mean that this finding should be considered preliminary dermal safety evidence, rather than definitive evidence of long-term safety.

Taken together, the findings suggest that the superior performance of F5 may result from the combination of favorable topical delivery characteristics and complementary biological activity of the two seed oils. The formulation demonstrated the highest release and skin permeation, followed by the strongest reduction in inflammatory paw edema and the greatest restoration of antioxidant parameters among the experimental groups. This convergence of formulation and pharmacological findings strengthens the biological plausibility of the proposed topical intervention.

The study is also relevant in the context of current research exploring plant-derived oils as topical therapeutic agents. Recent systematic evidence indicates that delivery systems can improve the topical performance of plant-derived oils by enhancing skin permeation, controlled release, and biological activity, although considerable variability remains among preclinical studies and further well-designed investigations are required before clinical translation. The present work therefore provides a useful proof-of-concept preclinical platform for the combined use of *B. papillosa* and *S. hispanica* seed oils in a topical formulation.

However, several limitations should be acknowledged. First, the present study used an animal model and therefore the findings cannot be directly extrapolated to humans. Second, although F5 performed better than the individual oil formulations, a formal synergy analysis was not conducted; consequently, synergistic interaction between the two oils cannot be conclusively established. Third, the mechanistic basis of the anti-inflammatory response was inferred primarily from edema and antioxidant biochemical parameters. Molecular investigations involving inflammatory mediators and signaling pathways such as TNF- α , IL-1 β , IL-6, COX-2, PGE₂, and NF- κ B, together with histopathological evaluation, would provide stronger mechanistic evidence in future studies. Finally, longer-term dermal toxicity, pharmacokinetic evaluation, and eventually well-designed clinical studies would be necessary to establish translational relevance.

5. CONCLUSION

The study demonstrated that the 1:1 *Buxus papillosa*–*Salvia hispanica* seed-oil gel (F5) showed favorable topical characteristics, enhanced release and permeation, and promising anti-inflammatory and antioxidant activity. F5 produced the best overall response among the tested formulations, supporting its potential as a plant-derived topical formulation for inflammation-associated oxidative stress. Further mechanistic and clinical studies are warranted.

ETHICS STATEMENT

All animal experiments were conducted in accordance with the institutional guidelines for the care and use of laboratory animals and were performed after obtaining approval from the appropriate Institutional Animal Ethics Committee (IAEC). The experimental procedures involving animals were conducted in accordance with applicable ethical guidelines for laboratory animal experimentation.

CONSENT FOR PUBLICATION

Not applicable. No identifiable human participant data or individual patient information were used in this study.

DATA AVAILABILITY STATEMENT

The datasets generated and/or analyzed during the present study are available from the corresponding author upon reasonable request, subject to institutional and ethical restrictions.

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AUTHOR CONTRIBUTIONS

Alka Sharma: [roles]. Ravinesh Mishra: [roles]. Bhartendu Sharma: [roles].

CONFLICT OF INTEREST

The authors declare that they have no competing interests or conflicts of interest that could have influenced the design, conduct, analysis, or interpretation of the study.

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