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Original Article

Dentistry Section

Formulation and Characterisation of a Hydrogel Infused with Scutellaria baicalensis Root Extract and Zinc Oxide Nanoparticles: An In-vitro Evaluation of Antioxidant, Anti-inflammatory, and Cytotoxic Effects

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ABSTRACT

Introduction: *Scutellaria baicalensis*, rich in bioactive flavonoids such as baicalin and baicalein, exhibits potent antioxidant, anti-inflammatory, and antimicrobial activities. However, its clinical translation is limited by poor solubility and bioavailability. Hydrogels offer a promising delivery platform, and incorporation of Zinc Oxide Nanoparticles (ZnO NPs) can further enhance therapeutic efficacy through their antimicrobial, antioxidant, and wound healing properties.

Aim: To formulate and characterise a *S. baicalensis* root extract-mediated ZnO NP-infused hydrogel and evaluate its in-vitro cytotoxicity, antioxidant activity, and anti-inflammatory potential.

Materials and Methods: This in-vitro experimental study was conducted at Department of Periodontics, Saveetha Dental College and Hospitals, Chennai, Tamil Nadu, India, from March 2025 to May 2025. ZnO NPs were synthesised via green synthesis using *S. baicalensis* extract. The hydrogel was prepared using sodium alginate, incorporating both extract and ZnO NPs. Cytotoxicity was assessed by brine shrimp lethality assay. Antioxidant activity was evaluated using 2,2-Diphenyl-1-Picrylhydrazyl (DPPH) radical scavenging and Ferric Reducing Antioxidant Power (FRAP) assays. Anti-inflammatory activity was determined via Bovine Serum Albumin (BSA) and membrane stabilisation assays. All assays were performed in triplicate, and statistical analysis was conducted using One-way Analysis of Varinace (ANOVA) and Independent t-tests. A p-value <0.05 was considered statistically significant.

Results: The hydrogel exhibited high biocompatibility, with no significant cytotoxicity at therapeutic concentrations. Antioxidant assays showed significant dose-dependent activity, reaching 73.72±1.86% DPPH inhibition and 0.74±0.04 FRAP absorbance at 50 µg/mL. Anti-inflammatory assays demonstrated up to 76.39±0.99% inhibition of protein denaturation and 73.14±2.39% membrane stabilisation. No significant differences were observed compared between the hydrogel formulation and standard controls (p>0.05) under the tested experimental conditions.

Conclusion: *S. baicalensis* - ZnO NP hydrogel demonstrated potent antioxidant and anti-inflammatory effects with excellent biocompatibility, supporting its potential for localised management of oxidative stress and inflammation.

Keywords: Free radical scavengers, Oxidative stress, Phytotherapy, Plant extracts

INTRODUCTION

The search for naturally derived therapeutic agents has accelerated in recent years, particularly in the field of inflammation and oxidative stress management [1]. Among various medicinal plants, *S. baicalensis*, commonly known as Baikal skullcap has emerged as a potent source of bioactive flavonoids, including baicalin, baicalein, and wogonin. These phytoconstituents are well-documented for their anti-inflammatory, antioxidant, and antimicrobial properties [2], making the plant extract an attractive candidate for incorporation into modern drug delivery systems. However, challenges such as limited solubility, poor bioavailability, and short retention at the target site restrict its clinical utility in conventional forms.

Hydrogels are three-dimensional, hydrophilic polymeric systems capable of absorbing significant amounts of water while maintaining structural integrity. Their biocompatibility, ease of application, and ability to sustain drug release make them ideal carriers for localised drug delivery, especially in mucosal or wound sites [3]. Incorporating plant-based therapeutics into hydrogels not only enhances their stability and delivery but also opens avenues for multifunctional bioactivity. The formulation of such hydrogels using naturally sourced polymers or additives aligns with the current trend toward biocompatible and eco-friendly biomedical materials [4].

Nanoparticles are ultrafine materials, possessing a high surface area-to-volume ratio and distinctive physicochemical and biological properties. These unique attributes have facilitated their extensive use in biomedical applications, including drug delivery, antimicrobial therapies, and tissue engineering [5-7]. In recent years, ZnO NPs have gained attention due to their broad-spectrum antimicrobial activity, low toxicity, and intrinsic anti-inflammatory and antioxidant effects [8-10]. When embedded in hydrogel systems, ZnO NPs can synergistically enhance the therapeutic action of herbal extracts by providing localised antimicrobial defense, and modulating oxidative stress [11]. Their inclusion in such formulations can also contribute to enhanced mechanical strength and structural stability of the hydrogel matrix [12-14].

Although several studies have explored plant-mediated synthesis of ZnO NPs, limited evidence exists regarding the use of *S. baicalensis* for nanoparticle fabrication and its integration into a hydrogel-based drug delivery platform. The novelty of the present study lies in the development of a *S. baicalensis* root extract-mediated ZnO NP-infused hydrogel and the comprehensive evaluation of its cytotoxic, antioxidant, and anti-inflammatory properties, representing a multifunctional strategy for the localised management of oxidative stress and inflammatory conditions. Accordingly, this investigation

aimed to formulate and physicochemically characterise a hydrogel incorporated with *S. baicalensis* root extract-derived ZnO nanoparticles, with specific objectives to green synthesise and characterise the nanoparticles, develop a hydrogel suitable for localised application, and assess its cytotoxicity along with antioxidant and anti-inflammatory activities.

The null hypothesis postulated that the developed hydrogel would not exhibit statistically significant antioxidant or anti-inflammatory activity across tested concentrations.

The alternate hypothesis proposed that the formulation would demonstrate significant dose-dependent antioxidant and anti-inflammatory effects while maintaining acceptable cytocompatibility.

Additionally, it was hypothesised that there would be no significant difference between the biological activity of hydrogel and selected standard controls formulations under the tested experimental conditions.

MATERIALS AND METHODS

This in-vitro study was conducted at the Department of Periodontics, Saveetha Dental College and Hospitals, Chennai, Tamil Nadu, India, from March 2025 to May 2025, following approval from the Institutional Human Ethics Committee (IHEC/SDC/PERIO-2303/25/15). All experimental assays were performed in three replicates (n=3) to ensure statistical accuracy and reproducibility of the results.

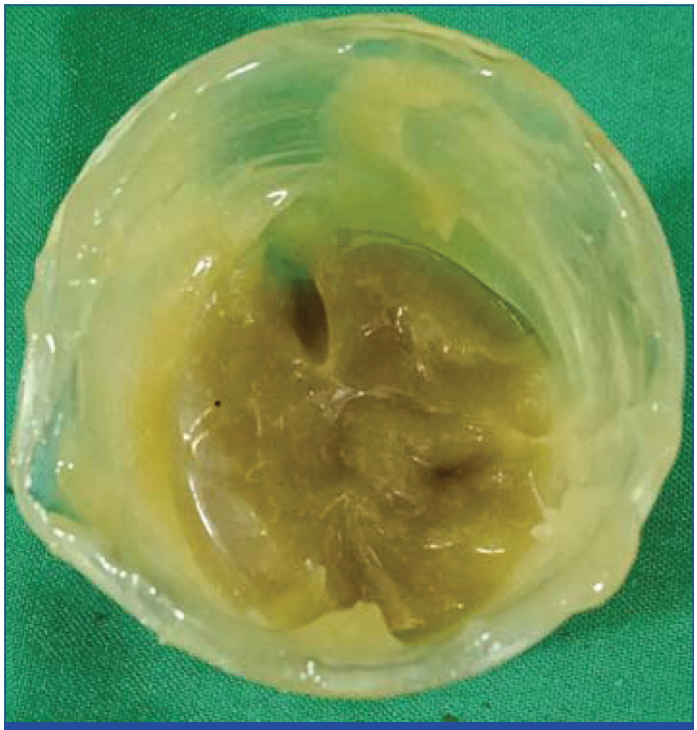
Study Procedure

Materials used were *S. baicalensis* root extract powder along with pharmaceutical-grade sodium alginate was procured from Himalayan Nutraceuticals Pvt., Ltd., India. Analytical-grade chemicals, including ZnO, Potassium Dichromate ($K_2Cr_2O_7$), BSA, 2,2- DPPH, Butylated Hydroxytoluene (BHT), acetate buffer,2,4,6-Tris (2-pyridyl)-s-triazine (TPTZ), and Ferrous sulphate heptahydrate ($FeSO_4 \cdot 7H_2O$) were obtained from Sigma-Aldrich®, USA. Diclofenac sodium, which was used as the reference standard, was supplied by TO Chemicals Ltd., Thailand.

Preparation of *S. baicalensis* root extract solution: To prepare the root extract solution, 1g of *S. baicalensis* root extract powder was dissolved in 20 mL of double-distilled water and stirred on a magnetic stirrer for 30 minutes at room temperature to ensure complete dissolution. The solution was then filtered through Whatman No. 1 filter paper to remove any undissolved particles. The clear filtrate was stored in a sterile amber bottle at 4°C until further use in the hydrogel formulation [15].

Green synthesis of ZnO NPs: ZnO NPs were synthesised via a green route using the prepared *S. baicalensis* extract. Briefly, 0.1g of ZnO powder was added to 100 mL of the extract solution and incubated on an orbital shaker at room temperature in the dark to prevent photoactivation. Visual changes in the solution's colour were monitored as an indicator of nanoparticle formation. After 36 hours, the mixture was centrifuged at 7000 rpm for 10 minutes. The resulting pellet containing the nanoparticles was collected, washed twice with distilled water, and stored at 4°C. Preliminary characterisation of the nanoparticles was performed using UV-Visible spectrophotometry (Model: UV-1800, Shimadzu Corporation, Kyoto, Japan) in the range of 200–600 nm [15].

Formulation of *S. baicalensis* and ZnO NPs-Infused Hydrogel: To prepare the hydrogel, 3 g of sodium alginate was gradually added to 20 mL of distilled water and stirred continuously with a magnetic stirrer until a homogeneous, viscous gel was formed. Subsequently, 1 mL each of the *S. baicalensis* extract and synthesised ZnO NPs suspension were incorporated into the alginate gel under continuous stirring. The mixture was homogenised for 10 minutes to ensure even distribution of the active components. The final volume was adjusted with distilled water if required, and the formulation was left to stand at room temperature for 24 hours to allow for ionic cross-linking and stable hydrogel formation [Table/Fig-1] [16]. Working solutions of the *S. baicalensis* and ZnO NPs-infused hydrogel were then prepared at concentrations of 10, 20, 30, 40, and 50 µg/mL using distilled water, and these concentrations were used for all in-vitro assays. Following synthesis, the hydrogel was subjected to in-vitro evaluation.



[Table/Fig-1]: *S. baicalensis* and ZnO NPs-infused hydrogel.

Characterisation

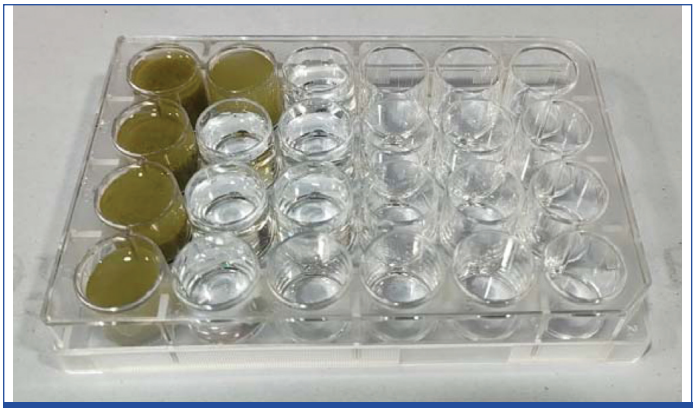
Cytotoxicity assessment - Brine Shrimp Lethality Assay: The biocompatibility of the synthesised *S. baicalensis* and ZnO NPs-infused hydrogel was evaluated using the brine shrimp (*Artemia salina*) lethality assay [17]. Brine shrimp eggs were procured from a local supplier (Aquatic Remedies, Chennai) and hatched in artificial seawater prepared with 40 g/L sea salt, supplemented with dried yeast (6 mg/L), and maintained under continuous aeration at 25±2°C. After 48 hours of incubation, actively swimming nauplii were collected using a Pasteur pipette, and 10 nauplii were transferred into each well of a 24-well plate containing 1 mL of artificial seawater.

S. baicalensis and ZnO NPs-infused hydrogel suspensions of different concentrations (10, 20, 30, 40, and 50 µg/mL) were added to the respective wells. Artificial seawater without hydrogel served as the negative control, while K2Cr2O7 was used as the positive control. The plates were incubated at room temperature for 24 hours under static conditions. Post-incubation, the number of viable and non viable nauplii was counted under a stereomicroscope [Table/Fig-2]. The percentage mortality was calculated using the formula:

Mortality (%)=(Dead nauplii/Total nauplii)×100

Antioxidant Activity

DPPH Radical Scavenging Assay: The antioxidant potential of the *S. baicalensis* and ZnO NPs-infused hydrogel was quantified using the DPPH assay. Various volumes (10-50 µg/mL) of the nanoparticle suspension were mixed with 1 mL of 0.1 mM DPPH solution (in methanol) and 450 µL of 50 mM Tris-HCl buffer (pH 7.4). The mixture was incubated in the dark for 30 minutes at room temperature. The absorbance was recorded at 517 nm using a UV-Vis spectrophotometer. BHT served as the standard reference antioxidant (control). The percentage of radical scavenging was calculated by [11]:



[Table/Fig-2]: A 24-well plate containing *Artemia salina* nauplii exposed to varying concentrations of *S. baicalensis* and ZnO NPs-infused hydrogel.

Inhibition (%) = (Absorbance of control - Absorbance of sample) / Absorbance of control x 100

Ferric Reducing Antioxidant Power (FRAP) Assay: The ferric-reducing ability of the formulation at various concentrations (10-50 µg/mL) was determined using the FRAP assay [18]. The working FRAP reagent was prepared by mixing 300 mM acetate buffer (pH 3.6), 10 mM TPTZ solution (in 40 mM HCl), and 20 mM $FeCl_3 \cdot 6H_2O$ (ferric chloride hexahydrate) in a 10:1:1 ratio. To the reaction mixture containing 3.6 mL of FRAP reagent and 0.4 mL distilled water, 80 µL of the nanoparticle sample was added. The mixture was incubated at 37°C for 10 minutes, and absorbance was measured at 593 nm. $FeSO_4 \cdot 7H_2O$ (ferrous sulphate heptahydrate) was used as the standard control to prepare a calibration curve (0.1-1.5 mM). The antioxidant capacity of the sample was expressed in terms of Fe2+ equivalents by comparing its absorbance to the standard curve.

Anti-inflammatory Activity

Membrane stabilisation assay: The anti-inflammatory potential of the synthesised *S. baicalensis* and ZnO NPs-infused hydrogel was evaluated by its ability to stabilise human Red Blood Cell (RBC) membranes under hypotonic stress [19]. Blood samples were collected from healthy adult volunteers after obtaining written informed consent. RBCs were isolated by centrifugation and washed thrice with phosphate-buffered saline, then suspended as a 10% v/v solution. In test tubes, 1 mL of RBC suspension was combined with varying concentrations (10–50 µg/mL) of hydrogel and incubated at 37°C for 30 minutes. After incubation, samples were centrifuged at 1,000 rpm for 10 minutes, and the absorbance of the supernatant was recorded at 540 nm. Diclofenac sodium served as the control. Percentage inhibition of haemolysis was calculated as [19]:

Inhibition (%) = (Absorbance of control - Absorbance of sample) / Absorbance of control x 100

Protein denaturation assay – Bovine Serum Albumin (BSA) method: To evaluate the ability of the nanoparticles to inhibit protein denaturation, the BSA assay was employed [19]. Different concentrations (10-50 µg/mL) of *S. baicalensis* and ZnO NPs-infused hydrogel were added to 2 mL of 1% BSA solution (pH adjusted to 6.8 using 1N HCl). The mixtures were incubated in a water bath at 37°C for 20 minutes, followed by cooling to room temperature. Absorbance was measured at 660 nm using a UV-Vis spectrophotometer. Diclofenac sodium served as the reference standard. The percentage inhibition of protein denaturation was calculated using the formula [19]:

Inhibition (%) = (Absorbance of control - Absorbance of sample) / Absorbance of control x 100

STATISTICAL ANALYSIS

All experimental assays were performed in triplicate, and data were expressed as mean±Standard Deviation (SD). One-way ANOVA was used to assess differences among the tested concentrations for each assay. In addition, an Independent t-test was carried out at each concentration to compare the hydrogel formulation with the respective standard control. A p-value of < 0.05 was considered statistically significant. Statistical analyses were performed using Statistical Package for Social Sciences (SPSS) software version 23.0 (IBM Corp., Armonk, NY, USA).

RESULTS

Brine shrimp lethality assay: The cytotoxic potential of the *S. baicalensis* and ZnO NPs–infused hydrogel was evaluated using the brine shrimp lethality assay. The hydrogel exhibited a concentration-dependent increase in brine shrimp mortality, starting at 5.3±1.19% at 10 µg/mL and rising to 10.0±1.02% (20 µg/mL), 19.4±1.11% (30 µg/mL), 29.6±1.50% (40 µg/mL), and 40.0±1.54% (50 µg/mL). One-way ANOVA revealed statistically significant differences among concentrations ($F=363.22$, $p<0.001$), confirming a dose-dependent effect. Overall, the hydrogel showed low cytotoxicity across the tested range, indicating good biocompatibility [Table/Fig-3]. Independent t-tests revealed no significant difference from the standard (potassium dichromate) at any concentration ($p>0.05$), confirming comparable activity [Table/Fig-4].

Assay	10 µg/mL (Mean±SD)	20 µg/mL (Mean±SD)	30 µg/mL (Mean±SD)	40 µg/mL (Mean±SD)	50 µg/mL (Mean±SD)	F-statistic	p-value[a]
Cytotoxicity (% mortality)	5.3±1.19	10.0±1.02	19.4±1.11	29.6±1.50	40.0±1.54	363.22	<0.001*
DPPH (% inhibition)	26.14±1.66	37.66±2.85	55.39±1.70	65.92±3.48	73.72±1.86	95.74	<0.001*
FRAP (mM Fe2+ equivalents)	0.20±0.01	0.33±0.02	0.49±0.02	0.61±0.01	0.74±0.04	107.96	<0.001*
BSA (% inhibition)	29.24±1.97	48.25±1.45	64.05±1.61	70.44±0.43	76.39±0.99	809.75	<0.001*
Membrane stabilisation (% haemolysis inhibition)	26.88±0.43	43.59±1.90	58.74±2.18	68.55±0.69	73.14±2.39	322.41	<0.001*

[Table/Fig-3]: Comparison of Mean±SD values for cytotoxicity, antioxidant, and anti-inflammatory activities of *S. baicalensis* and ZnO NPs-infused hydrogel across increasing concentrations.
[a]One-way ANOVA; *p-value <0.05 (Statistically Significant); DPPH: 2,2-diphenyl-1-picrylhydrazyl; FRAP: Ferric reducing antioxidant power; BSA: Bovine serum albumin

Assay	10 µg/mL (Mean±SD)	20 µg/mL (Mean±SD)	30 µg/mL (Mean±SD)	40 µg/mL (Mean±SD)	50 µg/mL (Mean±SD)
Cytotoxicity – Test	5.3±1.19	10.0±1.02	19.4±1.11	29.6±1.50	40.0±1.54
Cytotoxicity – Standard	6.0±1.41	11.2±1.32	20.1±1.29	30.3±1.91	41.0±1.69
Cytotoxicity – t-value	-0.66	-1.25	-0.71	-0.50	-0.76
Cytotoxicity – p-value[b]	0.66	0.21	0.36	0.27	0.52
DPPH – Test	26.14±1.66	37.66±2.85	55.39±1.70	65.92±3.48	73.72±1.86
DPPH – Standard	25.87±1.74	38.20±2.74	55.78±1.85	66.44±3.56	73.40±1.98
DPPH – t-value	0.36	-0.60	-0.28	-0.29	0.29
DPPH – p-value[b]	0.36	0.09	0.99	0.38	0.15

FRAP – Test	0.20±0.01	0.33±0.02	0.49±0.02	0.61±0.01	0.74±0.04
FRAP – Standard	0.21±0.01	0.34±0.02	0.51±0.02	0.63±0.05	0.77±0.03
FRAP – t-value	-1.00	-0.71	-0.71	-0.50	-0.66
FRAP – p-value[b]	0.17	0.08	0.81	0.06	0.18
BSA – Test	29.24±1.97	48.25±1.45	64.05±1.61	70.44±0.43	76.39±0.99
BSA – Standard	29.58±2.06	48.60±1.32	64.34±1.52	70.45±0.46	76.71±0.94
BSA – t-value	-0.36	-0.36	-0.42	-0.14	-0.27
BSA – p-value[b]	0.35	0.92	0.45	0.30	0.62
Membrane Stabilisation – Test	26.88±0.43	43.59±1.90	58.74±2.18	68.55±0.69	73.14±2.39
Membrane Stabilisation – Standard	27.02±0.46	43.84±1.85	59.02±2.04	68.78±0.72	73.40±2.28
Membrane Stabilisation – t-value	-0.30	-0.34	-0.14	-0.23	-0.13
Membrane Stabilisation – p-value[b]	0.47	0.55	0.07	0.07	0.48

[Table/Fig-4]: Independent t-test comparison between hydrogel test concentrations and corresponding standards.
[b]Independent t-test; p-value <0.05 (Statistically Significant); DPPH: 2,2-diphenyl-1-picrylhydrazyl; FRAP: Ferric reducing antioxidant power; BSA: Bovine serum albumin

DPPH radical scavenging assay: A dose-dependent increase in free radical scavenging was observed, from 26.14±1.66% (10 µg/mL) to 37.66±2.85% (20 µg/mL), 55.39±1.70% (30 µg/mL), and 65.92±3.48% (40 µg/mL), reaching 73.72±1.86% at 50 µg/mL. The trend was statistically significant (F=95.74, p<0.001) [Table/Fig-3]. No significant difference from the standard (BHT) was found at any concentration (p>0.05) [Table/Fig-4].

FRAP assay: Ferric reducing capacity increased consistently from 0.20±0.01 absorbance units at 10µg/mL to 0.74±0.04 at 50 µg/mL, with intermediate values of 0.33±0.02 (20 µg/mL), 0.49±0.02 (30 µg/mL), and 0.61±0.01 (40 µg/mL). The increase was highly significant (F=107.96, p<0.001) [Table/Fig-3]. Independent t-tests showed no significant difference from the standard (ferrous sulphate heptahydrate) at any concentration (p>0.05) [Table/Fig-4].

Protein denaturation assay: The hydrogel inhibited protein denaturation in a concentration-dependent manner, increasing from 29.24±1.97% at 10µg/mL to 48.25±1.45% (20µg/mL), 64.05±1.61% (30µg/mL), 70.44±0.43% (40µg/mL), and 76.39±0.99% (50 µg/mL). This effect was statistically significant (F=809.75, p<0.001) [Table/Fig-3]. No significant difference from the standard (diclofenac sodium) was detected at any concentration (p>0.05) [Table/Fig-4].

Membrane stabilisation assay: Haemolysis inhibition rose from 26.88±0.43% (10 µg/mL) to 43.59±1.90% (20 µg/mL), 58.74±2.18% (30 µg/mL), 68.55±0.69% (40 µg/mL), and 73.14±2.39% (50 µg/mL). The concentration-dependent trend was significant (F=322.41, p<0.001) (Table/Fig-3). No significant differences from the standard (diclofenac sodium) were found (p>0.05) [Table/Fig-4].

DISCUSSION

Inflammatory pathologies are often perpetuated by an intricate interplay of oxidative stress, excessive free radical accumulation, and heightened pro-inflammatory mediator release. Addressing these conditions requires therapeutic systems that combine strong antioxidant potential with anti-inflammatory efficacy, ideally within a safe, localised, and biocompatible delivery platform. In this context, the developed hydrogel integrating *S. baicalensis* root extract with green-synthesised ZnO NPs demonstrated low cytotoxicity within the therapeutic range, potent free radical scavenging, robust electron-donating capacity, and significant stabilisation of proteins and cell membranes, confirming its multifunctional bioactivity. The formulation demonstrated statistically significant dose-dependent antioxidant and anti-inflammatory activity (p<0.001), leading to rejection of the null hypothesis regarding absence of activity. However, no statistically significant difference was observed between the hydrogel and standard references (p>0.05), indicating comparable efficacy.

These results strongly resonate with earlier findings on *S. baicalensis* phytochemistry and therapeutic efficacy. Paczkowska-Walendowska M and Cielecka-Piontek J investigated *S. baicalensis* extracts incorporated into chitosan-based systems, demonstrating that extraction parameters and chitosan's degree of deacetylation significantly influenced the release profile, viscosity and antioxidant/anti-inflammatory potential [20]. They reported that higher deacetylation improved free radical scavenging and hyaluronidase inhibition, leading to enhanced resistance to biodegradation and prolonged local retention, principles echoed in the formulated hydrogel's performance, where the polymeric base likely aided in sustained bioactivity.

Similarly, Lee GY and Seo SH examined fractionated *S. baicalensis* extracts and revealed that specific fractions surpassed crude extracts in scavenging both DPPH and ABTS radicals [21]. In macrophage and keratinocyte models, these fractions reduced nitric oxide production, IL-6 release, and key chemokines such as IL-8 and RANTES, while downregulating ICAM-1 expression. These findings of mechanistic suppression of inflammatory signaling may partially correspond with the membrane stabilisation and protein-protective effects.

Dzięcioł M et al., focused on optimising extraction techniques for *S. baicalensis*, finding that reflux and Soxhlet methods yielded phenolic-rich extracts with high wogonin and oroxylin A content—compounds known for broad-spectrum bioactivity, including antioxidant and anti-inflammatory actions [22]. The strong FRAP and DPPH outcomes in the current work may similarly be attributed to these flavonoids, preserved during hydrogel formulation.

The therapeutic relevance of *S. baicalensis* in inflammatory modulation is further reinforced by Gao L et al., who demonstrated its efficacy in an in vivo inflammatory pain model. They found that *S. baicalensis* extract significantly reduced inflammatory cytokines [23]. This systemic anti-inflammatory capacity aligns with the present in-vitro demonstration of inflammation suppression through protein denaturation and membrane stabilisation inhibition.

The integration of ZnO NPs in the current hydrogel adds a synergistic dimension, supported by extensive literature. Rehman H et al., highlighted that phytosynthesised ZnO NPs exhibit a broad spectrum of biological activities including antioxidant, antimicrobial, antidiabetic, and anti-inflammatory effects, attributable to their phytochemical surface functionalisation [24]. They confirmed inhibition of COX-1, COX-2, 15-LOX, and sPLA2, which may explain the strong protein stabilisation effects seen in the current assays.

Nandhini J et al., further optimised microwave-assisted green synthesis of ZnO NPs using *Ocimum americanum* and *Euphorbia hirta* extracts, achieving nanoparticles with exceptional antioxidant (over 90% DPPH inhibition) and anti-inflammatory activities comparable to diclofenac [25]. They also demonstrated significant antibacterial and biofilm inhibition capabilities, as well as fibroblast

proliferation in wound healing models showcasing how plant-mediated ZnO NPs can be tailored for tissue repair, much like our hydrogel's envisioned role in oral inflammatory conditions.

Kirubakaran D et al., used *Acmella caulirhiza* to synthesise ZnO NPs, reporting notable antibacterial efficacy, strong radical scavenging (over 60% in DPPH assays), and measurable anti-inflammatory potential, with minimal haemolytic activity at relevant doses [26]. Their work illustrates how phytochemical diversity across plant sources influences nanoparticle functionality. While many botanicals have been explored for ZnO NP synthesis, this is, to our knowledge, the first study to employ *S. baicalensis* as the nanoparticle source and to integrate these NPs within a hydrogel delivery system. This dual novelty not only leverages the herb's rich flavonoid content but also harnesses the controlled-release and protective properties of the hydrogel matrix.

The principal strength of the study lies in its innovative combination of *S. baicalensis*-derived ZnO NPs with a hydrogel carrier, yielding a multifunctional system that addresses oxidative stress and inflammation concurrently. The multi-assay evaluation ensured a thorough characterisation of its bioactivity. In conclusion, the *S. baicalensis* root extract and ZnO NP-infused hydrogel demonstrated strong, concentration-dependent antioxidant and anti-inflammatory properties, coupled with low cytotoxicity. The novelty of sourcing ZnO NPs from *S. baicalensis* and embedding them within a hydrogel framework positions this formulation as a promising candidate for topical applications in oxidative stress- and inflammation-driven conditions. Clinically, such a formulation may warrant future investigation as a potential adjunctive in managing local inflammatory lesions, promoting tissue healing, and mitigating oxidative damage in oral or dermatological settings. Future studies should incorporate detailed mechanistic investigations and animal model validations to confirm the therapeutic potential, safety, and translational applicability of this formulation.

Limitation(s)

Limitations include the absence of mechanistic pathway analyses, such as gene or protein expression profiling, and the lack of in-vivo disease model validation, both of which are essential for translating these findings into clinical contexts.

CONCLUSION(S)

The *S. baicalensis* root extract-zinc oxide nanoparticle-infused hydrogel exhibited excellent biocompatibility and significant dose-dependent antioxidant and anti-inflammatory activities, with outcomes comparable to standard references. The synergistic combination of plant-derived bioactives and ZnO NPs within a hydrogel matrix may represent a potential approach for localised therapeutic interventions aimed at controlling oxidative stress and inflammation. These findings highlight its potential for further development in future biomedical investigations.

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