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In Vivo Study: An Innovative Spasm Simulation Utilizing Lactic Acid and Treatment with Baclofen loaded Transferosomal Gel

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***Abstract*—Baclofen, a GABA_B receptor agonist, is commonly used to manage muscle spasticity. Although systemic administration is effective, its use is limited by adverse effects. Topical application offers targeted delivery to affected muscles, potentially reducing spasticity while minimizing systemic side effects. To develop an innovative *in vivo* model for simulating muscle spasms using lactic acid, formulate a baclofen-loaded transferosomal gel suitable for topical application, and investigate its therapeutic efficacy in alleviating muscle spasms in the in vivo model. Twenty male rabbits (Lepus domestica) were divided into four groups. Muscle spasms were induced by intramuscular injection of 30 m Molar lactic acid. Baclofen nanoliposomes were incorporated into a Carbopol 934 gel for topical administration. Zeta potential of the formulation was determined, and the following clinical parameters were measured: lactate dehydrogenase (LDH), creatine kinase (CK), calcium ion (Ca^{2+}), potassium ion (K^{+}), phosphorus ion (P), alanine aminotransferase (ALT), aspartate aminotransferase (AST), blood urea, and serum creatinine. Baclofen nanoliposomes coated with Carbopol exhibited a high negative zeta potential (−63.3 mV). Significant differences ($P < 0.05$) were observed in LDH, CK, AST, ALT, Ca^{2+}, K^{+}, P, blood urea, and serum creatinine between the spasm-induced group and the baclofen gel–treated group. This study provides the first evidence of the safety and efficacy of topical baclofen gel in reducing muscle spasms, supporting its potential in nano-liposomal drug delivery applications and paving the way for future innovations in targeted spasm therapy.**

Keywords*— Lactic Acid, Transferosomal Baclofen Gel, Muscle Spasm, *Carpobol-934

I. INTRODUCTION

The transdermal drug delivery system (TDDS) represents a specialized pharmaceutical approach for drug administration, offering considerable advantages over conventional delivery methods. Traditional drug formulations often require high doses and prolonged treatment durations to achieve therapeutic efficacy, which may lead to serious adverse effects and reduced patient adherence [1]. Although oral administration is widely regarded as the most convenient and preferred route due to its practicality and patient compliance, it is associated with several limitations, including enzymatic degradation within the gastrointestinal tract, gastrointestinal disturbances, restricted absorption, and low bioavailability. Injectable drugs, on the other hand, present challenges such as needle phobia—a common psychological concern among both children and adults—as well as the risk of infection during administration [2]. To address these limitations, alternative drug delivery approaches have been explored, with TDDS emerging as an optimal strategy for enhancing therapeutic outcomes while minimizing systemic side effects [3]. This system has several advantages over oral and parenteral methods, including direct effect on the skin, delivery of active ingredients directly to damaged tissues, bypassing the first-pass effect of the liver, good absorption, maintaining stable drug concentrations in the blood, low incidence of side effects, and the ability to discontinue treatment quickly [4]. Additionally, transdermal delivery provides a non-invasive and convenient alternative for patients, improving adherence to therapy. It also allows for controlled and sustained release of drugs, which can enhance therapeutic efficacy over time.

Baclofen, primarily used to manage spinal spasticity resulting from spinal cord trauma or demyelination, acts at the spinal level and is generally better tolerated than centrally acting agents. Although drug biotransformation is considered oxidative, its specific metabolic properties remain unclear. Baclofen exhibits a high volume of distribution, low renal clearance at therapeutic levels, and minimal protein binding, making it unsuitable for removal via hemodialysis. For supraspinal spasticity, such as in cerebral palsy or stroke, intrathecal administration is preferred to reduce systemic side effects [5]. Baclofen is also considered a good candidate for transdermal drug delivery systems (TDDS) due to its low molecular weight, moderate lipophilicity, and relatively short half-life, which allow it to permeate the skin effectively while maintaining stable therapeutic levels over time. TDDS can provide targeted delivery, minimize systemic side effects, and improve patient compliance, making it a promising alternative to oral or intrathecal administration.

In developing new therapeutics, overcoming pharmacokinetic challenges—such as poor solubility, rapid metabolism, and limited barrier penetration—is critical for achieving optimal efficacy and tolerability [2]. Over the past two decades, nanotechnology-based delivery systems have provided innovative solutions to these limitations by enabling precise control of drug release and bio-distribution at the nano scale [7]. Among these, lipid-based nanovesicular carriers—including noisome, proniosomes, ethosomes, transferosomes, pharmacosomes, ufasomes, phytosomes, catanionic vesicles, and extracellular vesicles—have demonstrated particular success in enhancing drug stability, bioavailability, and tissue targeting, thereby addressing key

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obstacles in drug pharmacokinetics and improving therapeutic outcomes [6–8]. The versatility of liposome carriers, with a focus on their ability to encapsulate a variety of therapeutic agents, plays a significant role in drug delivery. The choice of components in liposome design can also affect drug release and targeting capabilities. Ultimately, polymers are incorporated into liposome structures to enhance their stability and functionality, thus expanding the scope of liposome applications in drug delivery [9]. Classification of liposomes based on morphology, dimensions, surface charge, and function [10]. Liposomal drug delivery systems constitute a highly versatile and sophisticated platform for therapeutic administration, offering notable advantages , including enhanced physicochemical stability, prolonged circulation, controlled release, and improved biodistribution of encapsulated agents [11]. By encapsulating active molecules within lipid bilayers, liposomes can protect labile drugs from enzymatic degradation, reduce systemic toxicity, and enable targeted delivery to specific tissues or cells. The pharmacokinetic profile and therapeutic efficacy of liposomal formulations are profoundly influenced by critical quality attributes, including particle size, morphology, surface charge, and the presence of conjugated ligands or functional moieties. These factors dictate key biological interactions, such as opsonization, cellular uptake, tissue penetration, and clearance pathways. To achieve optimal performance, a range of formulation and engineering strategies have been employed to precisely control these properties, enhance drug loading efficiency, and maximize therapeutic outcomes. However, these advanced approaches frequently involve the utilization of expensive excipients, highly specialized equipment, or multi-step procedures, which may pose challenges for scalability and clinical translation [5]. To further enhance the stability and therapeutic efficiency of the liposomal formulation, Carbopol-934 was incorporated due to its high viscosity, mucoadhesive properties, and ability to form stable hydrogels, which help maintain liposome integrity and provide sustained drug release.

Carbopol- 934 has a high molecular weight synthetic polymer of polyacrylipc acid, widely utilized in pharmaceutical formulations due to its excellent gelling, thickening, and mucoadhesive properties. When neutralized, it forms clear, stable hydrogels with pseudo plastic flow behavior and high swelling capacity, making it ideal for controlled and sustained drug release. Its bio-adhesive nature allows it to prolong drug residence time on mucosal surfaces, enhancing absorption and therapeutic effect [12-13].

Furthermore, Carbopol-934 can be effectively combined with soy lecithin—an amphiphilic phospholipid widely used as a natural emulsifier—to develop advanced drug delivery systems such as liposomal gels, emulsions, and transdermal formulations. The synergistic interaction between Carbopol and lecithin enhances the stability and permeation properties of formulations, particularly in transdermal and mucosal applications [14]. This combination offers improved encapsulation of hydrophilic and lipophilic drugs, extended release profiles, and superior bioavailability, making it an attractive strategy for the development of next-generation topical and systemic therapies such as~~,~~ neuromuscular disorders, analgesics, etc. [15]. This study explores the efficacy and clinical impact of topically applied, lipid- and gel-encapsulated baclofen in an animal model of explored spasticity.

II. Materials and Methods

Baclofen from baoji guokang (china), soya lecithin, carbopol-934, and phosphate buffer saline (pbs) from himedia (india), cholesterol from avonchem (uk), various surfactants (span-80, tween-80, sodium deoxycholate) from lobachemie (india), chloroform, and ethanol from chemlab (uk), lactate dehydrogenase (ldh) assay kit from biolabo (france), creatine kinase (ck) assay kit from biosystem (spain), aspartate transferase (ast) and alanine transferase (alt) from human (germany). kidney function tests were determined by b. urea and s. creatinine from randox (uk), phosphorus - spinreact (spain), and ca and k from biosam (france).

A. Ethical consideration

The current study adhered to the ethical standards set by the College of Science, and the study was approved by the Scientific Research Ethics Committee in the Department of Chemistry/University of Misan, ID no. 1117 dated February 3, 2025.

Preparation of Transferosomal Gel Loading with Baclofen Liposome Vesicles (BLVG)

Transferosomal gel was prepared by weighing (0.5%) g of Carbopol- 934 and adding it to 99.5 g of distilled water, stirring continuously with a mechanical mixer at 2000rpm for 60 minutes, as well as taking care to avoid clustering [16]. Then, it was left for two hours to hydrate and expand [17]. Glycerol (2%) was added to hydrate and improve the gel consistency. Tween 20 (5%) was also added to improve the homogeneity of distribution and stability of the liposomes. The previously optimized liposome formulation [5] was gradually incorporated into the gel base containing glycerol and Tween 20 under continuous stirring until a homogeneous gel was obtained. The pH of the gel was then adjusted to a range of 5–7 using triethanolamine, which was suitable for maintaining the stability of the Carbopol-934 gel. Finally, the drug-loaded gel was stored in sealed containers, protected from light, and kept at low temperatures ($^{0}5$) [17].

B. Experimental Animals

Twenty male Dutch breed (Lepus Lepus- domostica) rabbits weighting between 1900 and 2200 grams and aged 6-7 months, were purchased from the local market/Basra. Rabbits were housed in the animal facility of the Department of Biology, College of Sciences, University of Misan, for two weeks under controlled conditions (20–25 °C; 12 h light/12 h dark) in plastic cages.

C. Experimental Design

Rabbits were divided into four groups, with five animals in each group, as follows:

Control Group (A): This group received no treatment or spasm induction. Blood samples were collected directly and used as a reference for normal physiological conditions.

Spasm-Induced Group (B): Muscle spasms were induced using lactic acid at a concentration of 30 Mm. Blood samples were collected 6–12 hours after spasm induction.

Untreated Spasm Group (C): Rabbits in this group also underwent muscle spasm induction as described above, but received no treatment. Blood samples were collected after 3–5 days.

Treated Group (D): The affected area was treated topically with baclofen gel twice daily (every 12 hours) for 3–5 days.

Blood samples were collected at the end of the treatment period.

III. MUSCLE SPASM INDUCING PROCEDURE

The rabbit was placed in a 3-meter-long enclosed corridor, and visual stimuli such as a hand were used to guide. The rabbit is running intermittently for 15 minutes. Ten minutes after the end of the exercise, 30 mM lactic acid was injected into the rabbit's thigh muscle at a dose of (0.5-1) ml, according to the weight of the rabbit using a 1ml syringe. The motor behavior of the rabbits was relied upon, a biomarker for muscle spasm along with performing some biochemical analysis as shown in Figures 1, 2, and 3.

A. Samples Collection

Blood samples were drawn from all groups at the specified times from the rabbit's auricular vein using a 3 ml syringe. After that, the blood samples were placed in gel Tubes, left them to coagulate at room temperature for 10 minutes, and then transferred to a centrifuge at 5000 rpm for 10 minutes to separate blood components and obtain serum. After that, the serum was separated by micropipettes, placed in Eppendorf tubes, and stored for 24 hr–at 4 °C until biochemical tests were performed on it.

B. Statistical Analysis

Statistical Package for the Social Sciences (SPSS) program was used to analysis the data statistically, and data were expressed as mean ± standard deviation. One-way analysis of variance (ANOVA) was used to compare the mean of the four groups to find statistically significant differences [18].

IV. RESULTS AND DISCUSSION

The skin acts as a primary barrier to topical drugs, significantly limiting the absorption of pharmaceutical compounds [19]. Upon topical application, vesicle systems experience water evaporation, which increases the concentration of non-volatile actives and creates a hydration gradient that facilitates their diffusion across the stratum corneum [20]. Hydration, combined with the high elasticity and hydrophobicity of vesicles, drives penetration toward the water-rich inner layers; in contrast, occlusion disrupts this transport. The stratum corneum contains two hydrophilic pathways: inter-cluster (≈ 20%, lower resistance) and inter-corneal (≈80%, higher resistance) [21]. Conventional vesicles mainly accumulate in the upper stratum corneum with minimal deeper penetration, as shown in Figure4; thus, modifications with surfactants have been introduced to increase bilayer flexibility and deformability, enabling passage through narrow pores [19].

Nanocomposites help stabilize components. Recently, baclofen, a muscle relaxant of the GABA derivative family, began to be innovatively used for the symptomatic treatment of spasticity due to brain or spinal injury. Novel vesicular formulations of baclofen containing nanoliposomes were developed and characterized to improve the drug's effectiveness. Novel vesicular formulations of baclofen containing nanoliposomes were developed and characterized to improve the drug effectiveness, also physical–chemical and technological properties were assessed. All these were demonstrated through spectroscopic and morphological characterization tests and biological investigation of the resulting Baclofen liposome~~s~~ vesicles (BLVG).

Notably, BLVG was encapsulated with different proportions of Carbopol 934 gel in PBS solutions. The results indicated that a 0.5% concentration of Carbopol 934 gel was the most stable and non-toxic, increasing flexibility and water permeability and reducing stiffness [5].

The zeta potential is used to measure the surface charge and stability of particles. It is known that the greater the electrostatic repulsive forces between particles, the higher the zeta potential value, which leads to the absence of clumping, and thus the more stable these particles are. In our previous research [5], the results showed a zeta potential value of -65.6 mV, indicating that liposomes carry a high negative surface charge, which leads to the absence of sedimentation or agglomeration. Therefore, the liposomes exhibited good physical stability. The results of this study are consistent with those of (Neamah et al., 2023, and Flayah et.al.,2025) which showed that a high negative charge leads to increased particle stability and prevents agglomeration [17-18]. After encapsulating the liposomes with Carbopol- 934gel, the zeta potential value was observed to reach -63.3 mV , as shown in Figure 5 which is close to the zeta potential value of BLV_2, indicating that the encapsulation process did not negatively affect the surface charge and stability of the liposomes. This demonstrates the compatibility of Carbopol -934gel with BLV_2 [22].

TABLE 1: COMPARISON OF CALCIUM , POUTASIUM, AND PHOSPHOR PARAMETERS AMONG STUDY GROUPS AND CONTROL

	Mean ± SD			
	Group (A) **Control**	Group (B) **Spasm-Induced**	Group (C) **Untreated Spasm**	Group (D) **Treated with Baclofen Gel**
Ca mg/dl	10.24 ± 0.618 ^{a}	7.54 ± 0.594 ^{b}	8.14 ± 0.230 ^{c}	9.54 ± 0.673 ^{a}
K mEq/L	4.36 ± 0.403 ^{a}	6.58 ± 0.389^{b}	5.62 ± 0.258 ^{c}	4.51 ± 0.673^{a}
Phosphor mg/dl	4.5 ± 0.355 ^{a}	6.5 ± 0.228 ^{b}	5.24 ± 0.288^{c}	4.68 ± 0.402 ^{a}

Data are given as mean± SD; Different small letters indicate statistically significant differences (P<0.05) between groups; Similar small letters indicate no statistically significant differences between groups.

TABLE 2: COMPARISON OF BIO- MARKER ENZYMES AMONG STUDY GROUPS AND CONTROL

	Mean ± SD			
	Group (A) **Control**	Group (B) **Spasm-Induced**	Group (C) **Untreated Spasm**	Group (D) **Treated with Baclofen Gel**
CK mg/dl	267.4± 20.044 ^{a}	974.6± 21.442^{b}	515.8 ± 17.383^{c}	361.4 ± 21.524^{d}
LDH mg/dl	269.8 ± 28.839^{a}	807.6 ± 26.670^{b}	575.2 ± 30.408 ^{c}	376.4 ± 27.428 ^{d}
AST mg/dl	67.6 ± 7.127 ^{a}	122.6 ± 6.348^{b}	88 ± 7.615^{c}	74 ± 8.514 ^{a}
ALT mg/dl	51.2 ± 4.658 ^{a}	68.8 ± 5.167 ^{b}	61.4 ± 7.127 ^{c}	54.2 ± 3.898 ^{a}

Data are given as mean± SD; Different small letters indicate statistically significant differences (P<0.05) between groups; Similar small letters indicate no statistically significant differences between groups.

TABLE 3: COMPARISON OF KIDNEY FUNCTION TESTS AMONG STUDY GROUPS AND CONTROL

	Mean ± SD			
	Group (A) **Control**	Group (B) **Spasm-Induced**	Group (C) **Untreated Spasm**	Group (D) **Treated with Baclofen Gel**
B. urea mg/dl	27.6 ± 3.209 ^{a}	37 ± 2.549 ^{b}	31.8 ± 2.588 ^{c}	28 ± 2.588 a
S.creatinine mg/dl	0.78 ± 0.238 ^{a}	1.52 ± 0.496 ^{b}	1.26 ± 0.279 ^{c}	0.86 ± 0.260 ^{a}

Data are given as mean± SD; Different small letters indicate statistically significant differences (P<0.05) between groups; Similar small letters indicate no statistically significant differences between groups.

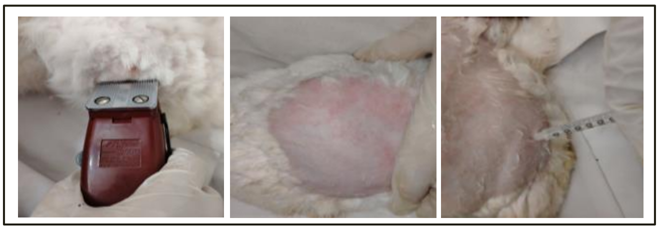


Fig. 1: Steps for lactic acid injection

Fig. 2: Steps to draw blood from the atrial vein

Fig. 3: Steps to treat muscle spasms with baclofen gel

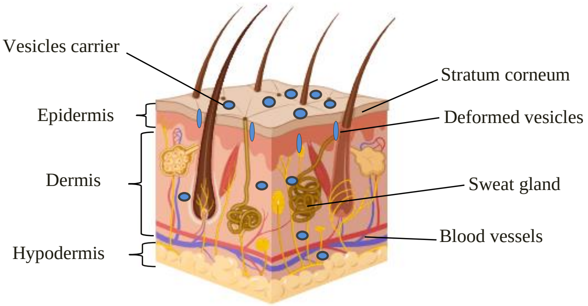
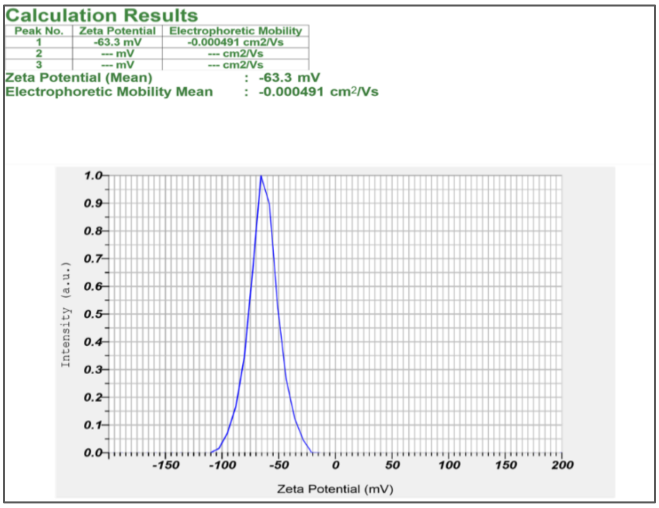


Fig. 4: Mechanism of penetration of modified liposomes across the skin layers



Calculation Results

Peak No.	Zeta Potential	Electrophoretic Mobility
1	-63.3 mV	-0.000491 cm2/Vs
2	--- mV	--- cm2/Vs
3	--- mV	--- cm2/Vs

Zeta Potential (Mean) : -63.3 mV
Electrophoretic Mobility Mean : -0.000491 cm^{2}/Vs

Figure 5: Zeta potential spectra of the obtained transferosomal gel loading with Baclofen liposome vesicles (BLVG)

Although nanocarriers with high negative zeta potential often face challenges in penetrating biological barriers due to electrostatic repulsion with the skin, this limitation does not apply to transfersomes (deformable liposomes). Studies have demonstrated that transfersomes efficiently traverse intercellular pathways in the skin, enabling them to overcome the skin barrier. The high negative zeta potential enhances their stability by reducing particle aggregation, while their remarkable deformability and interaction with skin lipids facilitate penetration into deeper skin layers. This deformability is attributed to the presence of surfactants such as Tween 80, Tween 20, Span 80, and sodium deoxycholate, which induce organized disruptions in the lipid bilayer, conferring flexibility and curvature that allow passage through pores smaller than their own diameter [23- 24].

The injection of lactic acid with strenuous exercise causes acid accumulation in the muscles causing an increase in the concentration of hydrogen ion in muscle cells as well as the loss of k+ from the muscle and its rise outside the cell and this leads to a decrease in pH, which makes the muscle environment more acidic and thus both factors, high extracellular k+ and low pH in the muscle, cause muscle spasms [23].

The results of the current study in Table 1, including a comparison of bio-ions parameters among study groups and control, illustrate the difference in Ca^{+2}, K^{+}, and Phosphorus inorganic (Pi) levels between the muscle spasms groups that were induced with treatment or without treatment . The obtained mean values were analyzed statistically showing a significant increase ($P < 0.05$) for K^{+} and P in both groups B and C compared with group A.

While there was a significant decrease ($P < 0.05$) in Ca^{2+} level among groups B, C, and A.

In addition, there were no significant difference in Ca^{+2}, K^{+}, and Pi concentration, which were recorded in our rapprochement data between groups A and D.

Prolonged or high-intensity muscular exertion results in a progressive decline in force generation, commonly termed muscle fatigue. This phenomenon is attributed to metabolic perturbations that compromise either the function of the contractile proteins or the processes underlying excitationcontraction (E-C) coupling [25]. A hallmark of fatigue-induced metabolic changes is the pronounced elevation of inorganic phosphate concentration within the myoplasm ($[Pi]_m y_o$), which exerts effects on both the contractile machinery and the regulatory pathways controlling muscle activation [26].

Emerging evidence indicates that a critical contributor to fatigue is the impaired release of calcium ions (Ca^{2+}) from the sarcoplasmic reticulum (SR) [24]. This dysfunction appears to be closely linked to increased $[Pi]_m y_o$ levels. Experimental observations in both chemically skinned and intact muscle fibers reveal that elevated $[Pi]_m y_o$ significantly attenuates SR Ca^{2+} release. In parallel, a reduction in SR Ca^{2+} content is consistently observed in fatigued fibers, aligning with the diminished release capacity [28].

Strikingly, muscle fibers deficient in creatine kinase exhibit blunted increases in $[Pi]_m y_o$ during fatigue and display a delayed onset of impaired SR Ca^{2+} release, suggesting a causative role for inorganic phosphate. The prevailing hypothesis posits that Pi permeates the SR membrane and forms calcium phosphate (Ca Pi) complexes or precipitates within the SR lumen, effectively reducing the availability of releasable Ca^{2+} [29-30].

This mechanistic framework underscores the pivotal role of inorganic phosphate in modulating SR function under conditions of metabolic stress. The implications of Pi-induced Ca^{2+} release inhibition are discussed in the context of various fatigue paradigms in human skeletal muscle, emphasizing its significance in the regulation of muscle performance during sustained activity [31-32].

V. CONCLUSIONS

This study provides the first evidence supporting the safe and effective use of topical baclofen gel, laying the groundwork for future innovations in nano-based drug delivery systems, including nano-fat technology.

The overall findings of this study indicate that topical baclofen gel is safe and well-tolerated, with no statistically significant alterations in renal function markers (serum creatinine and blood urea) compared with the control group. Furthermore, the assessment of biochemical enzymes (CK, LDH, AST, and ALT) demonstrated the absence of muscle or hepatic toxicity, confirming the protective profile of the formulation. In addition, the evaluation of electrolyte balance (Ca^{2+}, K^+, and phosphorus) revealed no detrimental disturbances among the treated groups, further supporting the systemic safety of topical baclofen gel. Collectively, these results suggest that topical baclofen gel may serve as a promising and safe therapeutic option for managing spasm without inducing systemic adverse effects. This study lays the groundwork for future research on advanced nanotechnology-based delivery systems to enhance both safety and therapeutic efficacy.

Conflict of Interest

The authors declare no conflict of interest.

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