**RESEARCH ARTICLE**

# Exploring Locust Bean Gum and Thiolated Chitosan Hydrogels for Sustained Delivery of Telmisartan in Hypertension Treatment: An *In Vivo* Study

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**ABSTRACT**

This article outlines development of a mucoadhesive hydrogel composed of thiolated chitosan and Locust Bean Gum for targeted transport of telmisartan. Hydrogel was prepared by tri-polyphosphate ionotropic gelation cross-linking method. Optimized formulation was evaluated in a preclinical study using a hypertension-induced rat model, comparing it to free telmisartan. Research showed that oil-entrapped beads loaded with TEL had a relative bioavailability of 151.59%, 1.52 times greater than the pure TEL suspension. It appears that the new method of drug delivery using oil-entrapped beads loaded with transdermal ligands improves patient compliance by increasing drug solubility, oral bioavailability, and therapeutic effectiveness. Further, the formulation's anti-hypertensive effects were verified to be substantially prolonged in animal models by *in vivo* pharmacodynamic tests.

**Keywords:** Locust gum, telmisartan, IPN hydrogel, anti-hypertertensive study, thiolated chitosan

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**Conflict of interest:** None

## INTRODUCTION

Oral route is commonly employed as its numerous advantages, factors including user-friendliness, patient choice, affordability, and the convenience of producing on a huge scale[1]. Despite these Significance, formulation of effective oral drugs can be complicated by the physicochemical properties of certain compounds, including poor water solubility and limited permeability through biological membranes[2]. To overcome these challenges and enhance bioavailability, variousprocess have been developed to increase solubility of drugs after oral administration. These contains use of amorphous solid dispersions with synthetic polymers, co-crystallization techniques, electrospinning, and formulation of floating hydrogel[3,4]. Notably, low-density floating hydrogel are designed to stay afloat on gastric contents, allowing them to stay in stomach for prolonged periods. This extended retention enables controlled, sustained drug release, leading to a more consistent release profile and reduced fluctuations in drug concentrations[5].

The oral hypertension medicine telmisartan (TEL) falls within the biopharmaceutical categorisation system's (BCS) class II drug category owing to its low water solubility[6]. Its uneven bioavailability is a result of its poor dissolving behaviour and restricted oral bioavailability, which ranges from 42% to 58%. TEL is poorly soluble[7]. Additionally, TEL's solubility is pH-dependent, with limited solubility observed at higher pH values (pH 3–9)[8]. To boost bioavailability, TEL can be formulated as a gastroretentive hydrogel, which increases the drug's solubility in acidic conditions and prolongs gastric retention time[9]. Surface solid dispersions and co-crystal formation are two of the many methods that have been investigated for increasing TEL's solubility[10]. Furthermore, the development of TEL in hydrogel formulations not only improves solubility but also contributes to sustained drug release, enhancing its therapeutic efficacy.

Hydrogels are very biocompatible, just like real tissue[11]. Contact lenses, biosensor membranes, synthetic skin, and medication delivery systems are some of the many uses for these materials. Natural hydrogels are often more cost-effective and biocompatible than their synthetic or mixed-polymer counterparts, however hydrogels derived from any source can be useful[12]. Multiparticulate hydrogel provide several benefits in drug delivery, including improved bioavailability, reduced variability in drug absorption, and extended or controlled release[13,14]. They offer targeted delivery & reduced local gastrointestinal irritation. Additionally, they minimize the risk of dose dumping, allow for flexible formulation strategies, and can incorporate multiple drugs with varying release profiles. These advantages make multiparticulate hydrogel ideal for drugs with poor solubility or those requiring sustained or targeted release[15].

Chitosan, a promising natural polymer, can be formulated into hydrogels, but its high solubility in the stomach and inability to effectively load hydrophobic drugs limit its use. To manage the medication release profile and associate bioactive molecules to polymers, chemically modifying chitosan can provide desirable features and expand the

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scope of possible uses of chitosan[16]. Chitosan modifications and their possible use in medication delivery systems have been the primary foci of research. One promising mucoadhesive drug delivery technique is thioated chitosan[17]. However, thiolated chitosan alone may not provide sustained drug release due to its high porosity. Combining it with other polymers, like locust bean gum, can create interpenetrating networks (IPNs) that improve controlled release[18]. This approach has been explored in hemostatic applications, but its use for poorly soluble drugs, such as telmisartan, remains underexplored.

Objective of current work is to inspect outcome of IPN hydrogel using thiolated chitosan & locust bean gum for sustained release of telmisartan to expand its release properties at GIT, pH value and theoretically its pharmacokinetic parameters. Optimized formulation was evaluated for its effect in hypertension induced animal model rats.

## MATERIALS AND METHODS

Telmisartan was donated by Chennai's Madras Pharmaceuticals. Spectrum Chemicals supplied locust bean gum and chitosan. Merck supplied all other chemicals. Double-distilled water and analytical solvents were employed in this work.

*Experimental Methods*

*Preparation of Thiolated Chitosan Polymer*

The EDAC click chemistry was used to create chitosan-cysteine conjugates. This was accomplished by adding 5 g of chitosan in 1 % acetic acid, addition of 5 g of cysteine & added 50 mM EDAC. 1N NaOH was used to bring pH down to 5. For three hours, the mixture was constantly mixed. EDAC and free cysteine were subsequently extracted from the solution using methanol by dialysing it in a dialysis tube that was shielded from the light. Before being used, the filtrate solution was kept in a tightly sealed container after being dried for 12 hours at 40 °C.

*Formulation of LBG:TC Hydrogels*

A 1% acetic acid solution was used to dissolve a exactly weighed quantity of thiolated chitosan. At 40°C, 25 millilitres of distilled water were used to dissolve locust bean gum (LG). After thoroughly mixing the two prepared polymer solutions, they were agitated for two hours at 250 rpm. pH was adjusted with 2 M NaOH to 5.8. Next, using ultrapure water, an aqueous solution of tripolyphosphate (TPP) was made on a concentration of 0.5 mg/mL. Five to seven percent of this solution was then kept and chilled in a refrigerator at 0 to 2°C for four hours. For ten minutes, the polymer solution was continuously stirred in preheated water bath to 60°C. TPP solution mixed right away to mixture & stirred for ten minutes after this solution had been moved to an ice bath. Dried & preparation of TEL trailed by adding telmisartan (Table 1).

Table 1: Composition of TEL loaded LBG:TC Hydrogel optimized formulation

| Ingredients | Quantity (%) |
|---|---|
| Telmisartan | 1% |
| Locust Bean Gum (LBG) | 1.5% |
| Thiolated Chitosan (TC) | 1.47% |
| Tripolyphosphate (TPP) | 5.52% |

Table 2: Pharmacokinetics parameters of pure TEL and TEL loaded LBG:TC hydrogel

| Pharmacokinetics Parameters | Pure TEL | TEL loaded LBG:TC hydrogel |
|---|---|---|
| Cmax (µg/ml) | 1.31 | 1.24 |
| Tmax (hr) | 2 | 4 |
| AUC ($\mu g.ml^{-1}.hr$) | 10.04 | 15.22 |
| Kel ($hr^{-1}$) | 0.125 | 0.085 |
| Relative bioavailability (%) | - | 151.59 |
| MRT (hr) | 2.03 | 10.07* |

All standards are stated as mean ± SD (n=3)

*Significant TEL loaded hydrogel when associated with Pure TEL ($p < 0.05$)

*In-vivo Study*

*Experimental Animals*

An animal model was utilised in a pharmacodynamics study conducted at the Invitox R&D Institute in Pune, India, to assess the optimised TEL loaded hydrogel's *In-vivo*

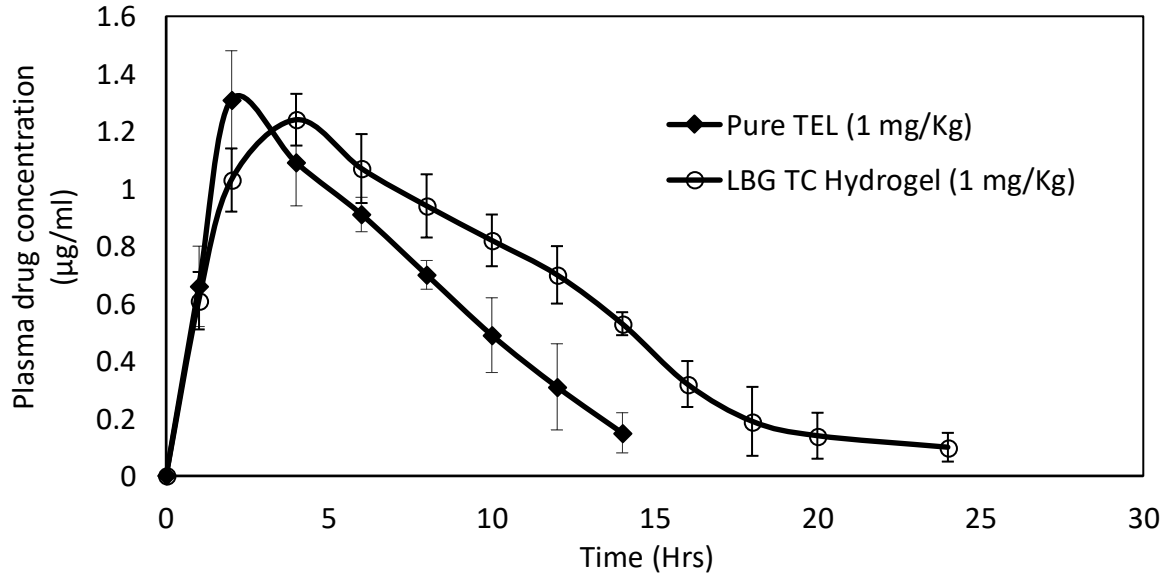


Figure 1: Plasma concentration-time curve afterward oral administration of pure TEL and TEL loaded LBG:TC hydrogel

performance. The Institutional Animal Ethical Committee (IAEC) gave their stamp of approval to all of the experimental techniques. Number of approval: IRDI/IAEC/M03/08/2023-24.

The animals housed at the Invitox R&D Institute in Pune, India, supplied the adult Wistar albino rats ranging in weight from 220 to 250 g. Rats were keptbelow typical laboratory settings, with a temperature of 25 ± 1°C, 55 ± 5% RH & a 12 h light/dark cycle. Each of 4 animals was housed in its own polypropylene cage and given an ordinary laboratory meal and unlimited access to water. Before treatment, the animals were maintained for 7 days to acclimatise.

*Pharmacokinetics Study (In Vivo)*

The six animals were separated into 3 equal groups. Optimal LBG:TC hydrogel was administered to Group III, while pure medication TEL was administered to Group I. One milligramme per kilogramme was given orally by gavage as a treatment. After that, rats were given anti-coagulantheparin, and blood samples were taken from their tail veins at 1, 2, 3, 4, 6, 10, 12, 20, and 24 hours after treatment. Centrifugation was used for 15 minutes at 3500 rpm to prepare plasma samples, which were then kept at 2-10°C. An HPLC method called reversed-phase analysis was used to measure the amount of drugs in plasma.

Rat tail vein blood samples were placed in centrifuge tubes that also contained sodium heparin, an anti-coagulant. At0.5, 1, 1.5,2,3,4, and 6 hours, blood samples were taken for both pure drug and cocrystals. Separating plasma for drug-content analysis required centrifugation at 3500 rpm for 15 minutes. With great care, the plasma was extracted and preserved at 2-10°C. With use of HPLC[19], the plasma samples were examined. Add 1 M HCl to approximately 0.5 ml of sample plasmato make it acidic. Four millilitres of ethyl acetate were vigorously vortexed with solutions for five minutes. Subsequently, they underwent centrifugation at 4000 g for 10 minutes. Following this, organic phase was moved to a separate tube & allowed to evaporate to dryness at 50°C with help of a mild stream of nitrogen. HPLC analysis was then performed.

Table 3: Antihypertensive Outcome of optimized TEL loaded LBG:TC hydrogel on Hypertension induced in rats

| Mean BP (mm/Hg) | I Control | II Positive Control | III Pure TEL | IV TEL loaded LBG:TC Hydrogel |
|---|---|---|---|---|
| Initial | 123 | 169 | 163 | 162 |
| 1hr | 122 | 165* | 128** | 142*** |
| 2 hr | 123 | 164* | 116** | 128*** |
| 3 hr | 125 | 162* | 132** | 120*** |
| 4 hr | 126 | 168* | 142** | 112*** |
| 5hr | 124 | 160* | 150** | 110*** |
| 6 hr | 120 | 164* | 152** | 101*** |
| 7 hr | 122 | 166* | 163 | 103*** |
| 8 hr | 123 | 165* | 160 | 106**** |
| 10 hr | 121 | 164* | 164 | 105*** |
| 12 hr | 124 | 163* | 161 | 110*** |
| 14 hr | 122 | 166* | 163 | 118*** |
| 16 hr | 123 | 167* | 165 | 136*** |
| 18 hr | 125 | 165* | 162 | 149*** |
| 20 hr | 124 | 164* | 163 | 151*** |
| 24 | 125 | 162 | 163 | 160 |

All standards are stated as mean ± SD (n=3)
*Significant when associated with control ($p < 0.05$)
**Significant when associated with positive control ($p < 0.05$)
*** Significant when associated with positive control ($p < 0.05$)

*In Vivo Pharmacokinetic Analysis*

To determine Cmax, Tmax & AUC0-∞, plasma samples were taken from six rats in each group. We directly estimated Cmax and Tmax from data of each plasma concentration[20,21]. To determine AUC0-∞, add (AUCt-∞) to

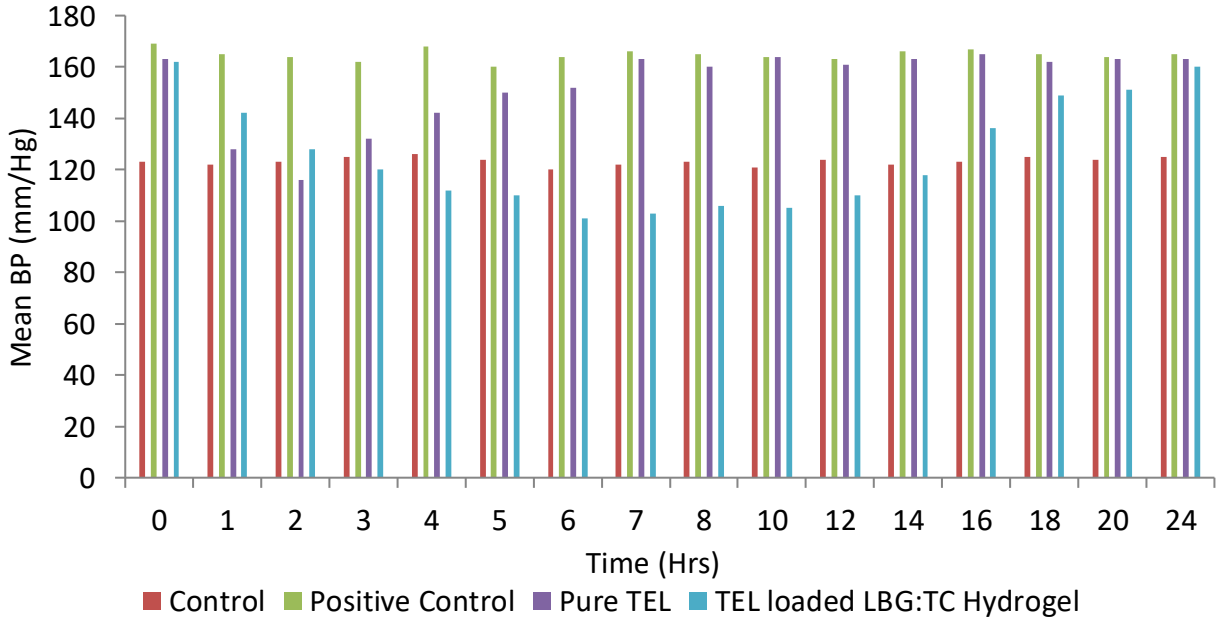


Figure 2: Antihypertensive Outcome of optimized TEL loaded LBG:TC hydrogel in Hypertension induced rats

(AUC0-t). Trapezoidal method was used to calculate AUC0-∞. Kel, total elimination rate constant, was used to get AUCt-∞[22]. Equation (5) was used to compute TEL Relative bioavailability (%), while equation (4) was used to calculate Kel.

$$K_{el} = \frac{Slope}{2.303} \dots (4)$$

$$\%\ Relative\ bioavailability = \left[\frac{AUC_{test}}{AUC_{ref}}\right] \times \left[\frac{Dose_{ref}}{Dose_{test}}\right] \times 100 \dots (5)$$

*Efficiency of Hydrogelalongside Hypertension Induced in Rats*

An animal blood pressure restraint of appropriate size and a sphygmomanometer are utilised to measure the systolic blood pressure using the Vet-Dop2 doppler blood pressure device[23,24]. After recording the rats' baseline blood pressure readings, hypertension was generated for two weeks using subcutaneous injections of Medroxy Progesterone Acetate (MPA, 10 mg/kg/week). Animals were deemed hypertensive if their minimal mean blood pressure was 167±3.2 mmHg. There were a total of six animals divided into five groups to assess the effects of therapies on hypertension. There were four groups: the first was a control, the second was a positive control group, the third was given TEL (5 mg/kg orally), and the fourth had optimised LBG:TC hydrogel. Sodium CMC was used to suspend the standard and formulation before oral gavage administration; blood pressure was monitored 1, 2, 4, 6, 10, and 12 hours after treatment.

*Statistical Analysis*

Mean ± SD; ANOVA; P<0.05 for statistical significance.

## RESULTS AND DISCUSSION

Hydrogel particles of LBG:TC IPN were utilized in both response formulation and assessment. A comparison was made between the expected and actual values of the design responses. In order to validate the technique, the percentage of mistakes was computed. The results were in agreement with the expected value. This proved that the optimization process was successful. Hydrogels with LBG:TC IPN can optimize drug release, mucoadhesive strength, and particle size. Using this technique, a collection of components based on central composite design were discovered. Hydrogel particles of LBG:TC IPN containing 1.5% LBG, 1.47% TC, and 5.52% TPP were predicted to have a size of 179.03 nm, a mucoadhesive strength of 75.00%, and a drug release of 99.96%. A appropriateness score of 0.917 for the LBG: TC IPN hydrogel particles was recorded, placing them within the satisfactory range of 0.8 to 1[25].

*Pharmacokinetics Evaluation Study (In-vivo)*

Main objective of TEL hydrogel that was made utilising biodegradable polymer in this study is to upsurge oral bioavailability of medication. In fact, when developing new dosage forms, evaluation of medication bioavailability is a crucial metric to think about. Figure 1 and Table 02 show the plasma levels of TEL hydrogel and TEL pure medication following a single dose of 1 mg/kg. In contrast to pure TEL (1.21 µg/ml), the Cmax for TEL hydrogel was 1.19 µg/ml. When testing pure TEL and LBG:TC Hydrogel, researchers discovered that time to attain maximum concentration, or Tmax, was 2 hours and 4 hours, respectively. One possible explanation for these findings is that the prepared hydrogel allows for the gradual diffusion of TEL. In contrast to pure drug Kel of 0.125 h-1, the TEL hydrogel's Kel of 0.085 hr-1 is significantly lower. Additionally, hydrogels loaded with TEL showed an improved AUC of 15.22 µg/ml.hr, while hydrogels without TEL had an AUC of 10.04 µg/ml.hr. Compared to the pure medication, TEL hydrogel had a greater AUC. When it came to TEL hydrogel, the relative bioavailability percentage was 151.59. The drug's delayed release and higher absorption rate are responsible for the observed boost in bioavailability. In comparison to pure TEL, whose MRT was 2.03 hours, hydrogel had a significantly longer MRT of 10.07 hours. The produced alginate hydrogel floats more than the pure medication, which could explain these observations. Improved bioavailability was a major finding in the medicinal application of TEL hydrogel. The development of a supersaturated solution may be responsible for this, since it greatly improves absorption and bioavailability[26].

*Anti-hypertensive Efficiency of Gastro-retentive Hydrogel*

The rat animal model was used to study antihypertensive action of hydrogel that was synthesised. Blood pressure was checked and recorded at various intervals after treatments. Table 3 and Figure 2 showed the research groups' pharmacodynamics outcomes.

The results demonstrated that hydrogel had an anti-hypertensive effect following MPA-induced BP induction in rats. A statistically significant increase in blood pressure (BP) was observed in all four groups following MPA administration; this finding supports the model's validity. Blood pressure was significantly controlled in the first hour after taking pure TEL orally ($P < 0.05$). Blood pressure increased progressively following the peak effect of pure TEL, which lasted for four hours.

The hydrogel formulation showed a slow reduction in blood pressure up to 6 hours after administration. The formulation had its greatest impact at the 6th hour, with a significant p-value ($P < 0.05$) indicating this. Blood pressure returned to its starting point after reaching its peak at the 6-hour mark. Hydrogel had a persistent effect on blood pressure (BP) for 20 hours after injection, with a marked decrease in the first hour. Peak effect was noted during the 20th hour. Results show that pure TEL administration rapidly and significantly lowers BP, however its effect wears off after 6 hours. A persistent anti-hypertensive effect was demonstrated by the hydrogel, which allowed for continuous medication release over 24 hours and successfully controlled hypertension during the duration. The drug's slow diffusion may have contributed to these effects, which lasted for 24 hours[27,28]. Results show that hydrogel formulation of TEL is superior to standard formulations in terms of bioavailability and solubility. By continually releasing the medication for 24 hours, the TEL encapsulated hydrogel formulation displays considerable impact over a longer duration.

## CONCLUSION

A telmisartan-loaded hydrogel was developed by an ionic gelation method & assessed for its *in vivo* pharmacokinetics parameters and anti-hypertensive efficacy. According to *in vivo* investigations, this formulation greatly improves the therapeutic efficiency of telmisartan by sustaining the anti-hypertensive action. Better patient compliance may result from increased drug efficacy, oral bioavailability, and solubility made possible by this innovative hydrogel delivery technology. The *in vivo* results also validated the formulation's long-term anti-hypertensive effects in animal studies. We find that our novel hydrogel technology significantly improves telmisartan's bioavailability and therapeutic efficacy.

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