



Research Paper

Development and pharmacological evaluation of floating drug delivery system of Tolbutamide

P SS Prasanna Kumar^{*1}, Srinivas Nandyala², A Anjali¹, Mullangi Poojitha¹

¹ Department of Pharmaceutics, A.K.R.G.College of Pharmacy, Affiliated to JNTUK, Nallajerla, East godavari, Andhra Pradesh, 534112

² Professor, Department of Pharmacology, Sandip Institute of Pharmaceutical Sciences, Nashik, Maharashtra 422213

ARTICLE INFO

ARTICLE HISTORY

Received 18 July 2024

Received in revised form 22 July 2024

Accepted 25 July 2024

Available online- 31 July 2024

Key words:

Tolbutamide, oral controlled release delivery systems, Diabetes, Alloxan

ABSTRACT

The floating drug delivery system (FDDS) is the most practical swelling and expanding, polymeric bioadhesive, high-density, and modified-shape method. The study created and tested floating Tolbutamide microspheres to prolong medication release. Tolbutamide -loaded microspheres (E1-E9) of EC: HPMC (5, 100, and 4000 cps) were synthesised employing polymer ratio, drug concentration, emulsifier concentration, and stirring speed. The formulations were examined for SEM, FTIR, in-vitro buoyancy, yield, entrapment efficiency, and drug release. Optimised formulation release data from all four batches was fitted to mathematical models to establish release mechanism.

All metrics for A2 formulation (EC: HPMC 5cps 1:2) were best, hence stability and in- vivo experiments were done. Stability at normal, rapid, and cold temperatures is studied for six months. Physical appearance, size, SEM images, buoyancy, residual drug content, and release were unchanged. In radiographic studies of barium sulfate-loaded A2 formulation floating, rats showed a mean stomach residency of almost 6 hours. In alloxan-induced diabetic rats, optimised formulations E2 and A2 (4mg/kg body weight dose of pure drug) were compared to normal, diabetic control, and pure drug for antidiabetic activity at various time intervals A2 was more effective than E2 against diabetes. After 15 days of administration, the formulation significantly maintains glucose levels ($p < 0.01$). After 15 days of the antidiabetic study, liver, pancreatic, heart, and kidney histology were done. Control, diabetic, pure drug, and formulation (E2 & A2) histology were compared. Microscopic studies showed that optimised formulations similar to pure medicine reduced and reversed alloxan-induced severe hepatic, renal, pancreatic, and cardiac lesions. Extended release and lower dose frequency may increase Tolbutamide patient compliance with A2.

Corresponding author :

P SS Prasanna Kumar ,Associate Professor, Department of Pharmaceutics,
A K RG College of pharmacy,Nallajerla, Andhra pradesh-534112
Ph.9908785447
E Mail-pssprasanna1@gmail.com



1. INTRODUCTION

In order to boost efficacy, eliminate adverse effects, and increase bioavailability, oral controlled release delivery systems were created. Oral drug administration is the safest, simplest, and most acceptable due to its cost-effectiveness, convenience of administration, and patient compatibility. Advancements in CRDDS technology can overcome the

disadvantages of conventional dose forms¹. These new methods revolutionise drugs and offer therapeutic benefits.

Short-half-life and GIT-absorbed drugs quickly leave the bloodstream and require repeated dose. Oral CRDDS formulation is emphasised to solve such difficulties². CR formulations limit drug release into the GIT and preserve serum therapeutic concentrations longer. This research aims to

develop and test a drug delivery system that reduces dosing frequency, improves absorption, reduces side effects, increases systemic bioavailability, and maintains a constant therapeutic plasma level. Tolbutamide floating microspheres will be developed and tested to improve GRT utilising ethylcellulose and hydroxypropyl methylcellulose as polymers³.

Short-lived, easily absorbed GIT drugs are often removed from blood circulation. This can be solved by developing a controlled release dosage form that gradually distributes the medication into GIT and maintains drug concentration. CRDD enhances drug bioavailability and predictability. Oral CRDDS reduces stomach retention and unexpected gastric emptying. Formulation evacuation from the stomach is unpredictable, thus dose forms that increase and control emptying stay in the stomach longer.

Tolbutamide is a first-generation potassium channel blocker, sulfonylurea oral hypoglycemic medication. This drug may be used in the management of type 2 diabetes if diet alone is not effective. Tolbutamide stimulates the secretion of insulin by the pancreas⁴⁻⁵.

It is not routinely used due to a higher incidence of adverse effects compared to newer, second-generation sulfonylureas, such as Glibenclamide. It generally has a short duration of action due to its rapid metabolism, so is safe for use in older people.

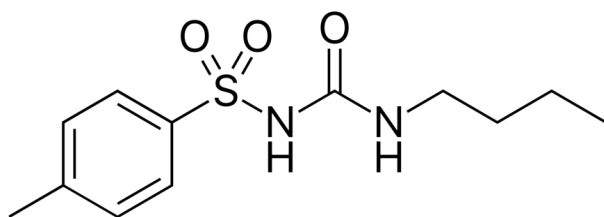


Figure no. 1 Structure of Tolbutamide

Any formulation's efficacy can be proven in-vivo. Thus, in-vitro performance can be validated by testing in animals like humans, microbes, cells, or biological molecules. In-vitro results cannot frequently anticipate in-vivo organism reactions. Most crucial is a dependable extrapolation approach from in-vitro to in-vivo. Certain problems can be solved using two methods:

To replicate tissues and interactions (human on chip system), increase in-vitro system complexity.

Several methods can evaluate floating dose forms in vivo. Rat, rabbit, dog, or human volunteers can generate floating system medication release, buoyancy, pharmacokinetic, and pharmacodynamic profiles. Antidiabetic floating dose formulations need in-vivo buoyancy studies to ensure medication release. Controlled medication release from floating system prolongs pharmacological response can be examined using these methods: The X-ray method, Gamma-Scintigraphy, Gastrosocopy, Ultrasonography, Magnetic resonance imaging⁶.

2. MATERIALS AND METHOD

Chemicals

S.No	Chemical	Brand names
1	Tolbutamide	Torrent Pharmaceuticals, Ahmadabad, India.
2	Ethylcellulose	Himedia Chemicals, India
3	Hydroxypropyl methyl cellulose	Himedia Chemicals, India.
4	Polyvinyl alcohol (PVA)	SD fine chemicals Mumbai, India.
5	Ethanol	SD fine chemicals Mumbai, India.
6	Dichloromethane	SD fine chemicals Mumbai, India.
7	Distilled water	Prepared in laboratory assembly
8	Polyethylene glycol	Sunchem, India

Table no.1 List of Chemical used in present research work

Tolbutamide appears as a white, crystalline, odourless powder. EC is odourless white powder. White, odourless HPMC powder came in several grades. Tolbutamide melts at 128-131°C. Basically insoluble, very slightly soluble, sparingly soluble, soluble, freely soluble, highly soluble.

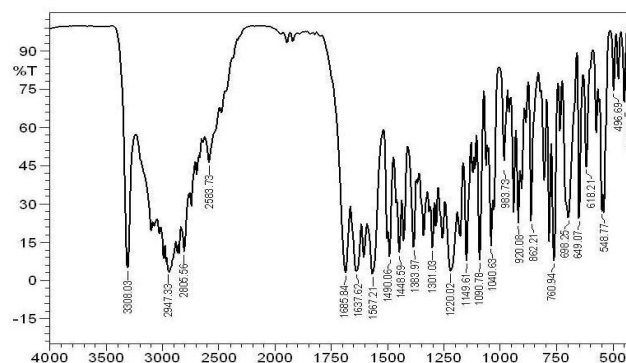


Figure no. 2 FTIR Spectra of Tolbutamide

A Shimadzu-1700 UV-Visible Spectrophotometer with 2 nm spectral bandwidth, ± 0.5 nm wavelength precision, and matched quartz cells of 1.00 cm were utilised for analytical measurements. To prepare a 1000 μ g/ml stock solution, 10 mg of Tolbutamide was dissolved in 10 ml of 0.1 N HCl. Dilute 1 ml of the solution with 9 ml solvent to create 100 μ g/ml sub-stock⁷⁻⁸.

Animal procurement and in-vivo floating behaviour experiment

The study employed 150-200 gm healthy albino rats. Individual rats were housed in polypropylene cages with a conventional 12-hour light/dark cycle at 25-30°C. The rats were fasted for 12 hours before being X-rayed to look for radio opaque stomach contents. Animals were given enough water but not food during the study⁹. After ingestion of radiopaque microspheres, rats were exposed to abdominal X-ray photography (Siregraph-B, Siemens, Karlsruhe, Germany) at 0, 2, 4, 6, and 8 hours to observe the formulation's type and position for anti-diabetic action.

Experimental procedure

This study used 25 healthy male albino rats (150-200 gm) in five groups of five. Group 1: Normal water-fed control.

Group 2: Water-provided diabetic control.

Group 3, diabetic animals received pure Tolbutamide (4 mg/kg body weight).

Group 4: Optimised E2 (EC alone) drug-dose comparable formulations.

Group 5: Tolbutamide-equivalent optimised formulations A2 (EC: HPMC 5cps).

Overnight starved rats of groups 2, 3, 4, and 5 received 120 mg/kg CDH in 0.9% w/v NaCl intraperitoneally to induce diabetes. To prevent hypoglycaemia, animals received 10% glucose solution bottles for 24 hours. Hyperglycemic animals (fasting blood glucose > 250mg/dl) were separated 72 hours following injection for further testing.

Blood Plasma glucose was obtained every 30 minutes for 4 hours, then every 6 and 8 hours for glucose estimation. Treatment was repeated for 15 days. Fasting glucose levels were estimated on 0, 7, and 15 days.

Histopathology

Histopathology was performed on antidiabetic rats. Animals were slaughtered and liver, pancreas, heart, and kidneys extracted on day 15. The organs were soon removed and preserved in 10% formalin for histopathology. Fixed in paraffin blocks, tissues were cut and placed on glass slides. After staining, slides were examined and photomicrographed¹⁰.

3. RESULT AND DISCUSSION

The discovered approach followed Beer's rule at 2-20 concentrations. The procedure was considered reproducible due to low RSD values (<0.44). Interference research results show no excipients employed in estimation. Over 97.40% recovery occurred. Thus, the suggested approach for Tolbutamide estimation in in-vitro dissolution tests and other goods proved suitable.

Tolbutamide-Loaded EC Floating Microsphere Preparation

A slightly modified solvent diffusion-evaporation approach produced floating microspheres. EC and 0.1% PEG (surfactant) were dissolved in 1:1 ethanol- dichloromethane at room temperature. This polymeric solution contained drug. The slurry was progressively added to 80 cc of polyvinyl alcohol emulsifier (0.46 % w/v) water. The organic solvent was evaporated by propeller agitation for 1 hour. The microspheres were washed 3-4 times with distilled water, dried at room temperature for 1 hour, and stored in fused calcium

In vitro buoyancy

50 mg of formulation was swirled at 100 rpm with a magnetic stirrer in 100 ml of SGF (pH 1.2) containing Tween 20 (0.02 w/v %) to assess in-vitro buoyancy. After 12 hours of filtration, floating microspheres were isolated from settled ones. The particles were dried and weighed

individually. Microsphere buoyancy was calculated using the formula below⁹.

$$\text{Buoyancy (\%)} = \text{Wf} / (\text{Wf} + \text{WS}) \times 100 \text{ (10)}.$$

Batch code	Buoyancy (%)	Drug Entrapment (%)	Yield (%)
E1	81.31±1.1	63.28±2.4	68.54±4.6
E2	80.41±2.4	68.03±2.2	76.15±2.5
E3	78.67±2.2	72.32±4.1	79.81±3.6
E4	84.31±3.2	65.47±2.2	72.42±2.3
E5	71.81±2.4	60.21±5.1	68.47±5.4
E6	77.12±3.3	62.45±1.5	74.12±2.3
E7	76.53±1.1	58.63±1.7	72.46±1.2
E8	76.24±3.2	71.47±2.2	76.94±3.2
E9	73.48±4.0	73.16±3.3	78.43±2.5

All values are represented as mean±SD (n=3)

Table no.2 Percent buoyancy, entrapment efficiency and yield of EC microspheres

In vitro drug release

Drug release from formulation was measured using a six-station paddle dissolving instrument (Veego, VDA-6DR, USP Std). Floating microspheres containing 16 mg of medication were preserved in 0.1N HCl with 0.02 w/v Tween 20. Temperature was kept at 37±0.5° C with 100 rpm rotation. Washbasin condition was maintained during study. A 1 ml sample was taken after 30 minutes, filtered over a 5µm membrane, and monitored at 247nm for spectrophotometry. Standard calibration curve used to calculate cumulative drug release.

SEM study

These SEM photos reveal that the microparticles were distinct, excellent spheres with smooth, dense surfaces. Microspheres have pores and inter-granular gaps. The torn surface shows microspheres' hollow interiors, which allow them float on GIT fluid .

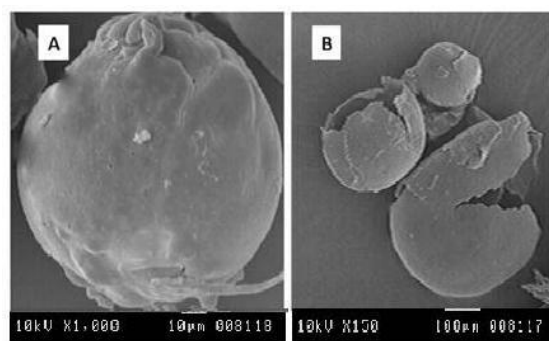


Figure no. 3 Spherical shaped EC microsphere and B) Ruptured surfaceshowing hollow nature of microspheres.

Drug-loaded EC microsphere FTIR spectra show distinctive peaks not seen in placebo microsphere spectra. Drug and Tolbutamide-loaded microsphere characteristic peaks. The results reveal that neither ethylcellulose nor formulation impact medication stability¹⁰.

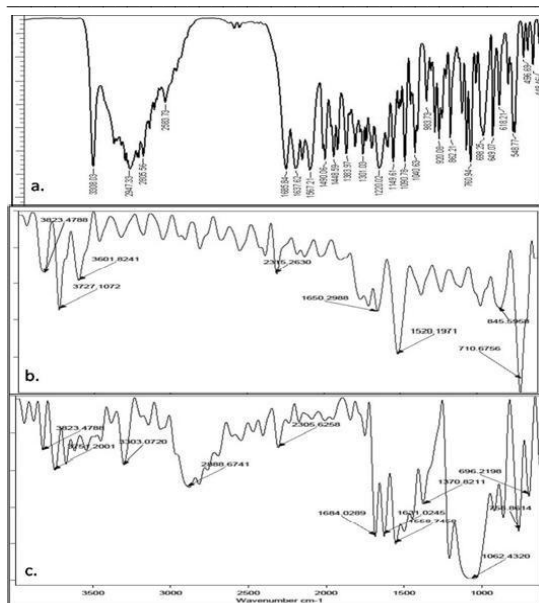


Figure no. 4 FTIR spectrum of (a) Tolbutamide, (b) Placebo EC microspheres and (c) Drug loaded microspheres.

EC prep: HPMC microspheres

Solvent-diffusion evaporation with EC and HPMC (5, 100, and 4000 cps) produced microspheres. Formulations with different HPMC concentrations and fixed EC ratios showed the effect of increasing

Polymer concentration on parameters. Pouring polymer and drug solution in ethanol and dichloromethane into PVA-containing water solution generated microspheres. Polymer precipitates around dichloromethane droplets due to fast ethanol dispersion into external aqueous phase. Dichloromethane evaporates, creating voids in floating microspheres. Thus, smooth, spherical microspheres that float above stomach fluid were created.

SEM

SEM images of optimised microspheres made from HPMC (5, 100, and 4000 cps). Unique, spherical microspheres with porous skin and smooth surfaces were created. Microspheres have small cavities on their surfaces due to solvent evaporation during drying and inter-granular gaps. Their hollow structure helps them float. Microspheres' flat surfaces show drug and polymer uniformity. Microspheres have dense, smooth exterior surfaces with discrete pores.

FTIR evaluation

Tolbutamide's peaks at 3308, 2947, 1685, and 1637 cm⁻¹ imply -NH, -CH, C=O stretching, and -NH bending vibrations. Drug-containing microspheres showed Tolbutamide's -CH and -C=O stretching vibrations at 2994 and 1645 cm⁻¹. Thus, drug microsphere formulation did not change chemically¹¹.

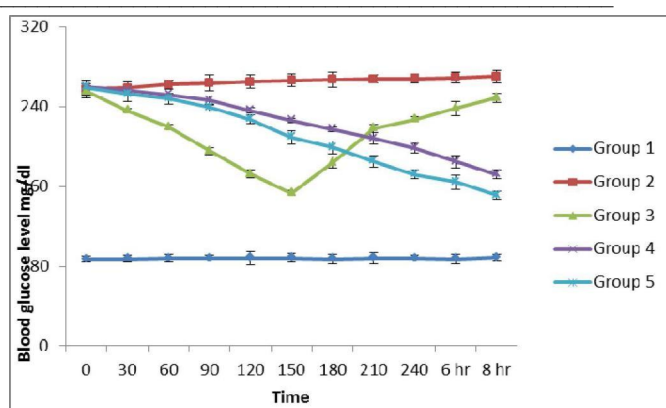


Figure no. 5 Blood glucose level of various groups of animals at different time interval on first day

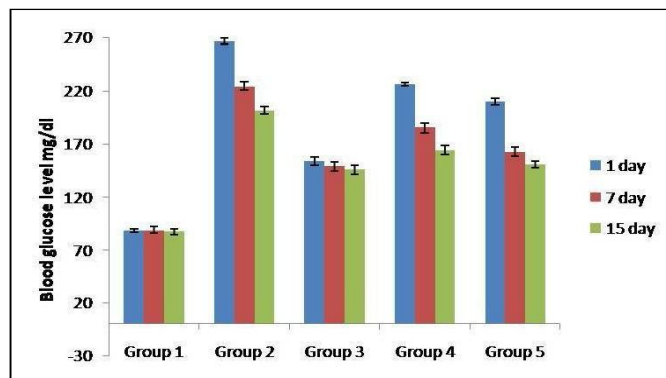


Figure no. 6 Comparison of blood glucose level of animals of three different days

Histopathology

Liver

The control rats' livers had healthy hepatocytes with central and portal veins. The microscopic architecture has hexagonal lobules and acini with well-preserved cytoplasm and nucleus. The same degenerative liver histological changes are caused by alloxan. In contrast, alloxan-treated livers lost their typical architecture and cellular arrangement around the central vein. In the absence of insulin, liver tissue showed hepatocyte death, periportal fatty infiltration, dilated portal vein (PV), leucocyte inflammation, and cell edema. Rat liver sections treated with Tolbutamide demonstrate normal microvasculature and healthy hepatocytes. Histological findings in liver segment improved with mild blood vessel dilatation¹²⁻¹³.

The therapy of A4 microspheres restored normal cellular organisation near the major vein and lowered necrosis in injured blood vessels. Besides minor cellular inflammation, alloxan did not harm hepatocytes. In rats, E2 microspheres (EC alone) had similar non-lethal effects on hepatic histoarchitecture. Fat buildup and cellular edema surrounding hepatocytes were moderate. The formulation-treated groups reversed liver cell damage similarly to the medication.

Pancreas

The pancreas synthesises insulin with a normal percentage of islet of Langerhans encircling the pancreatic acini with conspicuous nuclei. Such traits were absent in alloxan-induced diabetic rats. Alloxan-treated rats' pancreas showed cellular damage, with shrunken islets, irregular shapes, and a small number of cells and cytoplasmic vacuolization¹⁴.

Tolbutamide barely altered cell shape. Tolbutamide's non-apoptotic impact on islet cells supports this result. Visualising exocrine cells generates pancreatic cells, demonstrating the drug's insulin-boosting effects.

E2 formulation administration to rats showed pancreatic islet regeneration with slight cellular inflammation, while A2 microspheres administration showed marked improvement in cellular injury, as shown by partial restoration of islet cells, -cell damage, more symmetrical vacuoles, and an increase in islet cells.

Kidney

In the control group, kidney morphology showed healthy prominent glomeruli, tubules, Bowman's capsule, macula densa cell, and compact tissue. Diabetic rats showed glomeruli shrinkage, lipid accumulation, and lymphocyte infiltration. Diabetics pass urine, which causes glomerulus structural alterations.

These morphological alterations imply that hyperglycaemia dilates cortical distal and proximal tubules, while inflammation is the subsequent impact. Tolbutamide revealed membrane, capillaries, and very mild glomerular inflammation in those that reversed these pathological destructions and had normal basements. A4 microspheres improved kidney histo-architecture without glomerular or tubular pathology or inflammation. As shown by the low incidence of inflammation in rats after EC microsphere treatment, the situation improved.

Heart

Normal control animal heart tissues had regular cardiac myocyte organisation and striations. Cardiac myocytes have large, oval-shaped, central nuclei. Histology of cardiac tissue in alloxan-induced diabetes mice showed cardiac myofibril disarrangement and cardio myocyte nuclei distortion. This group showed significant infarcts and sub-endocardial necrosis with moderate lymphocytes and macrophages. Insulin insufficiency directly disrupts cardiac myofibrils¹⁵.

In alloxan-intoxicated Tolbutamide-treated rats, cardiac tissues showed only little cellular infiltration and muscle fibre disintegration. A2 formulation caused no significant injury, with typical striations and well-arranged cardiac myocytes and moderate cellular inflammation. While rats treated with E2 formulation showed moderate inflammation near cardiocytes and cellular edoema with scarring. Thus, formulations did not impair alloxanized animals' vital organ histoarchitecture and cellular morphology.

4. CONCLUSION

GRDDS is a unique controlled release formulation that is attracting attention in delivery system design. Classically, gastroretentive systems ensure that physiological parameters like gastric retention time support the system. The lack of GI motility effects makes floating systems more popular than other GRDDS.

To create, characterise, and assess floating microspheres of Tolbutamide, an antidiabetic medication for type II Diabetes Mellitus. Tolbutamide has an hour plasma half life. Short half-life and primary absorption from the gastrointestinal tract (GIT) eliminate it from blood circulation, requiring regular doses¹⁶. Control release and swellable polymers EC and HPMC

released medication effectively. Thus, solvent evaporation is used to microencapsulate Tolbutamide to prevent stomach adverse effects and control medication release. EC-only and HPMC-containing floating microspheres (5, 100, and 4000 cps) have good in-vitro buoyancy and gastric fluid release.

Tolbutamide-loaded microspheres (E1-E9) of EC (A1-A9, B1-B9, and C1-C9) of EC: HPMC (5, 100, and 4000 cps) were synthesised utilising polymer ratio, stirring speed, drug, and emulsifier concentration¹⁷.

Tolbutamide-loaded microspheres of different polymers were distinct, round, free-flowing, and uniform, according to SEM. The torn surface shows EC microspheres' hollow interiors, which allow them float in stomach fluid. The EC: HPMC 5, 100, and 4000 rpm microspheres had dense, smooth surfaces with identifiable pores. Drug and polymer dispersion is homogeneous on microspheres' flat surfaces.

The release study experimental condition matched physiological requirements. The release occurred in 0.1 N HCl for 12 hours. Increased EC and HPMC concentration slows medication release. All four batches' microspheres release more drug when rotated from 600 to 1200 rpm. Drug release was higher for HPMC 5cps formulations than 100 and 4000 cps HPMC formulations.

All parameters for A2 formulation (EC: HPMC 5cps 1:2) were excellent and best, hence stability and in-vivo investigations were done. Stability studies show how API or completed product quality changes with time under changing environmental factors including temperature, light, and humidity. ICH provides storage instructions to estimate medicine shelf life. ICH and WHO criteria were followed to test optimised formulation (A2) stability at normal and accelerated conditions. The best formulation (A2) was stored in amber-colored screw-capped borosilicate glass containers at normal ($25 \pm 2^\circ\text{C}/60 \pm 5\% \text{ RH}$), freezing ($5 - 8^\circ\text{C}/65 \pm 5\% \text{ RH}$), and oven ($40 \pm 2^\circ\text{C}/75 \pm 5\% \text{ RH}$) temperatures for 6 months utilising programmable environmental test chambers¹⁸.

Without in-vivo measurements and comparisons, in-vitro release of any preparation is unimportant. The effectiveness can be verified in-vitro and in-vivo. Out of all formulations tested in-vitro, A2 (EC: HPMC 5 cps) performed best. Thus, this formulation was chosen for in-vivo floating behaviour, antidiabetic activity, and histological tests. A2's antidiabetic activity was compared to the best EC-only formulation.

A similar radiopaque placebo formulation of barium sulphate radio contrast material was developed and tested radiologically to determine optimised formulation buoyancy in vivo. Radiopaque microspheres were characterised for particle size and in-vitro floating behaviour to establish this formulation's resemblance to optimised A2. Water was available ad libitum to healthy albino rats fasting for 12 hours. Radiopaque microspheres were eaten and the rat was retained for abdominal X-rays at 0, 2, 4, 6, and 8 hours to check the formulation's type and position.

During the investigation, rat blood drug pharmacological response was assessed for antidiabetic activity. In alloxan-induced hyperglycemic rats, optimised formulations E2 and A2 were compared to normal, diabetic control, and pure Tolbutamide for antidiabetic efficacy over time. Due to the drug's short biological half-life, pure drug-treated rats' blood glucose levels first decline and then climb after 150 minutes. The blood glucose

level of rats treated with optimised formulation E2 (EC alone) and A2 (EC: HPMC 5 cps) was slower than pure Tolbutamide treated rats in the first hours, but it increased after 3 hours and by the end of the trial. Compared to pure drug-treated rats, optimised formulation led to controlled Tolbutamide release.

E2 and A2 were compared for their in-vivo and in-vitro antidiabetic effects. A2 formulation is more promising than E2 because hydrophilic polymer HPMC enhances medication solubility in gastric medium and release. In A2, EC gives the formulation floating properties so it stays in the stomach fluid longer. The formulation led to a significant ($p < 0.01$) maintenance of blood glucose levels after 15 days of administration.

Histology of the control rats' livers revealed healthy hepatocytes, while alloxan administration caused architectural loss and degeneration. With normal hepatocytes, tolbutamide reversed normal microvasculature. Like the pure drug group, the optimised formulation repaired liver cell damage. Normal control pancreas has normal proportion of islet of Langerhans around pancreatic acini with conspicuous nuclei damaged by alloxan. In rats, E2 formulation caused pancreatic islet regeneration with modest cellular inflammation, while A2 formulation improved cellular injury and restored islet cells.

Control kidney showed healthy prominent glomeruli, tubules, Bowman's capsule, macula densa cell, and compact tissue. Alloxan reduced glomeruli, fat accumulation, and lymphocyte infiltration. Pure RG reversed these pathological damage. A4 microspheres improved kidney histo-architecture while EC alone caused little inflammation.

Alloxan-induced diabetic rats had disrupted cardiac myocyte organisation and normal striations. Pure drug delivery caused little cellular infiltration and muscle fibre fragmentation. A2 formulation had no negative effects, whereas E2 caused minor inflammation near cardiocytes and cellular edoema with scar formation. Thus, formulations did not affect alloxanized animals' vital organ histo-architecture and cellular morphology.

5. CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

6. ACKNOWLEDGEMENT

We are highly grateful to our Honorable Chairman, A.K.R.G. College of Pharmacy, Andhra Pradesh for providing the necessary facilities to carry out this research work.

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