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ORIGINAL ARTICLE **OPEN ACCESS**

Demonstrate the antidiabetic effect of polymeric microsphere formulation of Glibenclamide using Alloxan-induced diabetes mellitus model in rats

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ABSTRACT

The purpose of this study was to provide information on the effect of Glibenclamide microsphere formulation and to investigate the therapeutic effect in Sprague–Dawley (SD) rats. The test item formulation was administered as a single dose intramuscularly. SD rats were divided randomly and assigned to one of five subgroups. A single dose of Alloxan was used to induce diabetes in rats. The determination of blood glucose level after 48 hours confirmed diabetes. Blood glucose levels were measured up to 9 days after the test compound, standard drug and drug solution were administered in a single dose to each treatment group. Glibenclamide microsphere formulation group significantly reduced blood sugar levels from the fourth hour to the ninth day after formulation administration. Glibenclamide microsphere formulation consistently reduced blood sugar levels up to the ninth day, indicating that drug release from the microsphere formulation was controlled up to the ninth day. Based on the findings, it is possible to conclude that Glibenclamide microsphere formulation has an anti-diabetic effect when administered intramuscularly. According to the results, a single dose of 'Glibenclamide microsphere formulation' normalised blood sugar levels in rats.

Keywords: *Glibenclamide, alloxan, blood glucose, diabetes mellitus, microsphere.*

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INTRODUCTION

The novel drug delivery has drawn more attention in recent years. Polymeric systems include drugs for controlled and targeted medication release. [1] The release of drugs from these systems should be predictable, consistent, and at the desired rate. [2] There are various controlled release formulations available in tablet form, but over time, the Microsphere formulations have gained tremendous appeal since they outperform the others in many ways. The need for long-term treatments of chronic illnesses prompted the widespread development of long-acting parenteral formulations (LAPFs), with the aim of improving medication pharmacokinetics and therapeutic efficacy. [3] LAPFs have been shown to increase patient adherence and to lengthen the half-life of medicines, both of which improve treatment outcomes. [3]

Diabetes mellitus (DM) is one of the world's most significant health issues at the moment. [4] It has been documented that the diabetes prevalence is steadily increasing, and that 6.4% of individuals in the world suffer diabetes, with 285 million persons estimated to have the disease in 2010 and perhaps 439 million by 2030. In developing countries, the prevalence of adult diabetes will rise by 69% between 2010 and 2030, while it will rise by 20% in wealthy nations. [5] By 2030, diabetes will be the seventh for the most common cause of death, according to WHO forecasts. [6]

The pancreas produces insulin, which is the main hormone responsible for elevated blood sugar levels used as a type of protection. In type 2 diabetes, insulin is not produced enough in response to spikes in blood sugar, such as those that occur after meals. Glibenclamide is a widely used oral anti-diabetic medication for the treatment of non-insulin-dependent diabetes mellitus (type II). It primarily stimulates the pancreatic beta cells, which are responsible for producing insulin. The beta cells begin to produce more insulin as a result. Hence, people with type 2 diabetes benefits from a reduction in blood sugar levels.

The goal of Glibenclamide, a second generation sulphonyl urea, is to be more efficacious than first generation medications. As a result of its 4-6 hour biological half-life, it needs to be administered more than once to maintain plasma concentration. This results in discomfort for the patient and changes in plasma medication concentration, which may result in diminished therapeutic effects or harmful effects.

In order to prevent pointless and morally dubious investigations on human patients and to gain a thorough scientific understanding of this illness, animal models of DM are crucial. Additionally, it is preferable to mimic DM in experimental animals due to the variety of treatment approaches that may be used and the resulting complications that may occur; these models offer safer alternatives to which the therapeutic intervention can be applied. Alloxan and streptozotocin constitute the most significant and highly preferred experimental models for this pathological state, despite the fact that there are other ways to cause DM. [7]

The most frequent chemical used to cause experimental diabetes is alloxan (2,4,5,6-tetraoxypyrimidine; 2,4,5,6-pyrimidinetetrone), which selectively destroys beta cells in the pancreatic islets by a series of alterations that finally results in apoptosis. [7] For example, the dose of alloxan used to cause diabetes in rats ranges from 40 to 200 mg/kg administered intravenously (i.v.) or intraperitoneally (i.p.). [8,9] Alloxan has two separate pathological effects. First, selective inhibition of insulin production triggered by glucose and second, by producing reactive oxygen species (ROS) that cause necrosis of beta cells. [10]

By adjusting the dose of the drug employed, Alloxan has been widely used to cause experimental DM in animals like rabbits, mice, and dogs with variable degrees of disease severity, leading to "Alloxan Diabetic," an insulin-dependent diabetes mellitus that is similar to type-1 diabetes. [12, 13]

MATERIAL AND METHODS

Preparation of Microspheres

The microspheres of Glibenclamide were prepared by the o/w emulsion solvent evaporation/extraction method. In brief, the drug was dissolved in N-Methyl Pyrrolidone (NMP) which used as miscible solvent for preparation of drug phase, PLGA dissolved in dichloromethane. Drug phase prepared in NMP and polymer phase prepared in DCM. Drug phase is mixed with the polymer solution (O). The Drug polymer solution in organic solvents added to polyvinyl alcohol solution (W) through a long, narrow nozzle and agitated vigorously with a homogenizer. The resulting O/W emulsion was kept under stirring using magnetic stirrer to remove N-Methyl Pyrrolidone (NMP) and dichloromethane (DCM) by evaporation. The semi-dry microspheres are obtained. These microspheres filtered through sieve, washed twice with water and collected. The microspheres dried using lyophilizer. The micrographs of Glibenclamide microsphere generated using scanning electron microscopy refer figure 1.

Animals:

All healthy adult Sprague Dawley rats of either sex weighing between 220 to 270 gm were used for the study. A total of 30 rats (either sex) were selected for the study.

Test animals details

Species	: Rats (Rattus novergicus)
Strain	: Sprague Dawley
Sex	: Male/Female (Females Will nulliparous & Non Pregnant)
Range	: ± 20 % of the mean body weight of animal for each sex
Age	: Young healthy adult 8-10 weeks old
No. of Animals	: 30 (both sex)
Body weight Range	: 220 to 270 gm

Environmental Condition for test animals:

Animal was maintained under following environmental conditions.

Temperature	:	22 ± 3 °C
Relative Humidity	:	30- 70%
Photo Period	:	12 h light & 12 h dark cycle

Housing of animals

Animals were housed in groups (1 animals/cage) for each sex clean, sterilized steel cages. Cages having provision for holding pelleted feed & drinking water. Cages & Water Bottles were change.

Feed & Drinking Water

Animals were fed by Autoclaved *ad libitum* water & pelleted laboratory animal diet manufactured by Nutrivet Life sciences feed. Every batch of feed was analysed for microbial contamination. Fresh Potable water drinking water was processed by reverse osmosis system. Microbial contaminant analysis was being performed at regular interval. Certificate of analysis was included in final study report & in raw data.

Room Sanitation

During the conduction of study, the floor of the experimental room was cleaned daily. All worktops were mopped with disinfectant solution on day-to-day basis.

Acclimatization

All animals were acclimatized for minimum 7 days. During acclimatization all animals were observed once for clinical signs for mortality & morbidity. The individual animal was subjected to a detailed clinical examination before allocation to study to ensure that the selected animals were in good state of health. All observations to be conducted during acclimatization period were only ensuring the health status of animal prior to treatment.

Animal Identification

During acclimatization animals were identified by temporary animal number were given with marker pen on the fore limbs of each animal. Each cage label having study no, temporary Id, species, sex, strain, cage no, acclimatization start date, acclimatization end date, sign, and date.

Method for blood sampling: [14, 15]

The rat was anesthetized by anaesthetic ether in anaesthetic chamber. After small anesthetized rat was taken up from anaesthetic chamber. Now put animal on operation table and tail is squeezed with ethanol to dilate the vein and prick the tip of tail and drop of blood is collected on strip of Dr.Morepan Gluco One glucometer.

Estimation of blood glucose

Blood sugar level was measured by employing a Dr.Morepan Gluco One glucometer, All measurements were performed by the same operator, employing the same glucometer.

Induction of diabetes in rats:

Group of six albino rats of either sex weighing between 220 to 270 gm were selected forthe study.The animalswererandomlydistributedinto5 groups(n=6,I,II,III,IV, V);eachgroup was consistingof6 animals. SD rats were randomized in Group-I (Negative control- 10ml/kg p.o. Dist. water) Group-II (Positive Control-Alloxan 150mg/kg i.p. once), Group-III (Glibenclamide microsphere formulation i.m.) Group-IV (Glibenclamide drug solution i.m.) and Group-V (Glibenclamide tablet p.o.) and for evaluation of antidiabetic effect of Glibenclamide microspheres formulation, Diabetes in SD rats were induced by Alloxan monohydrate 150mg/kg i.p. After 48 hrs, diabetes was confirmed by the determination of fasting blood glucose level with the help of one-touch electronic glucometer. Rats with a fasting plasma glucose range of 250-350 mg/dl were selected for the study.

Evaluation of antidiabetic effect:

Test compound, standard drug & drug solution were administered in single dose to different treatment group, after successful induction of diabetes in rats and blood glucose level was measured at 0, 4, 10 hours and 1,3,5,7,9th day of the dose administration.

Group	**Treatment**
I	Negative Control (10ml/kg p.o. Dist. water)
II	Positive control (Alloxan 150mg/kg i.p.)
III	(Alloxan 150mg/kg i.p.) + Glibenclamide Microsphere formulation i.m.
IV	(Alloxan 150mg/kg i.p.) + Glibenclamide drug solution i.m.
V	(Alloxan 150mg/kg i.p.) + Glibenclamide tablet p.o.

Diluent for reconstitution was selected as based on solubility testing which was performed at the test facility before the commencement of dosing.

Treatment Procedure:

Required dose volume for each animal was taken from formulation and administered required dose into animal body. Intra muscular route was selected for the test item administration as it is clinically intended route of test item.

Blood sugar level was measured by employing a Dr.Morepan Gluco *One* glucometer, All measurements were performed by the same operator, employing the same glucometer. Blood sugar level were measured on different time interval blood glucose level were measured at 0, 4, 10 hours and 1, 3, 5, 7, 9th day after administration. Blood sugar level measurement at different time interval (mg/dL) as below

RESULTS AND DISCUSSION

Glibenclamide microsphere formulation group significantly decrease the blood sugar level from 4th hour to 9th day after administration of formulation. Glibenclamide drug solution group significantly lowers blood sugar level on 4th hour up to 24th hour and Marketed Glibenclamide tablet lowers level on 4th hour to 10th hour after drug administration and were compared with positive control group. Glibenclamide microsphere formulation was decreased blood sugar level consistently up to 9th day; it means that drug release from microsphere formulation was in controlled manner up to 9th day.

Based on the results, it was concluded that the test item 'Glibenclamide microsphere formulation' significantly decrease blood sugar level. It shows antidiabetic effect when administered through intramuscular route under the conditions and procedures followed in the study.

Based on the findings, single dose administration of 'Glibenclamide microsphere formulation' normalized blood sugar level in rats.

Table 1 Blood sugar level of Group I-Negative Control group

Animal No.	Time interval (Days)							
	0 hours	4 hours	10 hours	24 hours	3rd	5th	7th	9th
I	82	89	112	78	109	92	79	98
II	98	77	108	89	93	114	118	83
III	106	92	92	69	88	84	85	107
IV	79	104	65	108	73	96	112	94
V	91	81	94	90	77	88	83	87
VI	123	117	102	87	113	108	79	115
Mean	96.5 ±16.38*	93.3 ±14.92*	95.5 ±16.83*	86.8 ±13.11*	92.2 ±16.33*	97.0 ±11.71*	92.7 ±17.56*	97.3 ±12.08*

Table 2 Blood sugar level of Group II- Positive Control group

Animal No.	Time interval (Days)							
	0 hours	4 hours	10 hours	24 hours	3rd	5th	7th	9th
I	291	307	329	379	413	436	462	542
II	337	345	366	418	452	487	523	570
III	298	310	334	363	395	430	472	498
IV	315	352	367	389	413	435	481	503
V	326	334	369	394	418	447	469	492
VI	358	366	384	418	437	452	476	489
Mean	320.8 ±24.96*	335.7 ±23.48*	358.2 ±21.72*	393.5 ±21.73*	421.3 ±20.15*	447.8 ±20.86*	480.5 ±21.79*	515.7 ±32.83*

Table 3 Blood sugar level of Group III- Glibenclamide microsphere formulation

Animal No.	Time interval (Days)							
	0 hours	4 hours	10 hours	24 hours	3rd	5th	7th	9th
I	328	289	213	129	66	94	122	284
II	352	327	286	224	118	105	138	276
III	341	278	206	127	78	81	109	313
IV	321	305	262	214	92	99	126	291
V	340	308	264	144	85	76	146	292
VI	281	269	246	193	107	88	124	279
Mean	327.2 ±25.07*	296.0 ±21.39*	246.2 ±31.21*	171.8 ±3.74*	91.0 ±19.06*	90.5 ±10.97*	127.5 ±12.96*	289.2 ±13.29*

Table 4 Blood sugar level of Group IV-Glibenclamide drug solution

Animal No.	Time interval (Days)							
	0 hours	4 hours	10 hours	24 hours	3rd	5th	7th	9th
I	342	259	207	288	363	394	442	481
II	318	243	219	279	339	354	371	382
III	324	229	214	325	372	410	451	513
IV	336	247	221	304	343	352	373	392
V	330	272	178	259	375	422	456	496
VI	332	245	209	263	328	359	373	390
Mean	330.3 ±8.52*	249.0 ±23.65*	208.0 ±17.51*	286.3 ±11.41*	353.3 ±19.31*	381.8 ±30.79*	411.0 ±42.60*	442.3 ±60.47*

Table 5 Blood sugar level of Group V- Glibenclamide tablet marketed formulation

Animal No.	Time interval (Days)							
	0 hours	4 hours	10 hours	24 hours	3rd	5th	7th	9th
I	319	253	275	339	377	402	448	492
II	312	212	269	223	271	297	377	558
III	320	237	273	358	384	427	471	506
IV	308	224	307	321	344	467	489	423
V	315	209	271	318	338	389	482	493
VI	352	232	274	376	398	406	459	489
Mean	321.0 ±15.82*	227.8 ±18.10*	278.3 ±15.88*	322.5 ±53.51*	352.0 ±46.01*	398.2 ±48.11*	454.3 ±54.45*	493.5 ±66.03*

Table 6 Mean blood sugar level of all subgroups

Group	Time interval (Days)							
	0 hour	4 hours	10 hour	24 hour	3rd	5th	7th	9th
Negative Control	96.5 ±16.38*	93.3 ±14.92*	95.5 ±16.83*	86.8 ±13.11*	92.2 ±16.33*	97.0 ±11.71*	92.7 ±17.56*	97.3 ±12.08*
Positive Control	320.8 ±24.96*	335.7 ±23.48*	358.2 ±21.72*	393.5 ±21.73*	421.3 ±20.15*	447.8 ±20.86*	480.5 ±21.79*	515.7 ±32.83*
Glibenclamide Formulation	327.2 ±25.07*	296.0 ±21.39*	246.2 ±31.21*	171.8 ±3.74*	91.0 ±19.06*	90.5 ±10.97*	127.5 ±12.96*	289.2 ±13.29*
Glibenclamide drug solution	330.3 ±8.52*	249.0 ±23.65*	208.0 ±17.51*	286.3 ±11.41*	353.3 ±19.31*	381.8 ±30.79*	411.0 ±42.60*	442.3 ±60.47*
Glibenclamide tablet	321.0 ±15.82*	227.8 ±18.10*	278.3 ±15.88*	322.5 ±53.51*	352.0 ±46.01*	398.2 ±48.11*	454.3 ±54.45*	493.5 ±66.03*

*All values are expressed as mean ± SEM (n=6) * p<0.05 as compared with the Positive control group. All data are analysed by one way ANOVA followed by Dunnett's test.

Figure 1 Micrographs of Glibenclamide microsphere generated using scanning electron microscopy

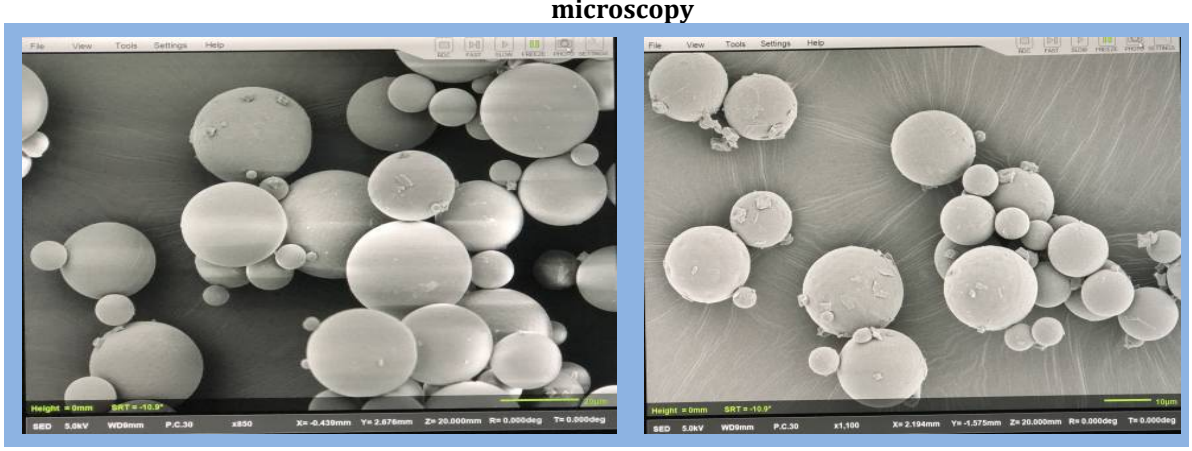
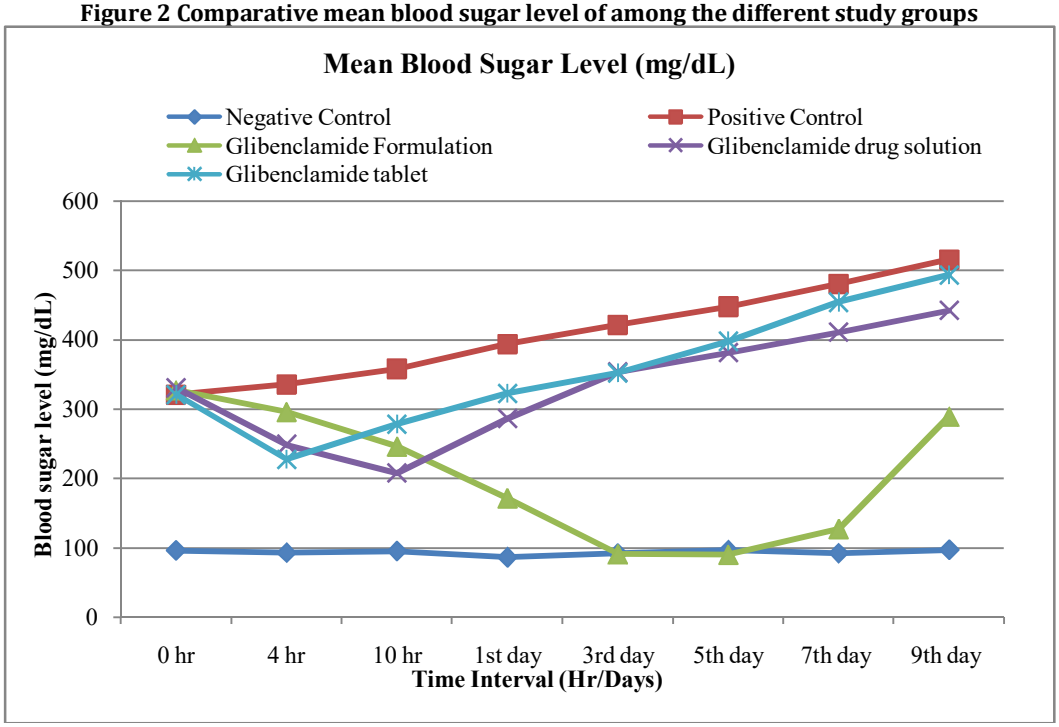


Figure 2 Comparative mean blood sugar level of among the different study groups



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CONFLICTSOFINTEREST

The authors declare no conflict of interest.

ETHICS STATEMENT

The study was conducted at Test Facility- Trans-Genica Services Pvt. Ltd.; Nagardeola, Tal- Pachora, Dist-Jalgaon 424104, Maharashtra, India.
Study facility is certified to compliance to below national standards.
CPCSEA, Government of India, Ministry of Environment & Forest, New Delhi: Certification for the purpose of "Research"

INFORMED CONSENT

The study was designed use to minimum number of animals to meet the scientific objectives, the goal of sponsor & in consideration of applicable regulatory requirements. Protocol for general procedures & use of animals for conducting this study was review & approved by Institutional Animal Ethics committee. All procedure related to animal experiment was performed as per recommendations of the Guide for the Care & Use of Laboratory Animals and committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) guidelines following all the ethical practices as laid down in CPCSEA guidelines for animal care.

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