

IN-VIVO EVALUATION AND ACCELERATED STABILITY STUDIES OF NATEGLINIDE FLOATING DRUG DELIVERY SYSTEM.

Mr. Sukumar M. Lande^{1a*}, Dr. Sampat D. Nawale², Dr. Santosh R. Tarke³, Dr. Mrunal K. Shirsat⁴

1a. Associate Professor, Shri Ganpati Institute of Pharmaceutical Sciences and Research, Tembhorni 413211

2. Principal, Delight Institute of Pharmacy, Pune 412216

3. Professor and HOD, SBSPM B. Pharmacy College, Ambajogai 431517

4. Principal, SBSPM B. Pharmacy College, Ambajogai 431517

1. Research Scholar, Sunrise University, Alwar, Rajasthan 301028

Abstract:

Among the many controlled release systems developed, GRDDS is one of the most unique and has received great attention in the design of delivery systems. One of the benefits of intestinal bacteria is that it helps the body's energy to improve the formation process, such as the time spent in the stomach. This study aims to design, characterize and evaluate floating microspheres of NG, a drug used in treating type II diabetes. The plasma half-life of NG is approximately one hour.

Due to its short half-life and easy absorption from the gastrointestinal tract (GIT), it is rapidly eliminated from the circulation, requiring frequent dosing. With repeated use of NG, some side effects may occur. Therefore, this research mainly focuses on the fabrication of floating microspheres to overcome these problems. EC and HPMC are used to control the release and swellable polymers to induce drug release. Therefore, an attempt has been made to microencapsulate NG by the solvent evaporation technique to prevent gastrointestinal irritation and induce drug release.

Floating microspheres were produced in a mixture containing EC alone as well as HPMC at different levels (5, 100 and 4000 cps).

Key Words: Accelerated Stability studies, floating drug delivery, nateglinide.

Introduction

The efficacy of each preparation can be seen by performing in vivo tests. Therefore, the results of in vitro study can be investigated by experiments on living organisms, primarily animals,

including humans, bacteria, cells or biomolecules. The results of in vitro experiments cannot generally be used to predict the in vivo response of the whole organism. The most important thing is to establish a reliable extrapolation from in vitro to in vivo results. Two methods can be used to solve some problems:

, increasing the complexity of the organism in vitro (human onon-chipistem) to reproduce tissues and their interactions). It numerically simulates the properties of complex systems using in vitro data using numerical models, with an emphasis on model parameters. Formvariable dosage can be evaluated in vivo by different types of studies. In vivo drug release, in vivo buoyancy, and pharmacokinetic and pharmacodynamic properties of floating systems can be determined using mice, rabbits, dogs, or human volunteers.

Antidiabetic floating dosage forms should have a valid in vivo flotation study to determine drug release. The controlled release of the drug from the floating system will also show the drug response (antidiabetic effect) in the long term. The internal buoyancy of Section

FDSDS can be studied by the following methods:

(a) X-ray method

In 1993, the first radiographic method was used to image the intestines of rats using barium sulfate. This method, which has been used by many researchers since then is the most popular and reliable method.

With the correct location of the formulation in the GI environment, the GI release of the drug and gastric emptying time can be easily predicted and correlated. A radiopaque substance (usually barium sulfate) is added during deposition to make it visible with X-ray radiation. An electronic method has also been chosen to study small objects and liquids using mice.

(b) Gamma-Scintigraphy

Short-lived gamma-emitting radionuclide isotopes are used as markers put into quantitative data to estimate dose location in healthy workers. The position of the dosage form can be monitored with a gamma camera. This work requires interpretation by a single analyst, and the low solubility, complexity, and expensive preparation of radiopharmaceuticals makes this approach less viable.

(c) Gastroscopy

The procedure includes intraoral endoscopy with the aid of fiber optic and video systems. This method allows you to see how much paper is stored in the stomach. After a while, the process can be evaluated in detail by removing the drug from the stomach.

(d) Ultrasonography

The use of ultrasound shows the various acoustic impedances of the interface and facilitates visualization of the abdomen. The use of ultrasound has been hampered by the inability of some designs to produce uniform noise when encountered in a physiological environment. It is necessary to study the position of the hydrogel in the stomach, the penetration of the gel by the solvent, and the interaction of FDDS with the gastric wall during peristalsis.

(e) Imaging via magnetic resonance

The location of the material in the intestinal tract of mice can also be seen using magnetic resonance imaging (MRI). But this method is not well designed compared to other methods of git images in different and different states., In vivo evaluation of formulation has a significant impact on drug dosage formulation. GRT measurement is very important for FDDS. Radiology is the oldest, easy, inexpensive and widely used method.

However, exposure to radiation during testing is problematic for humans and less of a concern for lab rats.

Therefore, the electronic method was chosen to monitor the fate of microspheres from the gut of mice. Based on in vitro studies, all A2 formulations (EC: HPMC 5 cps) showed the best and most satisfactory results. Therefore, this formulation was chosen for in vivo evaluation such as buoyancy, and antidiabetic activity followed by histopathological studies. The anti-diabetic properties of A2 were also compared with the best samples containing only EC.

Experimental

***In-vivo* floating behavior of radiopaque microspheres**

A. Preparation of radiopaque microspheres

Despite many studies, the radiographic technique presented by Tanwar et al has been modified and used to determine the anatomical location of selected specimens in the GI tract. The X-ray contrast agent barium sulfate (BaSO₄) is used locally and to detect floating microspheres.

BaSO₄ concentration ranged from 1 to 5% w/v and the best concentration was chosen according to the buoyancy behavior. Preparation of radiopaque placebo floating microspheres (RA2) by adding 3% barium sulfate to the polymer solution using an optimized process.

B. Procurement of animals and *in-vivo* floating behavior experimental procedure

Healthy albino mice weighing 150-200 g were used for this study. Mice were housed individually in polypropylene cages maintained according to the standard (12 h light and 12 h dark cycle; 25-30°C). Mice were fasted for 12 hours and x-rayed first to detect radiopaque material in the stomach. Animals were not allowed to eat during the study, but adequate water was provided. After ingestion of radiopaque microspheres in mice, the gastrointestinal tract was examined at 0, 2, 4, 6, and 8 hour intervals to assess the nature and location of the preparation (Siregraph-B, Siemens, Karlsruhe, Germany). Up to 8 hours (Figure 1)

Antidiabetic activity

A. Experimental protocol and method

25 healthy male albino rats (150-200 gm) were selected for this study and were partitioned into five groups of five rats each.

Group 1: Normal control (provided with water).

Group 2: Diabetic control (provided with water).

Group 3: Diabetic animals treated with NG in pure form (4 mg/kg body weight).

Group 4: Optimized formulations E2 (EC alone) treated equivalent to the dose of the drug.

Group 5: Optimized formulations A2 (EC: HPMC 5cps) treated equally to NG dose.

In groups 2, 3, 4, and 5, diabetes was induced by intraperitoneal injection of 120 mg/kg alloxan monohydrate (CDH) in 0.9% w/v NaCl in overnight fasted animals. The animals were given bottles of 10% glucose solution for the next 24 hours to avoid diabetes. At 72 hours after injection, hyperglycemic (fasting blood glucose above 250 mg/dl) animals were isolated and used for further research. During the study, animals were given water ad libitum. Fasting blood glucose was calculated by taking blood samples from the rats' veins, and blood glucose levels

were checked using Accu-check Active Glucose Test Strips and the Accu-check Active Test Meter. To measure blood sugar, blood samples are taken every 30 minutes to 4 hours, then every 6 and 8 hours. Continue to give similar treatment for 15 days.

Fasting blood glucose estimates were made on days 0, 7, and 15. Values are summarized in Tables 1 and 2, respectively.

Histopathological studies

Rats under antidiabetic study were used for histopathological assessment. On the fifteenth day, animals were sacrificed and organs such as liver, pancreas, heart, and kidney were isolated. The collected organs were excised quickly and fixed in 10% formalin for histopathological tests. Tissues were fixed in paraffin blocks, sliced into pieces, and kept on glass slides. Slides were observed and photomicrographs were captured by microscope after suitable staining (Fig. 2-5).

Results and discussion

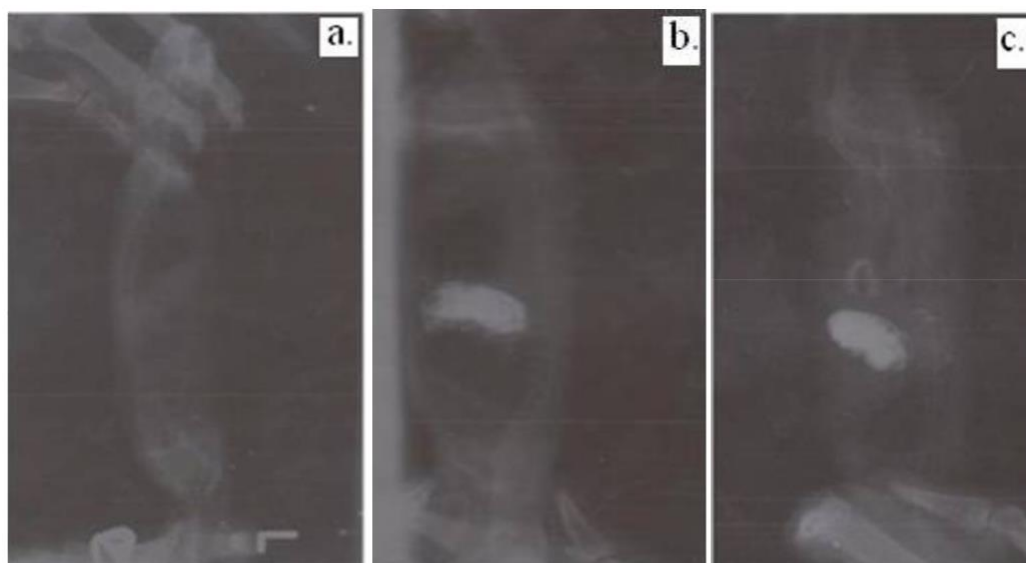
***In-vivo* floating behavior**

Radiopaque placebo floating microspheres A2 (EC: HPMC 5cps 1:2 ratio) were successfully prepared by the same method as described in the experimental section using barium sulfate. For *in-vivo* radiographical contrast, a sufficient quantity of barium sulfate is required to be encapsulated in microspheres. But at the same time, a higher quantity of barium sulphate affects the floatability of microspheres. Therefore, an optimized concentration of 3 % was used to attain the same. The prepared radiopaque microspheres were of particle size $219 \pm 3.1 \mu\text{m}$ (n=3) which is almost similar to the size of microspheres of normal A2 formulation $208.27 \pm 2.7 \mu\text{m}$ (n=3). The radiopaque microspheres were found to float over the selected medium, which indicates that their density is <1 , although some particles settled down due to high density than normal A2 formulation.

The characterized radiopaque microspheres were administered to rats by intragastric tube with a sufficient quantity of water. Since gastrointestinal motility under fasting conditions has characteristic housekeeping waves, which occur after every 1.5-2 hours approximately and may sweep away undigested substances from the stomach independent of their shape, size, and

density. Therefore working with a floating device will be better when a sufficient quantity of water is present in the stomach to avoid the effect of such waves. Thus, administration of formulation is done with approximately 100 ml of water. A series of X-ray photographs of radiopaque microspheres in the stomach of rats are shown in Fig. 1. Image at 0 hours clearly indicates the absence of any material before dosing of radiopaque microspheres. At the initial stage, within 4 hours after taking a dose, in the upper part of the stomach, clouds of particles were observed (Figs. 1 b, c) which indicates uniform distribution of formulation over the fluid.

At later stage 6 hours after dosing, the contents of the stomach surface were very much roughened owing to peristaltic movement as compared to the initial condition, but still some microspheres remained in such condition. Few microspheres in the form of clouds were seen in the upper portion of the stomach, even in the presence of peristaltic waves (Fig. 1 d). The prolonged residence of microspheres may be understood owing to floating characteristics and also by random emptying effect as it is a multiple-unit system. Due to their low density, floating microspheres always spread and reside in the upper part of the stomach and may have less chance of “fortuitous emptying” (Kawashima et al., 1991). As shown in Fig. 1 (e) almost complete emptying of microspheres from the stomach was observed at the 8th hour after dosing. As the density of drug-loaded microspheres, is lighter than barium sulfate-loaded formulation, their residence is prolonged further the gastric emptying time. Thus, results show that the mean GRT of optimized formulation was more than 6 hours in rats; it means that the formulation withstands the different phases without emptying.



d.**e.**

Fig. 1 X-ray images of formulation in gastric region of rat: (a) before dosing, (b) 2 hours, (c) 4 hours, (d) 6 hours and (e) 8 hours after dosing.

Antidiabetic activity

In-vivo evaluation was performed in male albino rats by determining blood glucose level after treatment with optimized formulation (equivalent to 4mg/kg body weight dose of pure drug) which is compared with pure drug treatment at same dose. The antihyperglycemic effect of two optimized formulations E2 (EC alone) and A2 (EC: HPMC5cps) was compared with pure diabetic rats at various time intervals (Fig.2). When NG solution (pure) was given orally, the blood glucose level starts to decrease within 30 minutes. At 150 minutes glucose level is at its minimum but afterward its level again starts to increase. Rapid decrease of glucose level of pure NG is due to faster rate of dissolution of drug in pure form in gastric fluid of the rat. The further increase in glucose level is due to elimination of drug as the biological half-life is short about 1 hour.

Treatment with formulation causes significant ($p < 0.01$) decrease in glucose level as compared to pure drug treated group of animals. Formulations E2 and A2 shows slower decrease in glucose level as compared to pure drug during the initial hours which increases after 3rd hour till the end of the study. Glucose level of both the formulations started to decrease significantly after 1 hour which is continued till eighth hour indicating controlled release of drug from the formulations.

On comparing the antidiabetic effect of E2 and A2 formulation it was observed that the effect was more promising for A2 formulation which contains EC: HPMC 5cps in 1:2 ratios. After three hours of the study glucose level of E2 formulation is 217.3 mg/dl whereas that of A2 is 199.3 mg/dl. Low effect of E2 formulation is due to insolubility of EC in gastric fluid. Presence

of hydrophilic polymer HPMC increases its dissolution in the gastric medium there by releasing the drug more effectively. Whereas use of EC was to impart floating characteristics so that drug will release slowly for longer duration in the fluid.

The assessment of blood glucose level was done on 7th and 15th day of the study. Blood samples were collected at 150 minutes (2.5 hours) from rats of each group and compared. The administration of formulation for a period of 15 days resulted in persistent significant value ($p < 0.01$) indicating maintenance of blood glucose level.

Table 1 Effect of formulations E2 and A2 on blood glucose level in alloxaninduceddiabetic rats on different days.

Group	Treatment	1 st day	7 th day	15 th day
		150 mins	150 mins	150 mins
1	Normal control	88.2	88.3	87.3
2	Diabetic control	266.4	224.2	201.3
3	Alloxan(120mg/kg, ip) + pure nateglinide (4mg/kg)	153.4	148.3	145.3
4	Alloxan (120mg/kg, ip) + E2 formulation (Equivalent to dose of drug)	226.3	185.3	163.8
5	Alloxan (120mg/kg, ip) + A2 formulation (Equivalent to dose of drug)	209.5	162.3	150.3

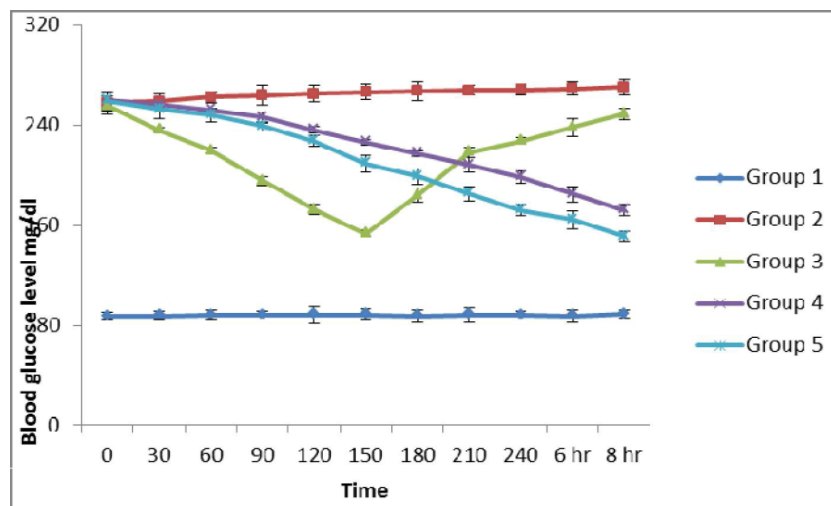


Fig 2 Blood glucose level of various groups of animals at different time interval on first day

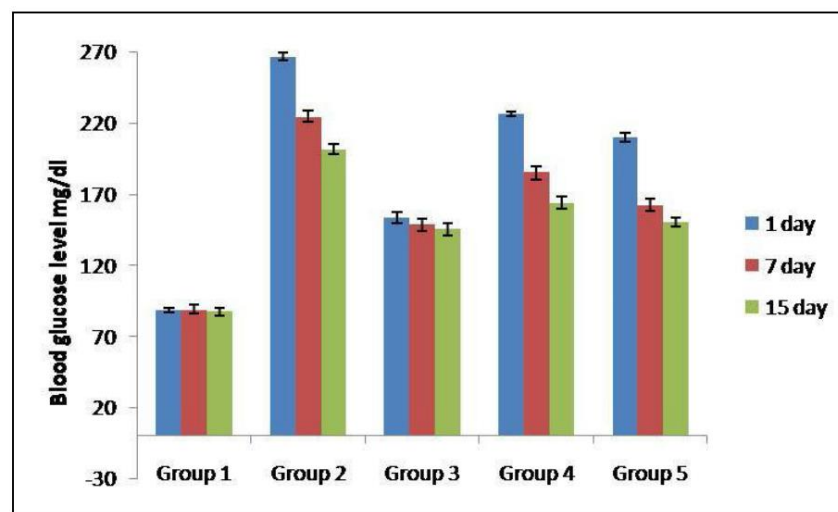


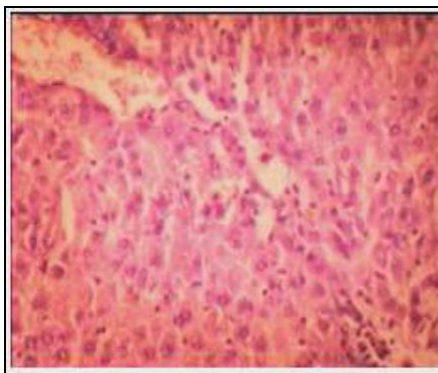
Fig 3 Comparison of the blood glucose levels of animals on three different days

Histopathological studies

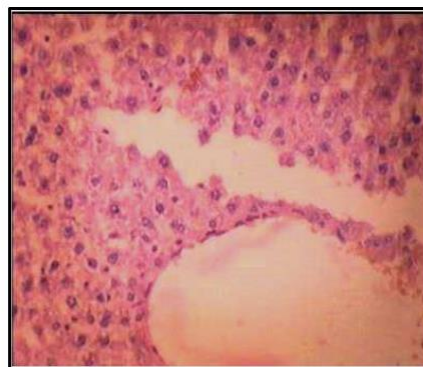
Diabetes is a chronic progressive disease characterized by hyperglycemia that leads to a deficit in insulin secretion/ action. During diabetes mellitus, alteration in the metabolism of carbohydrates, fats and proteins is observed. Various other complications associated with diabetes mellitus include neuropathy, retinopathy, nephropathy, and diabetic cataract. An assumption states that by 2025, about 8 billion people worldwide would be suffering from this disease.

Hyperglycaemia is known to cause harmful effects on the functioning of vital tissues and it seems to be necessary to evaluate the effects of antidiabetic drugs by biochemical and histopathological studies. Histopathological studies are necessary to ascertain the results after biochemical analysis. Antidiabetic activity of optimized formulation of EC and EC: HPMC 5cps

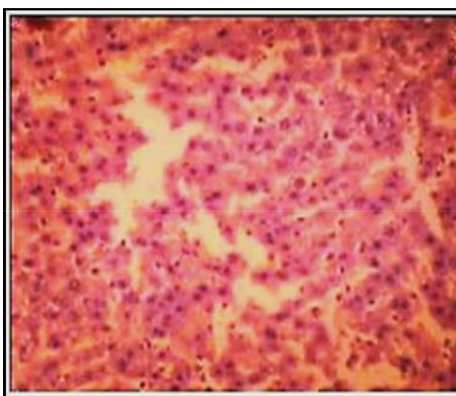
microspheres were performed by measuring the level of glucose in the blood of diabetic rats. Histopathological studies of various organs viz. liver, kidney, heart, and pancreas were performed at the end of the antidiabetic study to access the toxicological effect of this formulation on animals, if any.



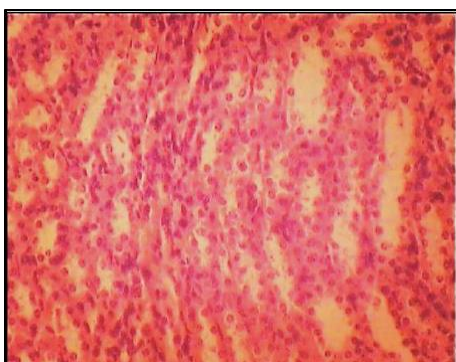
(a) Group 1: Normal control.



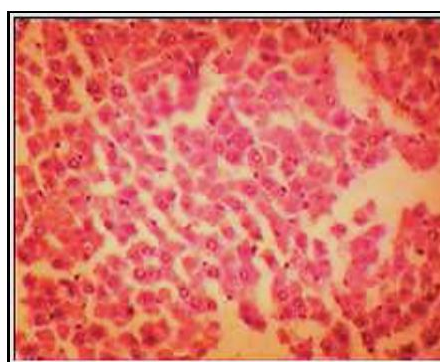
(b) Group 2: Diabetic control
(Alloxan 120mg/kg, ip)



(c) Group 3: Alloxan 120mg/kg, ip + Purenateglinide (4mg/kg)



(d) Group 4: Alloxan 120mg/kg, ip +
Formulation treated A2 (EC: HPMC)

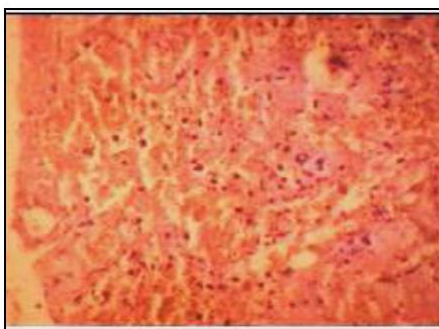


(e) Group 5: Alloxan 120mg/kg, ip +
Formulation treated E2 (EC)

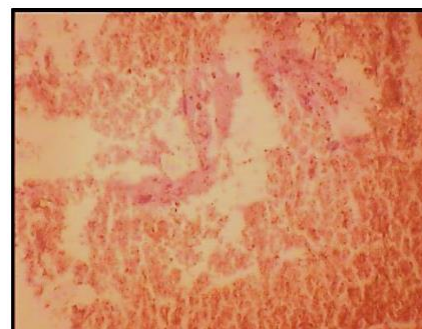
Fig.4 Liver histopathology

The liver of the control rats (Fig. 7.4a) presented typical histological organization, healthy hepatocytes containing central vein along with portal vein, matching the description of Teckman et al., 2002. The microscopic architecture is composed of hexagonal lobules, acini with well preserved cytoplasm and nucleus. The degenerative variations in the liver histology due to administration of alloxan are same as observed earlier (Shanmugasundarm et al., 1983). On the other hand, in alloxan treated section of liver, loss of the normal architecture with destruction in the cellular arrangement around the central vein was observed (Fig. 7.4 b). Due to absence of insulin, marked structural variations in liver tissue were observed such as necrosis of hepatocytes, periportal fatty infiltration, distended portal vein (PV), leucocytes inflammation with swelling of cells was seen. Section of liver of rat treated with nateglinide shows normal microvasculature and healthy hepatocytes. Improvement in histological features in liver section was observed with presence of very slight dilation of blood vessels (Fig. 4 c).

Normal cellular arrangement near the central vein and decreased necrosis was again obtained by treatment of A4 microspheres which also brought back the normal features of damaged blood vessels. No deteriorative effects due to alloxan were observed on hepatocytes apart from mild cellular inflammation (Fig. 4 d). Administration of E2 microspheres (EC alone) in rats also demonstrated similar non-lethal effects on hepatic histo-architecture. There was a mild degree of fat accumulation near hepatocytes with mild cellular swelling (Fig. 4 e). Reversal of damaged cells of liver for both the formulation-treated groups were similar to the drug.

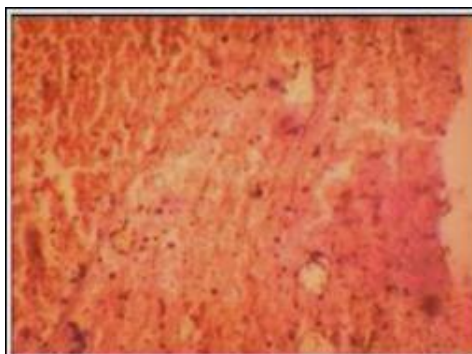


(a) Group 1: Normal control.

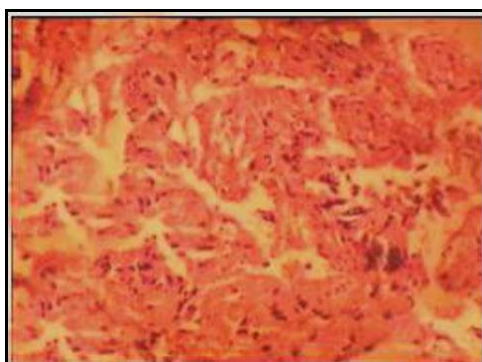


(b) Group 2: Diabetic control (Alloxan 120mg/kg,

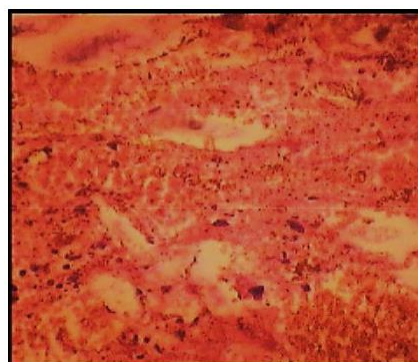
ip)



(c) Group 3: Alloxan 120mg/kg, ip + Purenateglinide (4mg/kg)



(d) Group 4: Alloxan 120mg/kg, ip +
Formulation treated A2 (EC: HPMC)



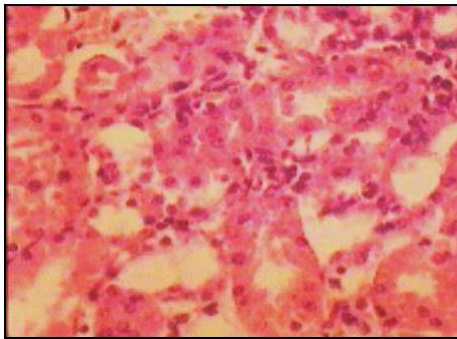
(e) Group 5: Alloxan 120mg/kg, ip +
Formulation treated E2 (EC)

Fig. 5 Pancreas histopathology

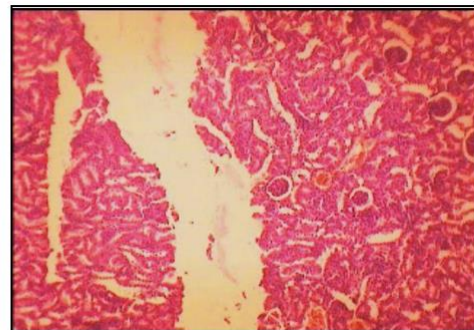
Pancreas is the principal organ for synthesis of insulin with normal proportion of islet of Langerhans surrounding the pancreatic acini containing nuclei which is perfectly prominent. Such features were observed in normal control group and is not present in alloxan induced diabetic rat (Fig. 5 a). Such observations were similar with the description of Tang et al. The cytoplasm of these cells confirming the presence of insulin granules, as described by Diani et al. (2004). Pancreas of alloxan treated rats demonstrated cellular damage, the islets have shrunk with considerable reduction in diameter and irregular shape, with a small number of cells and vacuolization of the cytoplasm (Fig. 5 b).

Nateglinide treatment caused virtually no change in cellular structure (Fig. 5 c). Such an effect is supported due to non-apoptotic effect of nateglinide over islet cells (Maedler et al., 2005). There generation of pancreatic cells by visualization of exocrine cells shows the positive effects of the drug on insulin production.

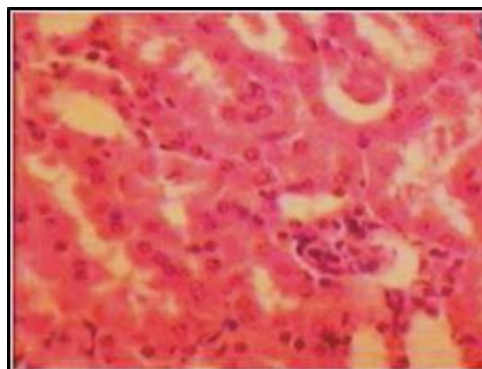
Administration of E2 formulation to rats showed pancreatic islet regeneration with slight cellular inflammation, while A2 microspheres administration showed marked improvement of the cellular injury, as evidenced from the partial restoration of islet cells, reduced β -cell damage, more symmetrical vacuoles and an increase in the number of islet cells (Fig. 5 d, e).



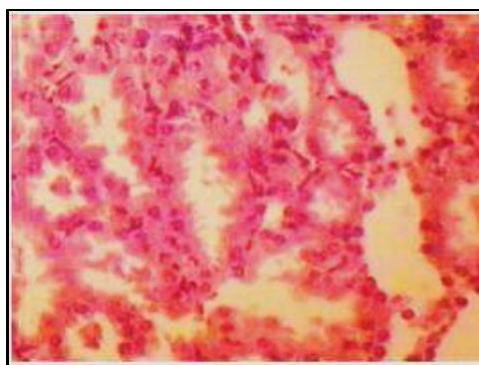
(a) Group 1: Normal control



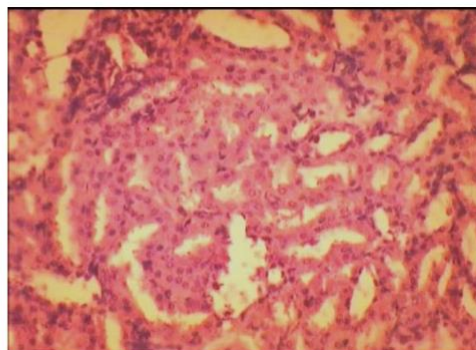
(b) Group 2: Diabetic control (Alloxan 120mg/kg, ip)



(c) Group 3: Alloxan 120mg/kg, ip + pure nateglinide (4mg/kg)



d) Group 4: Alloxan 120mg/kg, ip + Formulation treated A2 (EC: HPMC)

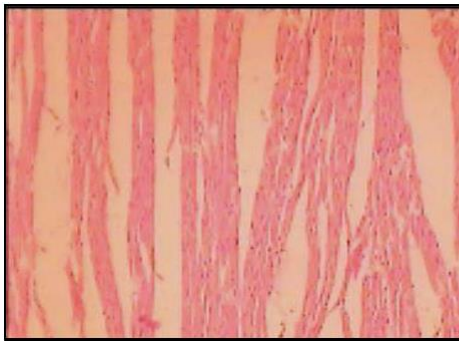


e) Group5: Alloxan 120mg/kg, ip + Formulation treated E2 (EC)

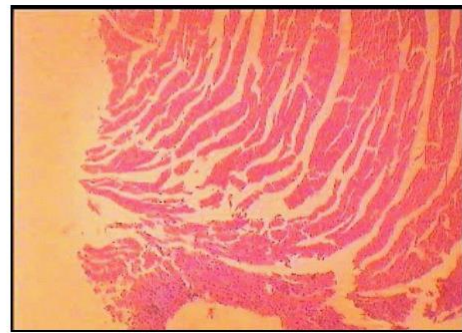
Fig. 6 Kidney histopathology

In the control group morphology of kidney (Fig.6 a) revealed the presence of healthy prominent glomeruli with normal baseline, tubules, Bowman's capsule, macula densa cell, and appearance of compact tissue.

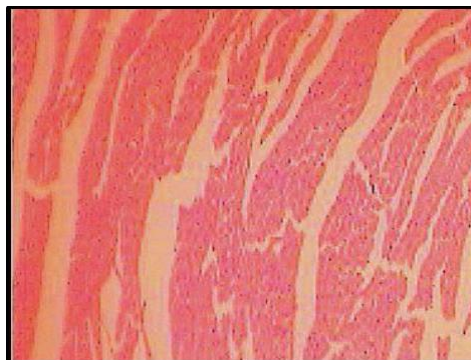
Shrinkage of glomeruli with deposition of fat on baseline and infiltration of lymphocytes were clearly observed in diabetic rats (Fig. 6 b). In diabetes, the passing of urine is common feature and is responsible for the observed structural changes in the glomerulus. These morphological changes indicate a primary effect related with hyperglycemia and causes dilatation of distal and proximal tubules in the cortex whereas the secondary effect was related to the inflammatory processes. Groups that reversal of these pathological destructions and show normal basement received nateglinide revealed membrane, and capillaries with very slight inflammation in the glomerular area (Fig.6 c). Treatment with A4 microspheres demonstrated notable improvements in kidney histo-architecture with no glomerular or tubular pathological alterations and absence of inflammation. Similarly, the administration of EC microspheres to rats aided to improve the condition as evident by only few incidences of occurrence of inflammation (Fig. 6 d, e)



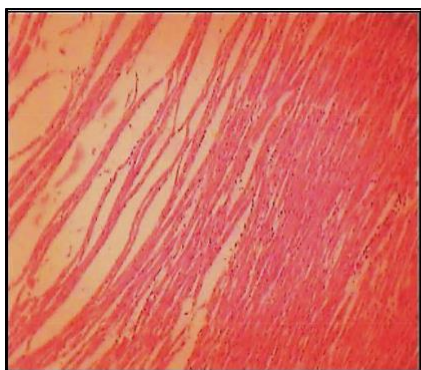
(a) Group 1: Normal control



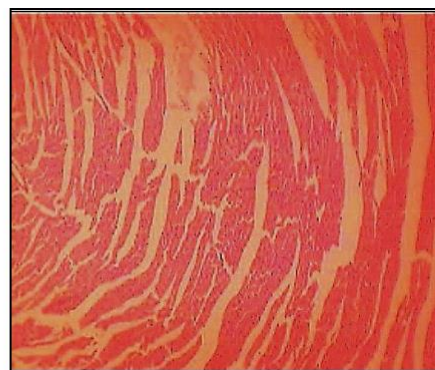
(b) Group 2: Diabetic control (Alloxan 120mg/kg, ip)



(c) Group 3: Alloxan 120mg/kg, ip + pure nateglinide (4mg/kg)



(e) Group 4: Alloxan 120mg/kg, ip +
+
Formulation treated A2 (EC: HPMC)



(f) Group 5: Alloxan 120mg/kg, ip +
Formulation treated E2 (EC)

Fig. 7 Heart histopathology

Heart tissues from normal control animals demonstrated regular arrangement of cardiac myocytes and normal striations. Cardiac myocytes contain nuclei that are prominent, single; oval-shaped, and is located centrally. In alloxan-induced diabetic rats, the histology of cardiac tissue shows disarrangement of cardiac myofibrils with deformation of nuclei present in cardiomyocytes. In this group of animals, a large infarct area of sub-endocardial necrosis with

moderate infiltration of lymphocytes and macrophages was observed. These modifications were related to a deficiency of insulin which leads to direct disruption in the cardiac myofibrils.

Cardiac tissues of alloxan-intoxicated nateglinide-treated animals demonstrated virtually no deterioration only slight cellular infiltration along with partial fragmentation of muscle fibers was seen. No noteworthy harmful effect due to treatment with A2 formulation was seen, and normal striations with well-arranged cardiac myocytes were observed apart from mild cellular inflammation. Whereas rats treated with E2 formulation demonstrated a mild degree of inflammation near cardiocytes and cellular swelling with scar formation. Thus it can be concluded that formulations exerted no harmful effect on histo-architecture and cellular morphology of vital organs of alloxanized animals.

Summary and Conclusion

The experimental target of the performed work was to design and develop floating microspheres containing significant buoyancy and dissolution properties. Four types of formulations of EC, EC: HPMC 5, 100 and 4000 cps were developed, optimized, and sequentially evaluated. Prepared formulations were suitably sized and had the best in-vitro buoyancy. Drug entrapment and product yield were high for all the formulations. The proportion of drug-polymer, speed of rotation, and emulsifier concentration affect the shape, size and other evaluated parameters during the study. Short half-life thereby fast elimination of NG makes it advantageous to be delivered through the gastro retentive system.

The cellulose polymers EC and different viscosity grades of HPMC were used to develop systems which will modify the release of NG. Owing to prolonged residence time at absorption site, enhanced bioavailability can be attained. Optimized formulations show satisfactory drug release up to 12 hours. The observed release mechanism from microspheres was diffusion and erosion controlled. The optimized formulation during stability studies does not show any variation in the physicochemical properties of microspheres.

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