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Acute toxicity studies of metformin microspheres prepared by two different methods

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ABSTRACT

Previously microspheres containing metformin hydrochloride were prepared by using two different methods viz. ionotropic gelation and solvent evaporation method. In this study, acute toxicity studies were investigated in male albino rats following oral administration of the microspheres. The rats were carefully observed for any clinical signs for seven days, and gross observations of the organs were performed at 14 days. The blood glucose level was sustained to normal values till 12 h with microspheres using carbopol 934P polymer (C3, prepared by using ionotropic gelation method) and the chitosan microspheres (CH3, prepared by using non-aqueous solvent evaporation method). No toxic symptoms or mortality were observed for all the formulations up to dose 2 g/kg body weight. Treatment with all formulations for fourteen days significantly attenuated ($p<0.01$) the elevated total cholesterol and triglyceride levels in comparison with the vehicle treated diabetic rats.

Keywords: Microspheres, metformin hydrochloride, drug delivery, toxicity

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INTRODUCTION

Diabetes is major causes of death and disability in the world. The latest World Health Organization (WHO) estimate for the number of people with diabetes worldwide, in 2000, is 171 million, which is likely to be at least 366 million by 2030. The focus of medical community is on the prevention and treatment of the disease, as is evident from the rising number of research papers on the subject [1]. Microencapsulation is an accepted process used to achieve controlled release and drug targeting for many years [2].

Metformin hydrochloride (MTF) is an antidiabetic drug used to treat Non-insulin dependent diabetic mellitus, NIDDM [3] and is indicated as an adjunct to diet to lower blood glucose in cases where hyperglycemia cannot be controlled satisfactorily on diet alone. MTF has got a short half-life of about two hours. The selected multi-unit MTF microspheric drug delivery system in previous studies is expected to provide clinicians with a new choice of an economical, safe and more bioavailable formulation in the management of type II diabetes mellitus. Although much literature is available on the *in-vitro* studies of metformin microspheres [4-5], comparatively less work has been done on assessing the *in-vivo* activity of the prepared microspheres [6]. Therefore, an attempt is made to assess the *in-vivo* studies of the prepared microspheres containing metformin hydrochloride on experimental animals.

MATERIALS AND METHODS

MTF was a gift sample from Sun Pharmaceuticals, Baroda. Sodium alginate (CDH, New Delhi), Calcium chloride (Qualigens, Mumbai), diSodium hydrogen phosphate (CDH, New Delhi), potassium dihydrogen phosphate (CDH, New Delhi), NaOH (Merck Ltd, Mumbai), Hydrochloric acid (Merck Ltd, Mumbai), Sodium Chloride (Merck Ltd, Mumbai), Acetone and Liquid paraffin (Merck Ltd, Mumbai), Ethylcellulose was commercially obtained from S.D. Fine Chemicals, Mumbai. Hydroxypropylmethylcellulose (HPMC-E15) and Carbopol-934P was procured from Central Drug House, Mumbai. Chitosan (medium viscosity grade) was procured from Central Institute of Fisheries Technology, Cochin.

Glutaraldehyde from Spectrochem Pvt. Ltd (Mumbai), Streptozotocin was purchased from Sigma Chemicals (Germany). All other reagents and solvents used in experiment were of pharmaceutical or analytical grade and purchased from their respective commercial sources.

Test Animals

Randomly bred male albino rats of Wistar strain, weighing about 250-300 g were purchased from Animal facility division, Defence Research and Development Establishment (DRDE) and used for the study. The animals were maintained in the animal colony of IPS, College of Pharmacy and further used for the experiments. Rats were allowed to acclimatize to the experimental room conditions for a period of seven days prior to pre-exposure examination for gross motor activity and appearance. During the acclimatization period, the rats were observed for clinical signs of disease. Prior to the experiment, a detailed physical examination was performed on the animals.

The animals were maintained in an environment-controlled room. They were housed in rooms maintained at a temperature of 25 ± 2°C with humidity at 50-55% throughout the experimental period. The experimental room temperature and humidity were monitored daily. A photoperiod of 12 h light and 12 h dark was maintained throughout the duration of experiment. They were housed in polypropylene cages, four per cage, with dry and dust-free rice husk as the bedding material. The rice husk was changed every alternate day. They were provided commercial pellet feed (Lipton feed, Bombay, India) and water *ad libitum* during the experimental period.

The care and maintenance of animals were as per the approved guidelines of the “Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), India (No. 1039/AC/07/CPCSEA). All animal procedures were approved by the Ethical Committee of the Establishment.

Preparation of microspheres

Metformin microspheres were prepared by using ionotropic gelation method and solvent evaporation method, respectively in our previous studies [7-8] using different polymers such as ethylcellulose, HPMC, carbopol 934P and chitosan. A comparative release profile for microspheres (at 1:3 alginate: polymer concentration) produced by using ionotropic gelation method showed that the formulation containing alginate: carbopol 934P (1:3) gave the most sustained effect [7]. Drug release rate at 1:3 drug: polymer for microspheres produced by solvent evaporation method at a stirring rate of 1200 rpm was in the following order: carbopol 934P > HPMC > ethyl cellulose > chitosan [8].

Induction of Diabetes

Experimental diabetes was induced in the rats using streptozotocin (60 mg/kg). Streptozotocin was dissolved in 0.5 ml of citrate buffer (pH 4.5) and injected intraperitoneally into rats that had been fasted overnight but with access to water *ad libitum.* The extent of diabetic induction was monitored and based on blood glucose levels and weight decrease. Blood glucose levels greater than 180 mg/dl were accepted as the basal level for diabetes. This blood glucose level was achieved after 2-4 day of treatment with streptozotocin.

Acute toxicity evaluation in rats

The selected formulations from both the methods used for the preparation of microspheres were tested for acute toxicity (if any) in rats. To determine the acute toxicity of a single oral administration, different doses of the formulation (0.5, 1.0, 1.5 and 2.0 g/kg body weight) were administered to different groups of rats (six rats were used for each group). Mortality and general behavior of the animals were observed continuously for the initial four hours

and intermittently for the next six hours and then again at 24 hrs and 48 hrs following dose administration. The parameters observed were grooming, hyperactivity, sedation, loss of righting reflex, respiratory rate and convulsions.

Antidiabetic activity evaluation

Diabetes was induced in albino rats with administration of single dose of streptozotocin (60 mg/kg body weight, i.p.). After 72 hrs, the animals with fasting blood glucose level > 180 mg/dl were considered diabetic. The diabetic rats were randomly divided into eleven groups containing six rats in each group. One group of six rats was kept without streptozotocin administration as normal control. The groups were made as follows:

Group I: Normal, receive normal rat feed and water, ad libitum
Group II: Diabetic control, receive streptozotocin 60 mg/kg body weight, with normal rat feed and water, ad libitum
Group III: Diabetic standard, treated with Metformin (500 mg/kg, p.o.)
Group IV: Diabetic test, treated with the microspheric formulation A3
Group V: Diabetic test, treated with the microspheric formulation B3
Group VI: Diabetic test, treated with the microspheric formulation C3
Group VII: Diabetic test, treated with the microspheric formulation D3
Group VIII: Diabetic test, treated with the microspheric formulation E3
Group IX: Diabetic test, treated with the microspheric formulation H3
Group X: Diabetic test, treated with the microspheric formulation CA3
Group XI: Diabetic test, treated with the microspheric formulation CH3

Biochemical parameters

After the completion of 14 days, blood samples were collected from retro orbital sinus in micro-centrifuge tube presaturated with heparin. It was centrifuged at 3000 rpm for 20 min; the serum thus obtained was separated and used immediately for the further estimation [9]. The following biochemical parameters such as total protein, total cholesterol and triglyceride levels were studied using diagnostic kits (Merck Limited, Mumbai).

Statistical analysis

The data obtained in present investigation was subjected to statistical analysis. All results were expressed as Mean± S.D. The data were analyzed using Analysis of variance (ANOVA) and the group means were compared by Student-Newman Keule's test. Values were considered statistically significant when $p<0.01$. Graph Pad Instat 3 was used for the analysis of data.

RESULTS AND DISCUSSION

***In-vivo* studies of microspheres**

Based on the *in-vitro* release profile for the selected formulations namely, A3, B3, C3 and D3 (prepared by ionotropic gelation method) and E3, H3, CA3 and CH3 (prepared by non-aqueous solvent evaporation method) were studied for their *in-vivo* effect (Table 1). Intravenous injection of 60 mg/kg of Streptozotocin in male adult Wistar rats, makes pancreas swell and at last causes degeneration in Langerhans islet beta cells and induces experimental diabetes mellitus in 2-4 days.

Table 1: Composition of metformin microspheres prepared using ionotropic gelation method (A3, B3, C3, D3) and solvent evaporation method (E3, H3, CA3, CH3).

Code	Drug: Polymer	Method
A3	Drug:Alginate:ethylcellulose (1:1:3)	Ionotropic gelation method
B3	Drug:Alginate:HPMC (1:1:3)	Ionotropic gelation method
C3	Drug:Alginate:carbopol 934P (1:1:3)	Ionotropic gelation method
D3	Drug:Alginate:chitosan (1:1:3)	Ionotropic gelation method
E3	Drug: ethylcellulose (1:3)	Solvent evaporation method
H3	Drug: HPMC (1:3)	Solvent evaporation method
CA3	Drug: carbopol 934P (1:3)	Solvent evaporation method
CH3	Drug: chitosan (1:3)	Solvent evaporation method

Key: *HPMC denotes hydroxypropyl methylcellulose, Drug used in the study is metformin hydrochloride*

Induction of experimental diabetes mellitus in male adult rats weighing 250-300 g is indeed the first step in decreasing Nicotinamide-adenine dinucleotide (NAD) in pancreas islet beta cells and causes histopathological

effects in beta cells which probably intermediates diabetes induction [10]. The use of Streptozotocin for diabetes induction was done as it is claimed to have advantages of greater specificity and lower toxicity over alloxan [11]. The weight and blood glucose level changes in the rats treated with 60 mg/kg body weight of streptozotocin were significant. This is a good way of assessing the extent of diabetes induction in the animals and this approach has been described by other workers [12].

Acute toxicity studies

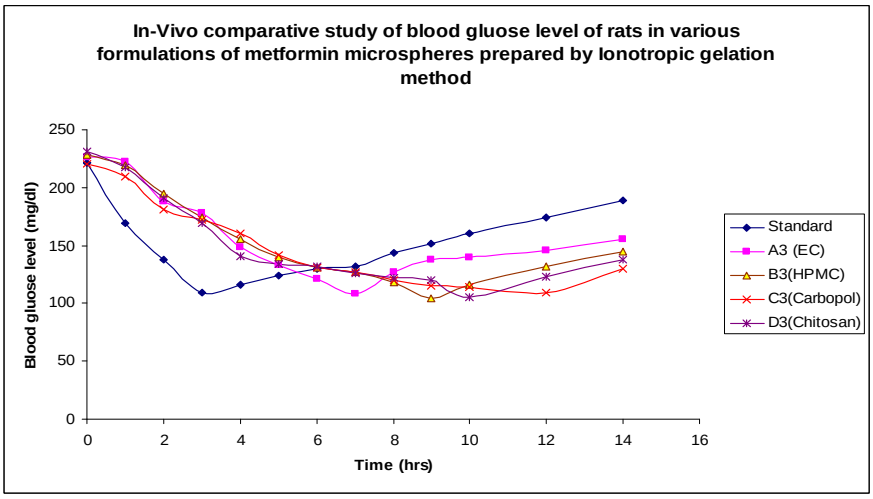
In acute toxicity study no toxic symptoms were observed for all the formulations up to dose 2 g/kg body weight. All animals behaved normally. No neurological or behavioral effects could be noted. No mortality was found up to 14 days study. Streptozotocin (60 mg/kg) administration resulted in significant reduction in body weight. Administration of various microspheric formulations at equivalent dose of 500 mg/kg administered were able to correct this aberration significantly ($p<0.01$). The change in body weight observed is represented in Table- 2.

Effect on blood glucose

Streptozotocin (60 mg/kg) administration resulted in significant elevation of glucose level. *In-vivo* evaluation of the selected formulations of metformin microspheres prepared by both the methods i.e. ionotropic gelation and non-aqueous solvent evaporation method were carried out in healthy normal male Wistar rats by measuring the hypoglycemic effect produced after their oral administration at a dose equivalent to 500 mg/kg of metformin in comparison with pure metformin hydrochloride at the same dose. When pure metformin was administered, a rapid reduction in blood glucose level was observed within 3 h which rapidly reached to the diabetic level requiring further dosing.

In case of microspheres prepared by the ionotropic gelation method, the reduction in blood glucose levels was slower which sustained over longer periods of time. Maximum reduction was observed with microspheres using carbopol 934P polymer (Figure- 1) whereas the blood glucose level was sustained to normal values till 12 h in case of the chitosan microspheres prepared by using non-aqueous solvent evaporation method (Figure- 2). The sustained hypoglycemic effect observed over longer period of time was due to the retardant effect of the polymers used in the microspheres.

Figure- 1



Effect on biochemical parameters

Diabetes leads to various metabolic aberrations in the animals namely increase blood glucose, decreased protein content and increased level of cholesterol and triglyceride. There was a marked decrease in the plasma protein content of untreated diabetic group ($p<0.01$) when compared with that of the control. All the selected formulations were able to correct this metabolic disturbance significantly. Total cholesterol and triglyceride levels were found to be significantly ($p<0.01$) increased in the vehicle treated diabetic group in comparison with the control group.

Treatment with all formulations for fourteen days significantly attenuated ($p<0.01$) the elevated total cholesterol and triglyceride levels in comparison with the vehicle treated diabetic rats given in Table- 2, Figure- 3.

Figure- 2

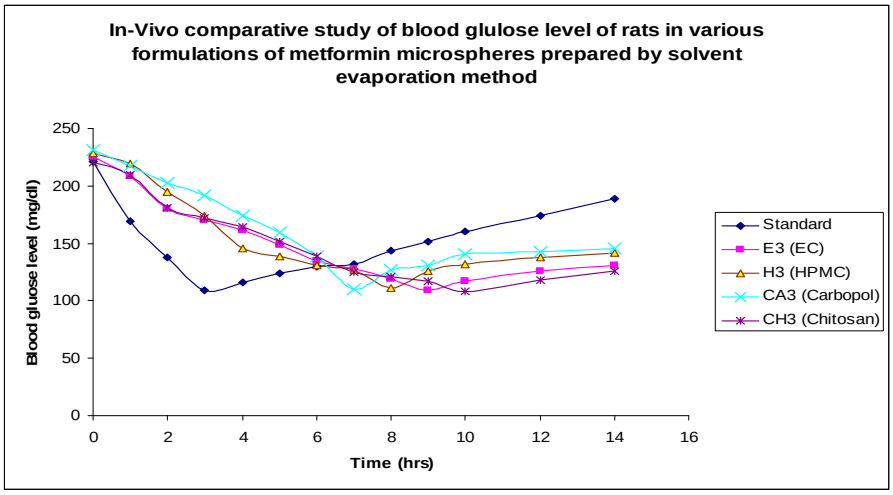


Figure- 3

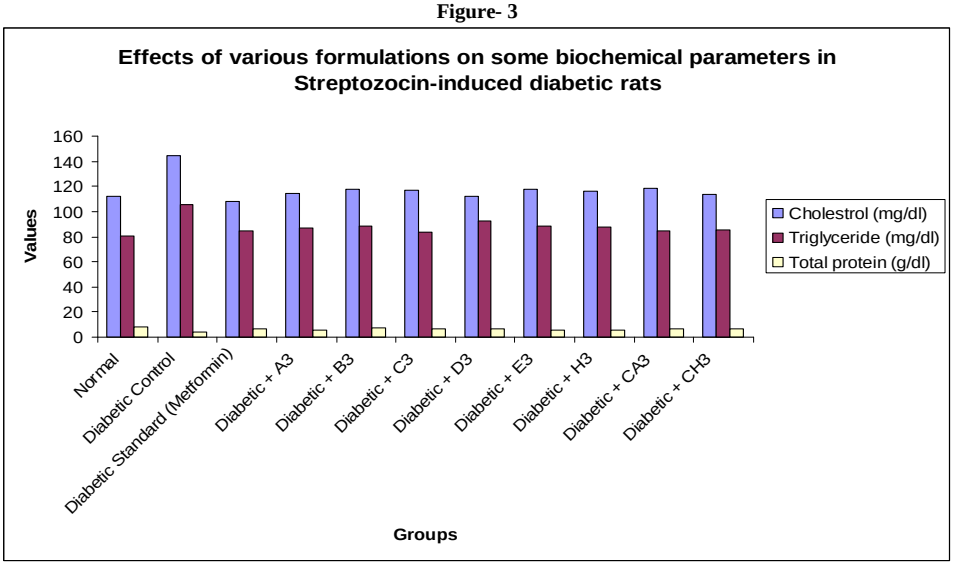


Table 2: Effects of various formulations biochemical parameters and change in body weight in Streptozocin-induced diabetic rats

S No.	Group (S)	Cholesterol (mg/dl)	Triglyceride (mg/dl)	Total protein (g/dl)	Change in body weight (g)
1	Normal	111.69 ± 5.32	80.52 ± 2.78	7.79 ± 0.48	+ 20.65
2	Diabetic Control	144.7 ± 5.02	105.7 ± 4.35	4.02 ± 0.24	- 33.75
3	Diabetic Standard (Metformin)	108.06 ± 3.1	84.35 ± 0.01	6.59 ± 0.13	+ 28.54
4	Diabetic + A3	114.4 ± 5.85	86.7 ± 0.28	5.51 ± 0.23	+ 10.12
5	Diabetic + B3	117.7 ± 4.57	88.5 ± 0.01	6.97 ± 0.12	+ 11.34
6	Diabetic + C3	116.9 ± 7.27	84.06 ± 0.02	6.4 ± 0.64	+ 14.85
7	Diabetic + D3	112.1 ± 8.13	92.4 ± 0.03	6.5 ± 0.44	+ 11.86
8	Diabetic + E3	117.9 ± 3.87	88.4± 0.12	5.73 ± 0.32	+ 14.91
9	Diabetic + H3	116.2 ± 5.87	87.5± 0.63	5.82 ± 0.52	+ 11.43
10	Diabetic + CA3	118.3 ± 3.19	84.6 ± 0.03	6.27 ± 0.27	+ 14.36
11	Diabetic + CH3	113.74 ± 0.6	85.6 ± 0.06	6.16 ± 0.25	+ 11.24

Values are given as Mean ±S.D.

CONCLUSION

The microspheres of metformin hydrochloride prepared by using ionotropic gelation and solvent evaporation method were capable to lower the blood glucose level for 12 h. The sustained hypoglycemic effect observed over longer period of time was due to the retardant effect of the polymers used in the microspheres. Microspheres using carbopol 934P polymer and chitosan demonstrated significant blood glucose reduction. Thus, the microspheres produced by both methods were not only promising for the sustained oral delivery of metformin hydrochloride *in-vitro* but also produced sufficient hypoglycemic effect in experimental animals.

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