ORIGINAL ARTICLE

BIOLOGICAL EVALUATION OF CHITOSAN-ANTIDIABETIC DRUG FORMULATIONS FOR THE TREATMENT OF DIABETES MELLITUS

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Abstract

The aim of this study was to investigate the biological effects of some chitosan-antidiabetic drug formulations on diabetes mellitus induced with streptozotocin to rats. The biochemical parameters such as glycaemia, glycosylated haemoglobin and hepatic, renal and lipid metabolism parameters were investigated. The food and water intake and body weight were also monitored as clinical parameters. The results showed that chitosan-antidiabetic drug formulations (chitosan-metformin: CS-M, chitosan-glibenclamide: CS-G, chitosan-metformin-glibenclamide: CS-MG) improved the biochemical and clinical parameters of diabetic rats which shows the beneficial effects of chitosan for diabetes mellitus treatment. The best results were obtained with CS-MG for which there were recorded the best hypoglycaemic effect in comparison with CS-M and CS-G. This polymeric system also showed good values for blood glycosylated haemoglobin (HbA1c), biochemical liver and renal function parameters: ALT, AST, LDH, total bilirubin, direct bilirubin and urea, creatinine and uric acid respectively.

Rezumat

Scopul acestui studiu a fost de a evalua efectele biologice ale unor formulări chitosan-medicament antidiabetic asupra diabetului zaharat indus cu streptozotocin la şobolan. Au fost investigaţi o serie de parametri biochimici şi anume: glicemia, hemoglobina glicozilată, precum şi parametrii funcţiei hepatice, renale şi ai metabolismului lipidic. Consumul de apă şi hrană precum şi greutatea corporală au fost de asemenea monitorizate ca şi parametrii clinici. Rezultatele au arătat că formulările chitosan-medicament antidiabetic (chitosan-metformin: CS-M, chitosan-glibenclamidă: CS-G, chitosan-metformin-glibenclamidă: CS-MG) au îmbunătăţit la şobolanii diabetici parametrii biochimici şi clinici, ceea ce arată efectele benefice ale chitosanului în tratamentul diabetului zaharat. Cele mai bune rezultate s-au obţinut cu formularea CS-MG, pentru care s-a înregistrat cel mai bun efect hipoglicemic în comparaţie cu CS-M Şi CS-G. Acest sistem polimeric a prezentat totodată valori bune şi pentru hemoglobina glicozilată (HbA1c) precum şi pentru parametrii funcţiei hepatice şi renale: ALT, AST, LDH, bilirubina totală, bilirubina directă şi respectiv uree, creatină şi acid uric.

Keywords: diabetes mellitus, chitosan, antidiabetic drugs, biochemical parameters, clinical parameters

Introduction

Diabetes mellitus (DM) is a one of the major diseases in the world that affects more than 8% of adults meaning approximately 380 million people. It is estimate that in less than 20 years the number of people with DM will reach 600 million [2]. The cost of DM treatment is huge and more than half is spent for its complications. This disease is associated with increased morbidity linked to blindness, renal failure, atherosclerotic vascular diseases and lower limb amputation [13, 14]. The glycaemic control is the main focus in the management of DM and although there are several hypoglycaemic agents in the current therapy, the treatment is far from ideal [12, 13]. Often the patients under treatment with antidiabetic drugs are unable to have optimal glycaemic control due to the side effects of drugs or severe hypoglycaemia [2, 4]. For example, up to 2.5% and 17.5% of sulfonylurea treated patients experience major and minor hypoglycaemia, respectively, while gastrointestinal problems affect up to 63% of metformin, 36% of thiazolidinedione, and 30% of acarbose treated patients [2]. Peripheral oedema is observed in up to 26% of thiazolidinedione treated patients and body weight gain of 1 to 5 kg are common with both sulfonylurea and thiazolidinedione therapy [5]. In order to improve the pharmacokinetic and safety profile of current antidiabetic drugs, new

polymeric systems based on chitosan-metformin-glibenclamide have been developed and characterized by our research team [1]. Chitosan is one of the most used biopolymers in drug delivery due its specific properties such as biodegradability, low toxicity, low immunogenicity and good biocompatibility [11]. Moreover, scientific data support chitosan for its important biological effects such as hypo-bilirubinemia and hypocholesterolemic effects, antacid and anti-ulcer activities, wound and burn healing properties, anti-inflammatory and neuroprotective effect [7]. Also, recent studies attribute to chitosan antioxidant and antidiabetic effects that could be very useful in the management of DM [6].

The aim of this study was to evaluate the hypo-glycaemic effect, the effect on hepatic and renal function as well as the effect on lipid metabolism of new chitosan-metformin-glibenclamide polymeric systems using type 2 diabetes mellitus rat model induced by streptozotocin.

Materials and Methods

Materials. *S*treptozotocin (SZT), metformin hydro-chloride, glibenclamide, sodium citrate buffer (pH 4.5) were purchased from Sigma Aldrich. Male Wistar rats were purchased from the Biobase of "Grigore T. Popa" University of Medicine and Pharmacy, Iaşi, Romania. The reagents used for assessing the biochemical parameters were purchased from Sigma Aldrich. Chitosan microparticles loaded with anti-diabetic drug (CS-M, CS-G, CG-MG) were developed within the laboratory of Pharmaceutical Chemistry, Faculty of Pharmacy, Iaşi, Romania.

Type 2 diabetes mellitus model. Type 2 diabetes mellitus was induced by streptozotocin according to the protocol described in the literature [9]. Male Wistar rats weighing 150 - 200 g were housed at 24 ± 2ºC, humidity of 40 - 70%, with natural day-night cycle, having *ad libitum* access to food and water, except the day of the experiment, when animals were used after 24 h fasting. A single dose of streptozotocin 65 mg/kg body weight (b.w.) as a solution of 30 mg/mL in citrate buffer (0.01 M, pH 4.5) was intraperitoneally administered [9]. A group which received only citrate buffer solution was used as control. The blood glucose level was measured using glucometer FORA G71a, Switzerland with FORA test strips (1st, 2nd, 4th and 7th day of the experiment). For further investigations there were selected only the animals with hyperglycaemia (blood glucose level > 250 mg/dL).

Experimental design. The diabetic rats were divided into 8 groups (8 animals per group): group 1 (M) received metformin (300 mg/kg b.w.), group 2 (G) received glibenclamide (10 mg/kg b.w.), group 3 (MG) received metformin (300 mg/kg b.w.) and glibenclamide (10 mg/kg b.w.), group 4 (CS-M) received chitosan microparticles loaded with metformin (300 mg/kg b.w.), group 5 (CS-G) received chitosan microparticle loaded with glibenclamide (10 mg/kg b.w.), group 6 (CS-MG) received chitosan micro-particles loaded with metformin (300 mg/kg b.w.) and glibenclamide (10 mg/kg b.w.), group 7 (healthy control) and group 8 (diabetic control) received the vehicle (Tween 80 – 2 mg/kg b.w.). The antidiabetic drugs (M, G) and chitosan-antidiabetic drug micro-particles (CS-M, CS-G, CS-MG) were orally administered, once per day, for a period of 45 days.

Biochemical parameters. Every three days, blood samples were collected from the tail artery and blood glucose value was measured using glucometer FORA G71a, Switzerland. At the 21st day of the experiment, retro-orbital blood collection was performed and the glycosylated haemoglobin was determined using Automatic Biochemistry Analyzer, Rx Imola. At the end of the experiment, overnight fasted rats were anaesthetized using ketamine (100 mg/kg b.w. i.p.) and sacrificed by cervical dislocation. For glycosylated haemoglobin (HbA1c) the blood samples were collected on anticoagulant (EDTA) and were analysed using Sysmex XT 1800 (Sysmex EuropeGmbh) analyser [14]. The blood samples used for serum biochemical parameters were collected in blood collection tubes without anticoagulant and centrifuged (14 min at 3500 rpm) and the serum was analysed using Cormay Accent 200 (Poland) analyser [12]. The experiments were performed in agreement with the current guidelines for laboratory animals having the consent of the Ethics Committee for Animal Research of "Grigore T. Popa" University of Medicine and Pharmacy, Iaşi, Romania (no. 91/05.01.2015).

During the experiment the rats were also monitored in view of food and water intake as well as of body weight. The average of water and food intake and body weight variation was calculated for every three days. The difference between body weight at specific time and initial body weight was also noted.

Results and Discussion

Blood glucose level

Oral administration, for 45 days, of chitosan micro-particles loaded with metformin (CS-M: group 4), glibenclamide (CS-G: group 5) and metformin-glibenclamide (CS-MG: group 6) significantly reduced the blood glucose levels in reference with the diabetic rats (group 8). The effect of chitosan microparticles loaded with antidiabetic drug was also improved in reference with the standard drugs (metformin – M: group 1; glibenclamide – G: group 2; metformin-glibenclamide – MG: group 3) that showed the beneficial effect of chitosan. At the end of the experiment the best hypoglycaemic effect was recorded for chitosan-meformin-glibenclamide formulation

(CS-MG, group 6). For this polymeric system the value of the glucose level was 167 mg/dL while for CS-M (group 4) and CS-G (group 5) the values recorded were 312 mg/dL and 220 mg/dL, respectively (Figure 1).

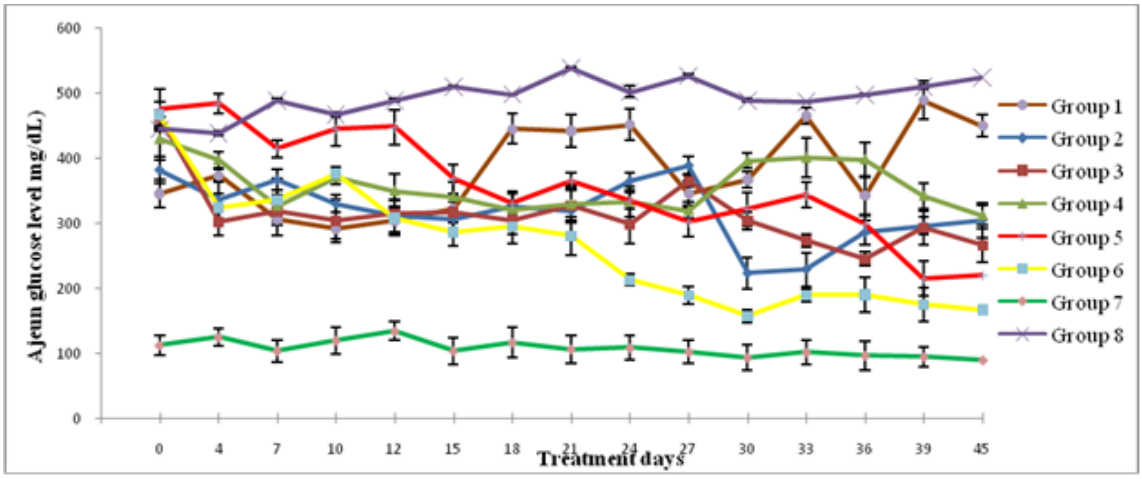

Figure 1.

Á jeun glucose levels of the diabetic rats treated with M (group 1), G (group 2), MG (group 3), CS-M (group 4), CS-G (group 5), CS-MG (group 6) in reference with non-diabetic (group 7) and diabetic rats (group 8)

Glycosylated haemoglobin level

Administration of chitosan microparticles loaded with antidiabetic drug (CS-M: group 4; CS-G: group 5 and CG-MG: group 6) was associated with a significantly reduction of the blood glycosylated haemoglobin (HbA1c) level in reference to diabetic rats (group 8) (Figure 2). It was also observed that the effect of chitosan formulations (CS-M, CS-G, CS-MG) was improved compared with the standard antidiabetic drugs (M: group 1, G: group 2 and MG: group 3). The best results were obtained with chitosan-metformin-glibenclamide formulation (CS- MG: group 6), for which the values of HbA1c recorded at the 21st day and 45th day of the experiment was 6.32% and 7.78%, respectively. In the similar conditions the values of HbA1c recorded for diabetic rats (group 8) were 11.50% and 12.07% respectively. It is also to be noted that the value of glycosylated haemoglobin recorded on 21st day of the experiment was less than the value recorded at the end of the experiment.

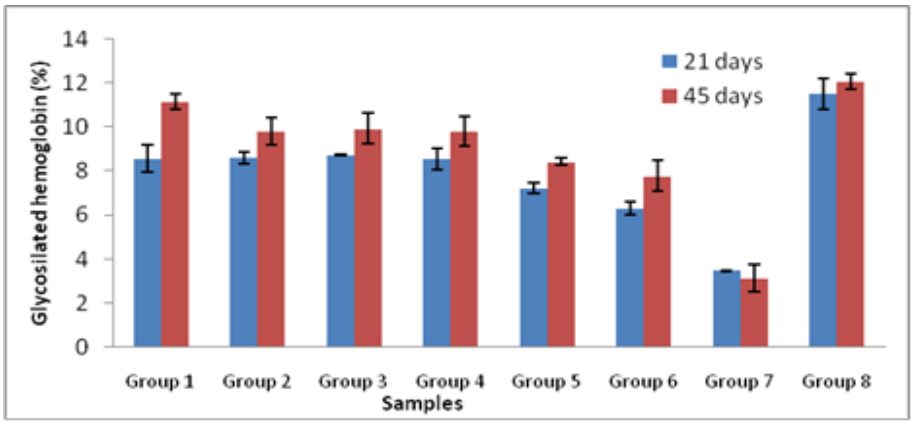

Figure 2.

The glycosylated haemoglobin (HbA1c) of diabetic rats treated with M (group 1), G (group 2), MG (group 3), CS-M (group 4), CS-G (group 5), CS-MG (group 6) in reference to the non-diabetic (group 7) and diabetic rats (group 8)

Biochemical markers of liver function

It is known that diabetes mellitus increases the risk of liver diseases such as hepatitis, steatohepatitis which can progress to cirrhosis, hepatocellular carcinoma and fulminant hepatic failure. Some antidiabetic drugs could also increase the hepatic pathologies risk. Although metformin is not metabolized by the liver and it is not commonly considered a hepatotoxin, several cases of hepatotoxicity have been reported [3]. The hepatic injury can be estimated through

biochemical markers such as alanine amino-transferase (ALT), aspartate aminotransferase (AST), lactate dehydro-genase (LDH), total and direct bilirubin levels [10]. Ours results (Table I) confirmed that diabetes mellitus induce hepatic injury, all biochemical markers recorded for diabetic rats (group 8) being higher than for the non-diabetic rats (group 7). It was also observed that chitosan reduced the hepatotoxicity of antidiabetic drugs, the values of ALT, AST and LDH for chitosan microparticles (CS-M, CS-G, CS-MG) being less than the values recorded for the antidiabetic drugs (M, G, MG), generally. The best results were obtained for CS-MG (group 6), for which the lowest values of ALT (122.3 ± 2.05 IU/L) and AST (721.2 ± 15.6 IU/L) have been recorded. These values were lower than those recorded for antidiabetic drugs (M, G, MG) and chitosan-anti-diabetic drug (CS-M, CS-G). In addition, for CS-MG, the value of LDH and total bilirubin were lower than the values recorded for metformin-glibenclamide combination (MG: group 3).

Table I

Biochemical markers used for evaluation of hepatic function

Biochemical markers	*M (group 1)*	*G (group 2)*	*MG (group 3)*	*CS-M (group 4)*
ALT (IU/L)	199.50 ± 2.52	183.50 ± 2.52	179.00 ± 2.52	155.60 ± 4.23
AST (IU/L)	1619.50 ± 13.98	1216.50 ± 13.98	1419.0 ± 13.98	1068.2 ± 26.75
LDH (IU/L)	5530.25 ± 25.89	2430.25 ± 25.89	7530.5 ± 25.89	2436.5 ± 25.71
Total bilirubin (mg/dL)	0.23 ± 0.09	0.13 ± 0.09	0.33 ± 0.09	0.69 ± 0.04
Direct bilirubin (mg/dL)	0.04 ± 0.00	0.04 ± 0.00	0.04 ± 0.00	0.04 ± 0.00
Biochemical markers	*CS-G (group 5)*	*CS-MG (group 6)*	*group 7*	*group 8*
ALT (IU/L)	162.30 ± 1.05	122.30 ± 2.05	101.10 ± 0.50	314.50 ± 4.60
AST (IU/L)	1292.80 ± 13.45	721.20 ± 15.60	239.93 ± 1.80	1688.2 ± 12.40
LDH (IU/L)	5491.00 ± 28.56	4701.90 ± 22.5	1800.36 ± 5.40	9075.3 ± 20.60
Total bilirubin (mg/dL)	0.68 ± 0.04	0.16 ± 0.03	0.34 ± 0.05	0.91 ± 0.04
Direct bilirubin (mg/dL)	0.04 ± 0.00	0.04 ± 0.00	0.04 ± 0.00	0.04 ± 0.00

M = metformin, G = glibenclamide, CS = chitosan

Biochemical markers of renal function

The renal dysfunction, especially the risk for diabetic nephropathy, was assessed using creatinine, uric acid and urea parameters. Serum creatinine, the indicator of glomerular filtration rate, is still the most frequently used biomarker of renal function. The results (Table II) showed that diabetic rats (group 8) presented increased values of urea, creatinine and uric acid in reference with the non-diabetic rats (group 7), which showed that the renal function was injured. For chitosan formulation (S-M, CS-G, CS-MG) the values of urea, creatinine and uric acid were lower than those recorded for diabetic rats (group 8) which means that the renal function of diabetic rats was improved. Also, it is important to be noted the beneficial effects of chitosan formulations in reference with the standard antidiabetic drugs (M: group 1, G: group 2 and MG: group 3).

Table II

Biochemical markers used for evaluation of renal function

Biochemical markers	*M (group 1)*	*G (group 2)*	*MG (group 3)*	*CS-M (group 4)*
Urea (mg/dL)	71.65 ± 1.48	55.24 ± 1.97	65.92 ± 1.52	67.5 ± 1.60
Creatinine (mg/dL)	0.72 ± 0.09	0.67 ± 0.05	0.55 ± 0.16	0.81 ± 0.04
Uric acid (mg/dL)	1.04 ± 0.45	1.41 ± 0.17	1.22 ± 0.72	1.55 ± 0.28
Biochemical markers	*CS-G (group 5)*	*CS-MG (group 6)*	*group 7*	*group 8*
Urea (mg/dL)	48.11 ± 1.74	46.51 ± 1.65	34.00 ± 1.41	107.00 ± 1.03
Creatinine (mg/dL)	0.56 ± 0.02	0.62 ± 0.02	0.58 ± 0.01	0.63 ± 0.01
Uric acid (mg/dL)	1.12 ± 0.31	1.16 ± 0.41	1.45 ± 0.39	4.92 ± 0.11

M = metformin, G = glibenclamide, CS = chitosan

Biochemical markers of lipid metabolism

It is known that the alteration of the lipid metabolism associated with hyperglycaemia increase the cardio-vascular risk in diabetes patients [15]. The lipid profile was assessed by estimating the serum levels of total cholesterol (TC), LDL cholesterol, HDL cholesterol and triglycerides (TG) [16]. It was observed that the total cholesterol, LDH cholesterol, HDL cholesterol and triglycerides had increased values for diabetes rats (group 8) compared to non-diabetic rats (group 7) (Table III). Antidiabetic drugs administered as own substances or chitosan formulations improved all these biochemical parameters. The best results were obtained with chitosan-metformin-glibenclamide formulation (group 6) for which the value of triglycerides (69.60 ± 1.39 mg/dL) was comparable with healthy rats (71.25 ± 0.86 mg/dL) (group 7) and 2.9 times lower than the diabetic rats

(201.11 ± 2.26 mg/dL) (group 8), which means this formulation reduce the hyperlipidaemia risk and implicitly the cardiovascular risk. For this formulation there were recorded also good values for LDH cholesterol (34.33 ± 0.35 mg/dL), HDL cholesterol (21.70 ± 0.95 mg/dL) and the total cholesterol (67.30 ± 1.65 mg/dL).

Table III

Biochemical markers used for evaluation of lipid metabolism

Biochemical markers	*M (group 1)*	*G (group 2)*	*MG (group 3)*	*CS-M (group 4)*
Total cholesterol (mg/dL)	100.50 ± 1.69	94.00 ± 1.34	89.45 ± 1.65	68.00 ± 1.96
LDL cholesterol (mg/dL)	38.50 ± 0.24	22.50 ± 1.07	19.00 ± 1.02	30.50 ± 0.25
HDL cholesterol (mg/dL)	27.20 ± 1.02	21.50 ± 0.71	24.00 ± 0.50	19.61 ± 0.96
Triglycerides (mg/dL)	120.13 ± 2.68	116.50 ± 2.67	221.80 ± 2.95	170.20 ± 1.26
Biochemical markers	*CS-G (group 5)*	*CS-MG (group 6)*	*group 7*	*group 8*
Total cholesterol (mg/dL)	62.36 ± 1.51	67.30 ± 1.65	64.00 ± 0.75	86.55 ± 1.34
LDL cholesterol (mg/dL)	25.63 ± 1.15	34.33 ± 0,35	20.50 ± 0.75	23.50 ± 0.96
HDL cholesterol (mg/dL)	29.50 ± 0.71	21.70 ± 0.95	18.00 ± 1.26	20.50 ± 0.50
Triglycerides (mg/dL)	144.15 ± 2.45	69.60 ± 1.39	71.25 ± 0.86	201.11 ± 2.26

M = metformin, G = glibenclamide, CS = chitosan

Body weight, food and water intake evaluation

During the experiment, the intake of food and water was increased for diabetic rats due the polyuria and polyphagia which characterize this disease. The excess of blood glucose will be eliminated through urine and consequently the water intake will be increased. For diabetic rats (group 8) the water intake was elevated from the start of the experiment (582.14 ± 14.15 mL/kg b.w./24 h) and it remained at the fairly constant level throughout the experiment. The antidiabetic drugs (M, G, MG) and the chitosan formulations (CS-M, CS-G, CS-MG) reduced the water intake compared with the diabetic rats (group 8), the most improvement being observed for CS-MG formulation (group 6). In this case the water intake was higher at the beginning (556.78 ± 22.12 mL/kg b.w./24 h) but it reduced during the experiment, so at the end the water intake was 336.78 ± 26.32 mL/kg b.w./24 h (Figure 3). The antidiabetic drugs and chitosan formulations have also reduced the food intake (Figure 4).

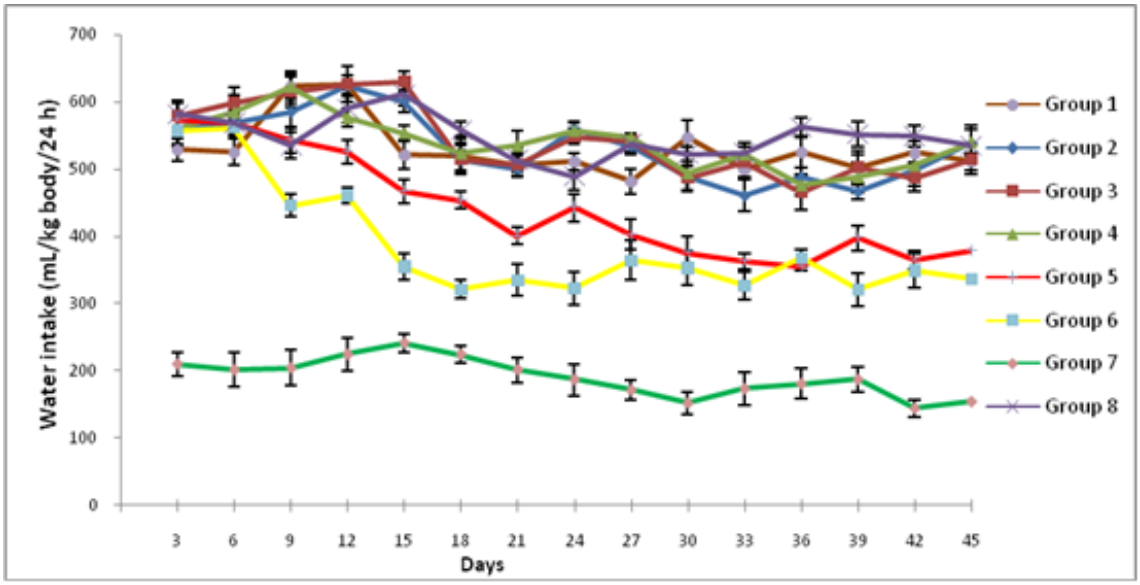

Figure 3.

The water intake of rats during the experiment

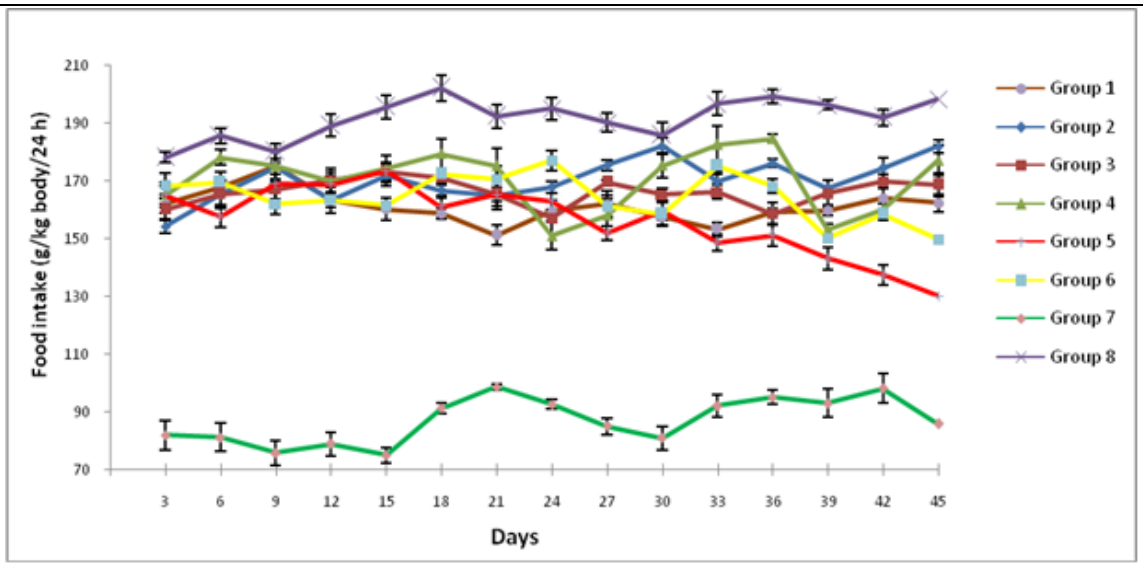

Figure 4.
The food intake of rats during the experiment

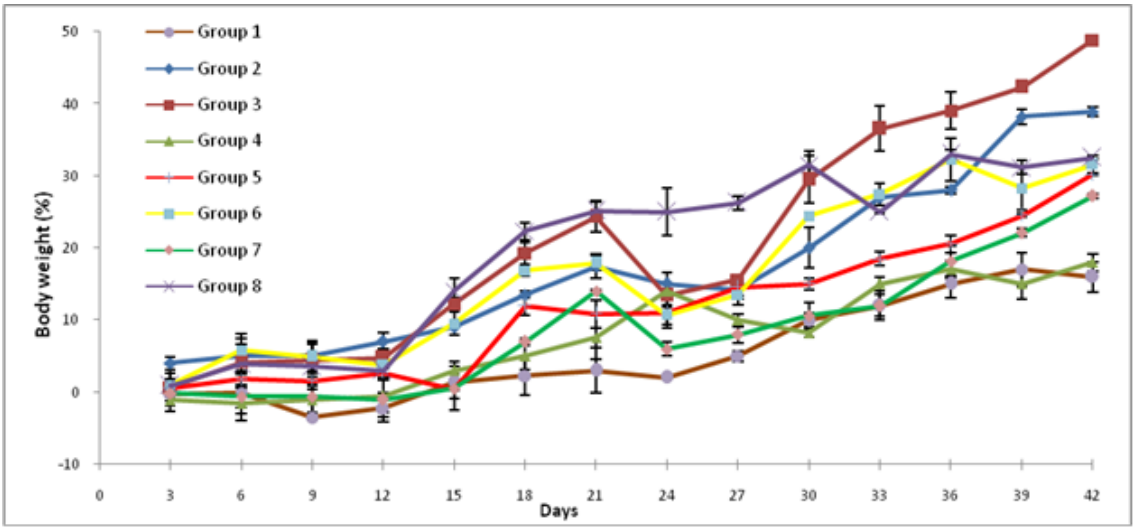

Figure 5.
The rats' body weight monitorization during the experiment

It is known that antidiabetic drugs increase the body weight. In our experiment it was observed that chitosan formulations (CS-M: group 4; CS-G: group 5; CS-MG: group 6) reduced the body weight in reference with diabetic rats (group 8) and also compared with standard antidiabetic drugs (G: group 2 and MG: group 3) (Figure 5).
Our results support the beneficial effect of chitosan to diabetes mellitus and are in agreement with other scientific data that proved the antidiabetic effects of chitosan oligosaccharide [17] as well as the effect of chitosan to reduce the weight and blood lipids to obese patients [8].

Conclusions

The biological effects of chitosan-antidiabetic drug formulations have been performed. The best results were obtained with chitosan microparticles loaded with metformin and glibenclamide (CS-MG). This formulation showed improved antidiabetic effects measured as blood glucose and glycosylated haemoglobin values in comparison with standard antidiabetic drugs (metformin and glibenclamide) and their chitosan formulations (CS-M, CS-G). This polymeric system showed also improved effect on hepatic and renal function as well as on lipid metabolism injured in diabetes mellitus conditions.

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