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COMPARATIVE STUDY OF ANTIDIABETIC ACTIVITY OF ZINC OXIDE NANOPARTICLES AND GLIPIZIDE IN STREPTOZOTOCINE INDUCED DIABETIC WISTAR RATS

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ABSTRACT

Background: Nanopharmacology has exceeded the pace of pharmacological and toxicological research on the effects of nanoparticles in the biological environment. The behavior of the Pharmacological parameters of nanoparticle in biological systems still require much investigation before they can be effectively utilized in human treatment paradigms. **Aims:** To compare safety and anti-diabetic activity of Glipizide and ZnONP in Streptozotocin - induced diabetic Wistar rats. **Settings and Design**: Total 36 adult, male wistar rats of comparable developmental age and weight; were incorporated in the study in six groups. Induction of Type 2 DM was done in 30 animals by administration of Streptozotocin 45 mg/kg subcutaneously. **Methods and Material:** Group one and two animals were administered with normal saline and group three was administered with Glipizide at a dose of 5 mg/kg/day/animal. Group four, five and six received ZnONP at a dose of 10 mg/kg, 50 mg/kg and 100 mg/kg respectively/day per animal. Group six also received Glipizide at a dose of 5 mg kg/day/animal. Animal were treated till 28 days and then blood samples were tested for hematology, biochemistry. Selected organs were sent for histopathology. **Statistical analysis used:** ANOVA. **Results:** There is additional weight reduction effect of ZnONP with higher doses of ZnONP more anti-obesity activity. Persistently low BSL were achieved by group six which received both

ZnONP and Glipizide as treatment and Group four which received ZnONP (10 mg/kg) only shows steady fall in BSL levels. Combination of ZnONP (100 mg/kg) with Glipizide has maximum reduction in BSL indicating combination has achieved highest glycemic control as compared. All groups receiving ZnONP as a treatment have shown almost similar smooth glycemic control on OGTT avoiding acute hypoglycemic or hyperglycemic episodes. **Conclusions**: ZnONPs have affinity towards pancreatic tissue and ZnONP (< 50 nm) show antidiabetic activity at graded concentration. When administered along with oral antidiabetic drug, ZnONP have shown added antidiabetic effect.

KEYWORDS: Antidiabetic, Zinc oxide, Nanoparticles

INTRODUCTION

The field of nanotechnology is rapidly expanding with the development of novel nano-pharmaceuticals that have potential for revolutionizing medical treatment. The rapid pace of expansion in this field has exceeded the pace of pharmacological and toxicological research on the effects of nanoparticles in the biological environment.[1] Current standards in biomedicine and environmental contamination define nanoparticles as being <100 nm in at least one dimension.[2] The behavior of the Pharmacological parameters of nanoparticle in biological systems still require much investigation before they can be effectively utilized in human treatment paradigms. Dosing parameters, absorption, distribution, metabolism and excretion require considerable further studies. In contrast to delivering a drug, which is an organic molecule, we are delivering somewhat of a discrete entity in a nanoparticle – comprised of atomic scale parts due to the quantum effects and electronic interactions that predominate at the nanoscale.[1]

Nanopharmacology is further complicated by the need to establish the behavior of nanoparticles within the traditional pharmacological parameters of absorption, distribution, metabolism and excretion (ADME). Nanoconstructs, in many cases, have limited metabolism and excretion and persist in biological systems. This poses a need to carefully examine our common ADME parameters and revise them if necessary.[1] Most of the nanomaterials submitted for preclinical evaluation either have unacceptably high toxicities during in vitro or in vivo testing, or fail to meet the minimum criteria for bioavailability according to their adsorption, distribution, metabolism, and excretion (ADME) profile. Nevertheless, it is becoming clear that nanoengineered pharmaceuticals and drug delivery platforms have issues that are distinct from those of conventional pharmaceutical ingredients.[3]

Nanotoxicology is emerging as an important branch of nanotechnology and is the study of interactions of nanostructures with biological systems to elucidate the relationship between physical and chemical properties such as, size, shape, surface, chemistry, composition, and aggregation of nanostructured materials with induction of toxic biological responses.[4] Recently, it has been realized that nanocarrier systems can cause serious harmful effects and several studies have reported harmful effects on organ systems.[5]

So, objectives of the study are

To study effects of Zinc oxide nanoparticles (ZnONP) on blood sugar levels (BSL) in Streptozotocin - induced diabetic Wistar rats using three graded dose levels.

To study effects of ZnONP on body weight of Streptozotocin - induced diabetic mellitus (DM) in Wistar rats.

To compare anti-diabetic activity of Glipizide (oral hypoglycemic agent) and ZnONP in Streptozotocin - induced diabetic Wistar rats.

MATERIALS AND METHODS

Institutional Animal Ethics Committee approval was taken. Total 36 adult, male Wistar rats of same developmental age and weight; bred at National Toxicology Centre, Pune, weighing 180- 200 gm were incorporated in the study. Induction of Type two Diabetes Mellitus was done in 30 animals by administration of Streptozotocin 45 mg/kg subcutaneously. Animals were observed for induction of Diabetes defined as achievement of BSL of 300 mg%. None of the animals required repeat dosing of Streptozotocine.

Animal Husbandry: Animals were housed in separate metabolic stainless steel cages measuring 34 x 23 x 15 cm with two animals in each cage. Cages were kept in a ventilated animal room maintained at relative humidity of 50 %, temperature of 25 - 30 C with 12 hour light/dark cycle. Distilled water and sterilized food for rats was made available ad libitum. Body weights of all animals were recorded weekly. Animals were housed according to their groups with each group containing six animals as follows.

Group 1: Normal control group (No DM, No Treatment)

Group 2: DM with control group (DM, No Treatment)

Group 3: DM with standard treatment group (DM with Glipizide Treatment)

Group 4: DM test group I (DM with ZnONP 10 mg / kg body weight /day /rat Treatment I)

Group 5: DM test group II (DM with ZnONP 50 mg / kg body weight /day /rat

Treatment II)

Group 6: DM experimental group (DM with ZnONP 100 mg / kg body weight /day /rat + OHA Treatment).

Treatment to Test and Control group: Group one and two animals were administered with Normal saline of the volume proportional to body weight. Group three was administered with Glipizide (second generation sulphonylurea) at a dose of 5 mg/kg/day/animal. Group four, five and six have received Zinc oxide nanoparticles at a dose of 10 mg/kg, 50 mg/kg and 100 mg/kg respectively per day per animal. Group six also has received Glipizide at a dose of 5 mg/kg/day/animal.

ZnONP saline suspension was prepared as follows. A stock formulation of ZnONP saline suspension was prepared in normal saline by sonication for 30 seconds The suspensions was kept on ice for 15 seconds and was sonicated again on ice for a total of three minutes at a power of 400 W. Before use, Zinc oxide NPs were diluted to desired concentrations in fresh saline. All samples were prepared under sterile conditions. Proper mixing of sample was done with vortex method before administering the dose. 100 mg mixed with sterile water to make volume 10 ml. Each of two ml administered to animal. After mixing with a vortex, a single dose of ZnO NP saline suspension was administered orally through the gavage technique.

All groups received treatment daily till 28 days with same dose and route of administration as mentioned above.

Body weight measurements were taken on 0^{th}, 7^{th}, 14^{th}, 21^{st} and 28^{th} day of the experiment. The blood samples for weakly BSL of animals of all groups were drawn from the retro-orbital plexus on 0^{th}, 7^{th}, 14^{th}, 21^{st} and 28^{th} days to observe efficacy and unwanted recovery from DM schedule as follows.

Behavior, symptoms and mortality: Neuro-behavior, body weights and mortality of animals were monitored and recorded carefully on 0^{th} and 28^{th} days after treatment. Neuro-behavioural features observed such as temperature, convulsions/tremors, lacrimation, piloerection, salivation, urination/defection, gait, mobility (depression/ anxiety), righting reflex and other significant findings. Differences in eating and drinking patterns, their physical activity and other parameters were observed.

On 28th day, blood samples were collected from the retro-orbital plexus. Serum was obtained by centrifugation at 3500 rpm for ten minutes and stored at -20 ˚C until usage. Neurobehavioural and clinical symptoms of animals studies and urine and fecal sample collected.

Heamatology and Biochemistry: One time heamatology, biochemistry and lipid profile was done at the end of study before sacrifice. Blood samples of all animals were withdrawn from retro-orbital plexus on 28th day and were subjected for blood biochemistry and heamatology. Hematological examination included Complete Blood Count. Total proteins, SGOT, SGPT, Alkaline Phosphatase (ALP), total Bilirubin, blood Urea and Creatinine was done as blood biochemistry. Total cholesterol, Triacylglycerol (TGL) and HDL – cholesterol was done as a part of lipid profile.

One time Oral Glucose Tolerance Test (OGTT) was done on 28th day of all animals at the end of study before sacrifice. OGTT was done as follows: After overnight fasting, 0 min blood samples (0.2 ml) were taken, then glucose solution (2 gm /kg of 25% w/v) was administered orally. Three more samples were taken at 30 min, 60 min and 120 min after glucose administration.

Anesthesia and necropsy: After 28 days of treatment with ZnONP saline suspension, both test and control group animal were anesthetized with Ketamine and Xylazine 1:1. All the animals of all groups were alive and well clinically. The animals were then euthanized by cervical dislocation. Photoghraphy of gross dissection of animals was done.

Organ weight/Body Weight coefficients: All major organs of both test and control group animals were weighed. After weighing the organs, the organ weight/BW coefficients of were calculated as organ weight (wet weight, mg)/BW (gm) x 100%.

All major organs were preserved after sacrifice and specimens of liver and pancreas were subjected in formaline for histopathology.

Statistical analysis- It was done using ANOVA test.

RESULTS AND OBSERVATIONS

Body weight: There was no significant difference in body weights on 0th day that is after induction of DM with Streptozotocine but before administration of test substance. There was

no significant difference in body weights till 14th day of administration of test substance. Difference in body then started appearing from 14th day (t = 4.462) between group one and group two and it continued on 21st day (t = 5.190) observation with increasing t value suggesting that difference in body weight is increased. Further the difference in body weight is persistent on 28th day between group one and group two (t = 5.423). Significant difference appeared between group one and group five (t = 2.910) and also between group one and group six (t = 4.796). The t value between group one and group six is more than group one and group five suggesting that difference in statistically more significant between group one and group six. Suggesting that higher doses of ZnO NP more anti-obesity activity.

Percentage difference (Table 1) is also seen with 10 % rise in 0th day weight in normal control group. The group four, five and six who received ZnONP as a treatment showed percentage reduction in weight of 0.10%, 1.48% and 3.69% respectively as compared to baseline on 0th day. This also showed maximum reduction in group six which received both ZnONP and Glipizide as treatment. Also higher dose of ZnONP has higher reduction in weight. Group five; which received 50 mg of ZnONP; has reduction of 1.48% as compared to that of group four (0.10%) which received 10 mg of ZnONP. Reduction in weight is more with group five which received 50 mg of ZnONP as compared to standard treatment group which received Glipizide only. This shows that is additional weight reduction effect of ZnONP.

BSL: Statistically significant difference was observed with ANOVA test on 0 day BSL when group one was compared with rest of the groups. This suggests that optimum induction of DM was achieved in all other groups as compared to group one which is no DM, no treatment group. Similarly no significant difference appeared when other groups are compared with each other suggesting post induction groups are homogenous before initiating the study. Trend of significant difference only between group one and other groups and contrary no difference when other groups compared with each other was observed on 7th, 14th, 21st and 28th day. The pattern (Graph 2) of BSL control shows that persistently low BSL levels (on all days 7th, 14th, 21st and 28th day) were achieved by group six which received both ZnONP and Glipizide as treatment as compared group three which received Glipizide only. Group four which received ZnONP (10 mg/kg) only shows steady fall in BSL levels and achieving lower BSL than group three.

Intra group comparison (Table 3) of BSL among different groups as compared to baseline 0^{th} day BSL showed that there is persistent reduction in p value in group six from 14^{th} to 21^{st} to 28^{th} day of study. On 28^{th} day of the study when all groups were compared to their baseline 0^{th} day BSL, it was found that significant intra group difference was in group three, four, five and six as compared to their respective baseline BSL levels at 0^{th} day. Further p values in group four, five and six which received higher doses of ZnONP showed statistically more significant difference as compared to group three which received Glipizide only. Group six which received ZnONP at highest dose among three ZnONP receiving groups (100 mg/kg) with Glipizide, showed p value of 0.005 which statistically highly significant compared to its baseline on 0^{th} day. There is no significant difference in group one and two as compared to their respective baseline BSL. This shows that persistently increasing difference of significance that is reduction in p value in group six indicates persistent reduction in BSL level achieving normoglycemia in DM rats due to ZnONP (100 mg/kg) with Glipizide. Also, persistently increasing difference of significance that is reduction in p value in group six as compared to groups three, four and five indicates ZnONP has antidiabetic activity which is marginally more significant than group three which received Glipizide only. And combination of ZnONP (100 mg/kg) with Glipizide has maximum reduction in BSL indicating combination has achieved highest glycemic control as compared to other groups.

Considering the trends of glycemic control over a period of two hours in Oral glucose tolerance test (Graph 4), it is observed that administration of ZnONP causes consistent glycemic control. Especially hyperglycemic effect at 60 minutes observed in group three which is administered with Glipizide only is dampened in groups three, five and six administered with ZnONP. All groups receiving ZnONP as a treatment have shown almost similar smooth glycemic control avoiding acute hypoglycemic or hyperglycemic episodes. Group four which received 10 mg/kg of ZnONP has shown lowest BSL in OGCT in all time points.

Lipid profile changes: Serial reduction in Cholesterol and TGL was also observed though statistically not significant. Statistically significant increase in HDL was observed between group one and group three ($t = 3.861$) and also between group three and group six ($t = 3.336$). HDL levels were high in all three groups who received ZnONP as test substance.

Biochemical changes: There statistically significant reduction in Urea and non significant reduction in liver enzymes like ALP, SGOT, SGPT as compared to standard treatment group.

Urea levels were more in group three as compared to group one and six with significant statistical difference. ALP levels were more in group three as compared to group one and four with significant statistical difference.

Hematology: No significant differences observed in RBCs, WBCs, Haemoglobine, Heamatocrit and Platelet concentration.

Histopathology: There were no histopathological changes in group one which was no diabetes no treatment group. Pancreas of group two with DM but no treatment showed changes like congestion, hemorrhage in exocrine and endocrine glandular section with focal degeneration all of minimal severity. Mild to moderate severity loss of beta cells of islets, Vacuuolar changes in islets were observed with reduced size of islets of Langerhans due to loss of beta cells along with degenerative and necrotic changes in Islets.

Pancreas of group three Diabetic animals receiving Glipizide which is known Antidiabetic have shown same changes but all these changes were of minimal intensity with some changes were focal.

In Pancreatic samples of animals in Group four, five and six which received graded concentration of ZnONP showed no abnormality as congestion, hemorrhages in exocrine and endocrine glandular section with no degenerative changes. Changes such as loss of *Beta cells of Islets*, vacuuolar changes in Islets, etc. or reduced size of islets of Langerhans due to loss of beta cells or degenerative and necrotic changes in Islets were seen as that of minimal intensity and mainly they were focal.

None of the specimen belonging to any group of animals showed infiltration of mononuclear cells in pancreatic interstitial tissue of pancreas. Overall histopathological lesion score in group two was moderate (3+) to mild (2+), in group three was mild (2+) to minimal (+) and in Group four, five and six was minimal (+).

Tables and Figures

Table 1: showing actual and percentage changes in weights in different groups.

Group	Weight 28th Day	Weight 0th Day	Difference	Percentage difference
NC	330	300	30	10
DC	288.7	289.5	-0.8	- 0.28
STD	252.2	245.2	-2	- 0.81
T1	278.7	279	-0.3	- 0.10
T2	286.2	290.5	-4.3	- 1.48
T3	261.2	271.2	-10	- 3.69

Table 2: showing actual and percentage changes in BSL in different groups.

	Pretreatment	Post-treatment	Difference	Percentage difference
Group 1	101.8	97.5	- 4.3	- 4.22
Group 2	392.2	374.5	- 17.7	- 4.51
Group 3	373	275.7	- 97.3	- 26.08
Group 4	372.5	258.8	- 113.7	- 30.52
Group 5	374	293.4	- 80.6	- 21.55
Group 6	371	245.8	- 125.2	- 33.75

Table 3: showing p values for intra-group comparison of BSL levels in different groups.

	BSL of 7th day	BSL of 14th day	BSL 21st day	BSL 28th day
Group 1	0.282	0.398	0.666	0.530
Group 2	0.791	0.233	0.037	0.196
Group 3	0.604	0.206	0.043	0.020
Group 4	0.921	0.179	0.068	0.017
Group 5	0.662	0.406	0.129	0.040
Group 6	0.084	0.032	0.030	0.005

Table 4: showing changes in Lipid profile in different treatment groups.

	Cholesterol	Triacylglycerol	HDL
Group 1	54.8	116	16.2
Group 2	71.2	122.5	16.5
Group 3	64.2	190.5	22.7
Group 4	55.8	158.8	17.8
Group 5	53.6	151.4	19.2
Group 6	54.5	130.5	19.8

Table 5: showing biochemical changes in different groups.

		ALT / SGPT	AST / SGOT	ALP	Urea
Group 1	Mean	58.0	103.3	278.0	33.1
	SD	15.0	20.1	94.5	3.2
Group 2	Mean	88.5	154.5	720.5	41.0
	SD	14.8	21.7	274.3	8.1
Group 3	Mean	81.2	156.5	1049.0	48.9
	SD	13.9	27.5	244.1	10.9
Group 4	Mean	71.3	161.0	521.7	34.8
	SD	18.6	29.5	207.1	6.9
Group 5	Mean	74.0	153.0	535.8	36.2
	SD	15.8	52.4	190.7	4.0
Group 6	Mean	63.6	136.8	627.2	31.9
	SD	10.7	21.3	226.0	9.1

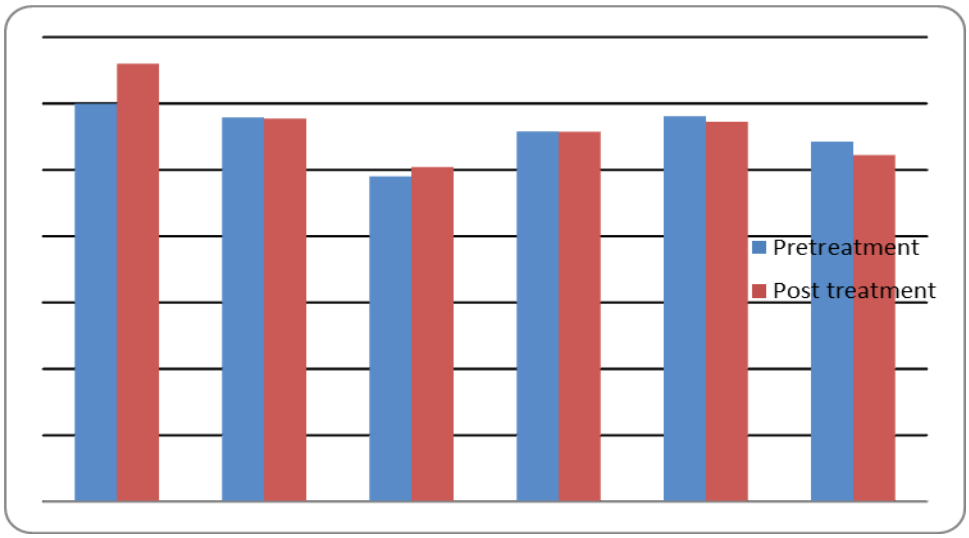

Fig. 1: Showing pretreatment and post treatment changes in weights in different groups.

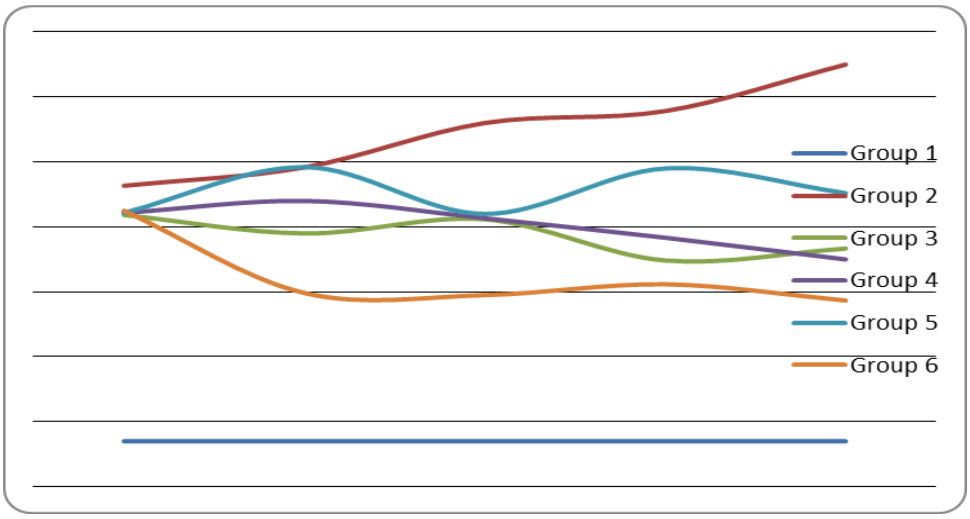

Fig. 2: Showing pattern of BSL control in different groups.

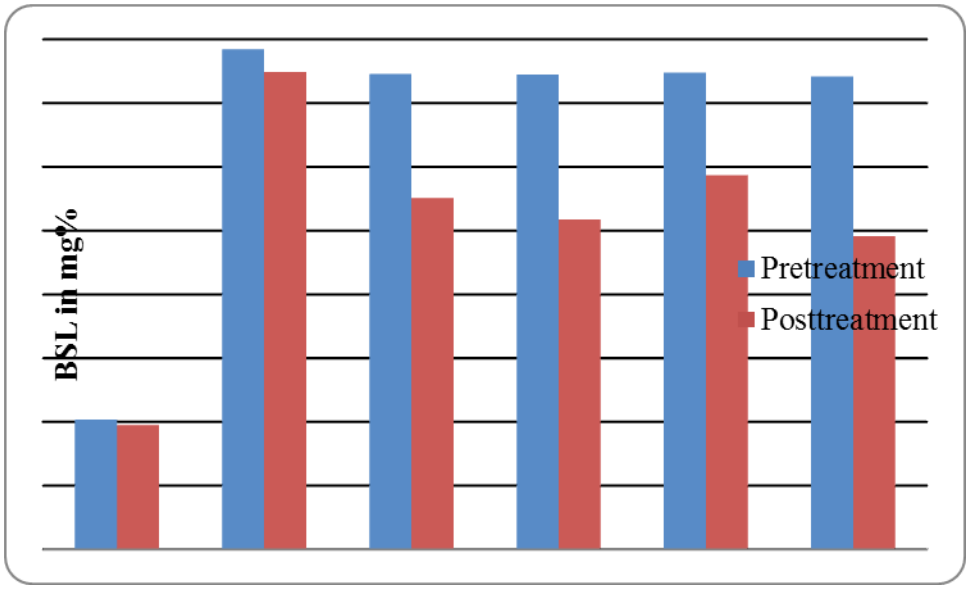

Fig. 3: Showing pretreatment and post treatment changes in weights in different groups.

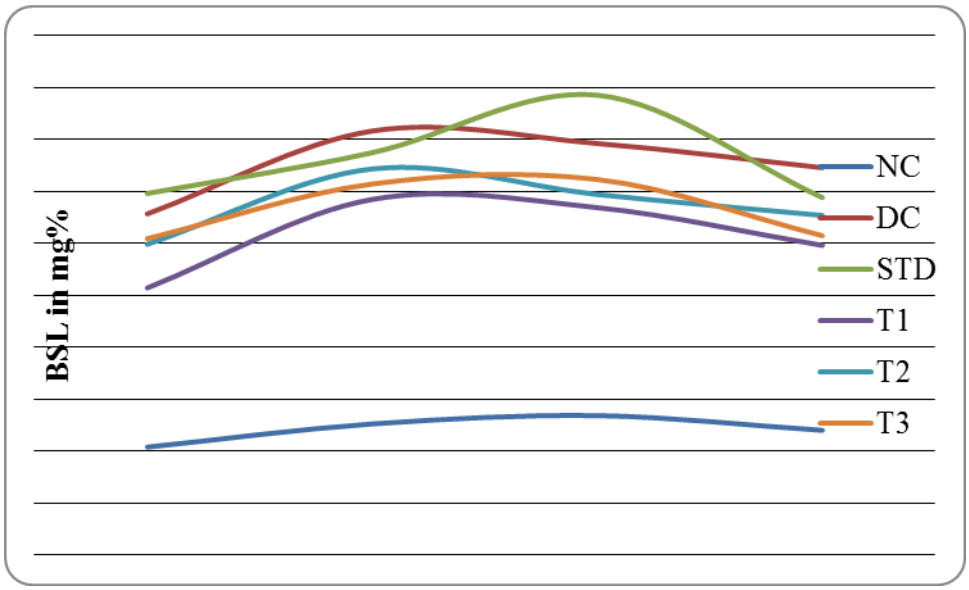

Fig. 4: Showing pattern of BSL control during OGCT.

DISCUSSIONS

Diabetes refers to a group of metabolic disorders characterized by high blood glucose levels. The correlation of DM and an imbalance in zinc homeostasis is well known. Zinc enhances hepatic glycogenesis through action on the insulin signaling pathway by increasing insulin receptor tyrosine phosphorylation enhanced PI3K activity and inhibition glycogen synthase kinase and thus improves glucose utilization.[6] Zinc is known to inhibit glucagon secretion thus reducing gluconeogenesis and glycogenolysis.[8,9] Zinc is also known to enhance the structural integrity of insulin and plays important role in insulin synthesis, storage and secretions. Insulin mimetic action of zinc is also known that is increase of lipogenesis and

inhibition of Nonesterified fatty acid release from adipocytes.[10] Clinically zinc deficiency is positively correlated with diabetes and may also affect progress of type two DM. Decreased zinc in the pancreas may reduce the ability of islets beta cells to produce and secret insulin. Considering its antioxidant activity reduced zinc may exacerbate the oxidative stress complications of diabetes. Thus there is complex inter relationship between zinc, DM and diabetic complications. In this study antidiabetic activity of ZnONP has been compared with known antidiabetic drug that is Glipizide in streptozotocine induced DM Wistar rats.

Insulin secretory activity of ZnONP is demonstrated in a study where it was found that there is dose dependent increase in insulin secretion by RIN-5F cells treated with 10ug/ml concentration of ZnONP as compared to untreated cells maintained at 25 nM glucose concentration. Further it was also demonstrated that this insulin secreting activity of ZnONP is dependent on concentration of glucose in the media but this activity is absent in euglycemic or normoglycemic conditions in the media.[11]

Animal study conducted by same group in strepazotocine induced DM rats using ZnONP (10 – 15 nm size) using 1, 3, 10 mg/kg dose showed significant antidiabetic activity. Single dose resulted in suppression of glucose levels in type one and type two Diabetic rats in which statistical significance observed in type two DM animals with dose of 3 and 10 mg/kg. Repeated dose study showed dose dependent decrease in fasting and non fasting BSL, suppression of glucose levels in OGTT, increase in non fasting serum insulin but no effect on fasting Insulin levels.[11]

In the study conducted with 90-day repeated dose, subchronic, oral toxicity; investigation of ZnONP of 20 nm in rats explored the actions of the NPs at three dose levels: 125, 250, and 500 mg/kg of bodyweight. It was observed that there is apoptosis of pancreatic acinar cells and infiltration of periductular lymphoid cells were observed as dose-dependent changes in both male and female experimental groups. Minimal to marked ductular epithelial hyperplasia and increased numbers of regenerated acinar cells was seen. These lesions in the pancreas were resolved during the recovery period, but they are considered to be toxicologically significant because they seemed to be severe enough to induce functional abnormalities. Bodyweight changes in the experimental and control groups were not significantly different for either sex, despite the fact that statistically significant differences were observed with increased in food and water intake in all test groups as compared to control group. But no significant difference was seen in BSL.[12]

Similar toxicity study conducted with 100 nm ZnONP for a 90-days with different surface charges (negatively charged and positively charged) to determine their no observed adverse effect level (NOAEL) and to identify target organs. Compared with the control groups, the group of male rats receiving 31.25 mg/kg of ZnONP (−) showed significantly decreased blood urea nitrogen levels ALP levels were significantly higher in the male group of rats receiving 500 mg/kg. In the female 500 mg/kg ZnONP (−) group, the levels of total proteins, albumin, and glucose significantly decreased compared with those in the control groups. Similarly, in the male 500 mg/kg group of ZnONP (+), the levels of the same were significantly decreased compared with those in the control groups. In addition, compared with the control groups, the female 500 mg/kg group showed significantly decreased levels of total proteins, albumin and cholesterol, but significantly increased levels of ALP. On histopathology, acinar cell apoptosis and chronic inflammation in the pancreas were seen. Most these lesions observed in the 500 mg/kg group but not observed in the 31.25 mg/kg and 125 mg/kg groups.[13]

Another study conducted with ZnONP (< 40 nm size) administetred orally for 13 weeks showed that, the no observed adverse effect level (NOAEL) of ZnONPs in rats is 268.4 mg/kg. As compared to present study, which has used < 50 nm sized ZnONP with lesser dose of upto 100 mg/kg for shorter duration of four weeks, this study has caused higher exposure to ZnONP which probably have resulted into pancreatitis and anemia in study animals. The NOAEL value 268 mg/kg resulted in the study is extremely high when considering that the tolerable upper intake level for zinc as a bulk material is 40 mg/day for an adult. So study has concluded that, oral intake of high dose ZnO NPs can cause pancreatitis and anemia, likely as a result of the absorption of ionized Zn owing to the complete dissolution of ZnO NPs in the acidic gastric fluid. This study results imply that ZnONPs are safe for application.[14]

CONCLUSIONS

ZnONPs have affinity towards pancreatic tissue and ZnONP (< 50 nm) show antidiabetic activity at graded concentration, When administered along with oral antidiabetic drug, ZnONP have shown added antidiabetic effect.

Along with antidiabetic activity, ZnO have shown antiobesity and lipid lowering property in nanoparticle form which is suggesting that ZnO is playing important role in the metabolic pathways regulating Obesity, Carbohydrate and Lipid metabolism.

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