Original Research Article

Effects of diabetogenic agent Streptozotocin on hematological parameters of albino wistar rats

"An experimental study"

Abstract:

Background: Diabetes mellitus has remained the major concern for medical sciences researches due its deleterious effects on general, physical and mental health of patients. To understand the pathophysiology and to explore better treatment options for such kind of metabolic disorders it is necessary to generate the experimental animal models. To create diabetic animal models, streptozotocin has shown predominance in selectivity as a diabetogenic agent. While studying effects of any intervention in the diabetic animal models, being a cytotoxic drug streptozotocin may affect the study results by inhibiting highly replicating cells especially hematopoietic cells.

Aims: The aim of study was to analyze the effects of streptozotocin on various cellular components of blood such as RBCs, WBCs (Lymphocytes, Neutrophils, Eosinophils), Hb%, HCT and Platelets, at baseline,5th day and 15th day without any intervention.

Study design: Animal based Experimental study.

Place and duration of study: The study was conducted at animal house of faculty of Pharmacy Ziauddin University Karachi, while laboratory work was performed at MDRL-1 Ziauddin University.

Methodology: In Group A normal saline and in group B and C 60mg / kg streptozotocin diluted in normal saline was administered intraperitoneally. After the confirmation of induction of Diabetes in rats, on fifth day blood samples were drawn from Group A and B and were analyzed. While blood samples from group C were drawn on fifteenth day.

Results: Analysis of various hematological parameters on 5th day revealed that there was a decrease in the levels of Hb, HCT, RBCs and WBCs with an increase in platelet count in group B in comparison to group A (control). On the other hand, in Group C (15th day), blood cell counts (Hb, HCT, RBCs, WBCs, Lymphocytes, Neutrophils and platelets) seemed to recover from streptozotocin induced decline that was observed in group B, however did not reach the baselines as in group A(control).

Conclusion: It is concluded that change in hematological parameters of rats after administration of streptozotocin is reversible. The blood parameters may recover near to base line values without any intervention within two weeks.

Key Words: Streptozotocin, Animal Model, Hematological parameters

Introduction:

Diabetes mellitus has remained the major concern for medical sciences researches not only due to its high incidence and prevalence rate but also due its deleterious effects on general, physical and mental health of patients (1). To understand the pathophysiology and to explore better treatment options for such kind of metabolic disorders it is necessary to generate the experimental animal models (2). To create diabetic animal models, surgical (pancreatectomy) and pharmacological (alloxan monohydrate and streptozotocin) options have been used in research but pharmacological options particularly use of streptozotocin has shown predominance in selectivity as a diabetogenic agent (3) (4). Chemically, streptozotocin is a derivative of synthetic Nitrosoureido Glucopyranose and has been used for cancer chemotherapies,(5) being its potential to inhibit DNA synthesis in bacterial and mammalian cells (6).While its diabetogenic effect is thought to be attributed to its ability to cause pancreatic β cells' death by DNA alkylation and hence used to induce diabetes mellitus in experimental animals (7) (8).

The methods to induce diabetes in animal models by streptozotocin fall under three categories 1. Multiple small doses (i.e. 40mg/kg) of streptozotocin over a period of several days 2. A single moderate dose (i.e. 60mg/kg) of streptozotocin or 3. A single large dose (100mg/kg) of streptozotocin produce diabetes in 48-72 hours. Usually a single large dose of streptozotocin is used to induce diabetes in experimental models as reported by Ito et al. 100mg / kg of streptozotocin produced non-insulin dependent diabetes mellitus in experimental animals (9). Streptozotocin can be administered by various routes including subcutaneous and intramuscular routes but intraperitoneal and intravenous administration routes are preferred. (10). After 3-4 days of streptozotocin administration fasting blood glucose levels are obtained to confirm the accuracy of procedure (11) and on 5th day when 180-500 mg/dl serum glucose levels are obtained experimental animals are considered as diabetic (12).

Though streptozotocin is preferred pharmacological method for induction of diabetes (13), many studies have reported spontaneous recovery from hyperglycemia due to reactive hyperinsulinemia insulinoma (14) (15) (16). Streptototozin, not only affects pancreas and cause diabetes in experimental animals but also have a potential to produce toxic effects on other body tissues as well. It has been learnt through a number of studies that streptozotocin is associated with high incidence of hepatic and renal tumors (17), increase in permeability of blood brain barrier (18), renal hypertrophy (19) and retinal damage in experimental animal models (20). As already discussed that streptototozin damages DNA by alkylation and produces free radicals, therefore it may harm any organ system of animals(21). Despite of aforementioned, streptozotocin is still employed in various researches for the induction of diabetes mellitus all over the world. While studying effects of any intervention (eg drugs, herbs, dietary modifications etc.) in the diabetic animal model, being a cytotoxic drug streptozotocin may affect the study results by inhibiting highly replicating cells especially hematopoietic cells. Moreover it is also unknown whether streptozotocin induced changes are corrected over the time or permanent. Hence in order to achieve unbiased results in the diabetic model it is necessary to analyze the immediate and delayed effects of streptozotocin on various hematological parameters before any intervention. Therefore, this study was conducted to analyze the effects of streptozotocin on various cellular components of blood such as RBCs, WBCs (Lymphocytes, Neutrophils, Eosinophils), Hb%, HCT and Platelets, at baseline,5th day and 15th day without any intervention.

Materials and Methods:

Study design:

It was an Animal based Experimental study.

Study settings and Duration:

The study was conducted at animal house of faculty of Pharmacy Ziauddin University Karachi, while laboratory work was performed at MDRL-1 Ziauddin University.

Animals:

Eighteen, male albino wistar rats of 12 weeks age, weighing 300- 400g were purchased from Animal house of Agha Khan University.

Ethical approval:

The study was approved by Animal Ethics committee Ziauddin University and Protocol No. 2018-003 was allotted. All the animals were given twelve-hour light and dark cycle, and before start of treatment animals were acclimatized with the environment. Animals were dealt through all procedures according to CARE guidelines 2010 (22).

Induction of Diabetes Mellitus:

60mg / kg (6mg / 100g) streptozotocin diluted in normal saline was administered intraperitoneally. (23). Rats were kept deprived of their feed and water for twelve hours before administration of streptozotocin. Blood glucose levels were obtained after 72 hours by using @Abbott Free Style Optium Xceed glucometer. Rats with blood glucose level >180mg/dl were considered as diabetic.

Blood sample collection:

1 ml blood were drawn from lateral tail vein of all the rats in EDTA containing vacutainer tubes, and was transferred to MDRL-1 for the analysis of RBCs, WBCs, Hb, HCT, Platelets, Lymphocytes, Neutrophils and Eosinophils

Grouping of Animals:

Animals were randomly selected for grouping.

Group A: Control group (streptozotocin untreated)

Group B: Streptozotocin Treated Diabetic Group 1(5^{th} day)

Group C: Streptozotocin Treated Diabetic Group 2 (15^{th} day)

Experiment:

In Group A normal saline was administered intraperitoneally as this was our control group, and in group B and C 60mg / kg (6mg / 100g) streptozotocin diluted in normal saline was administered intraperitoneally. After the confirmation of induction of Diabetes in rats, on fifth day blood samples were drawn from Group A and B and were analyzed. While blood samples from group C were drawn on fifteenth day and were analyzed by @sysmex automated cell counter.

Statistical analysis:

Data entry and analysis were conducted on SPSS version 20. Anova followed by post hoc tukey's test was applied for inter and intra group comparison of various hematological parameters. P value less than 0.05 was considered as significant.

Results:

We found that after the administration 60mg/kg streptozotocin, diabetic profile was achieved in group B and C, when compared with controls with a significant p value (i.e. 0.000). Analysis of

various hematological parameters on 5th day revealed that there was a decrease in the levels of Hb, HCT, RBCs and WBCs with an increase in platelet count in group B in comparison to group A (control). On the other hand, in Group C (15th day), blood cell counts (Hb, HCT, RBCs, WBCs, Lymphocytes, Neutrophils and platelets) seemed to recover from streptozotocin induced decline that was observed in group B, however did not reach the baselines as in group A(control) as shown in Table 1. While monocytes and eosinophils remained unchanged in Group C. Intergroup comparison of all animal groups showed significant P values i.e.<0.05 for FBS, Hb, HCT, RBCs, WBCs, Lymphocytes, Neutrophils and Platelets count, while the difference among all groups for Eosinophils and Monocytes was non-significant, p values (1.00 and 0.905) respectively as shown in Figure 1.

Table 1. Means of variables in all groups.

Hematological Parameter	Group A Control (mean ± sd)	Group B STZ* treated 5th day (mean ± sd)	Group C STZ* treated 15th day (mean ± sd)	P value
FBS Levels (mg/dl)	79.83 (± 8.7)	296.6 (±24.8)	285.83 (±8.9)	0.000
Hb (g/dl)	16 (± 1.2)	12.8 (±1.1)	15 (± 0.632)	0.000
RBCs / µl	10.41 x 10^6 (± 0.81)	7.12 x 10^6 (± 0.35)	9.8 x 10^6 (± 0.46)	0.000
	8.85 x 10^3 (± 0.89)	6.95 x 10^3 (± 0.50)	8.41 x 10^3 (± 0.86)	0.002
Platelets x 10^3 / µl	708.16 +- (± 16.4)	879.33 x 10^3/µl (± 30.14)	676.5 x 10^3 / µl (± 26.48)	0.000

Table 1. Represents the means of variables (i.e. sum of values of all samples / n= 6) in all groups and p value after Anova. Graphical representation for each variable is shown through mean plots.

Following are the means plots of hematological parameters of Albino wistar rats.

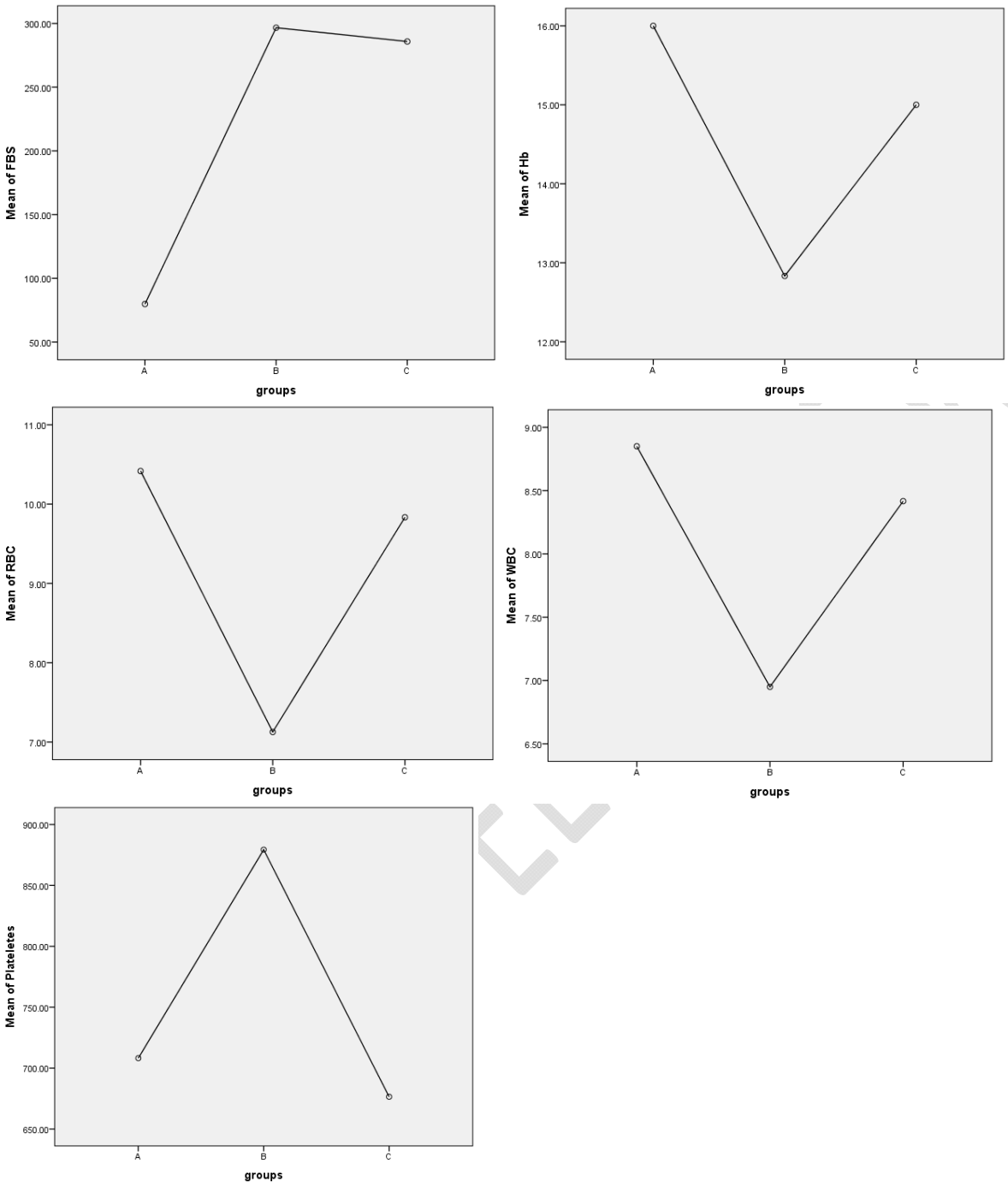
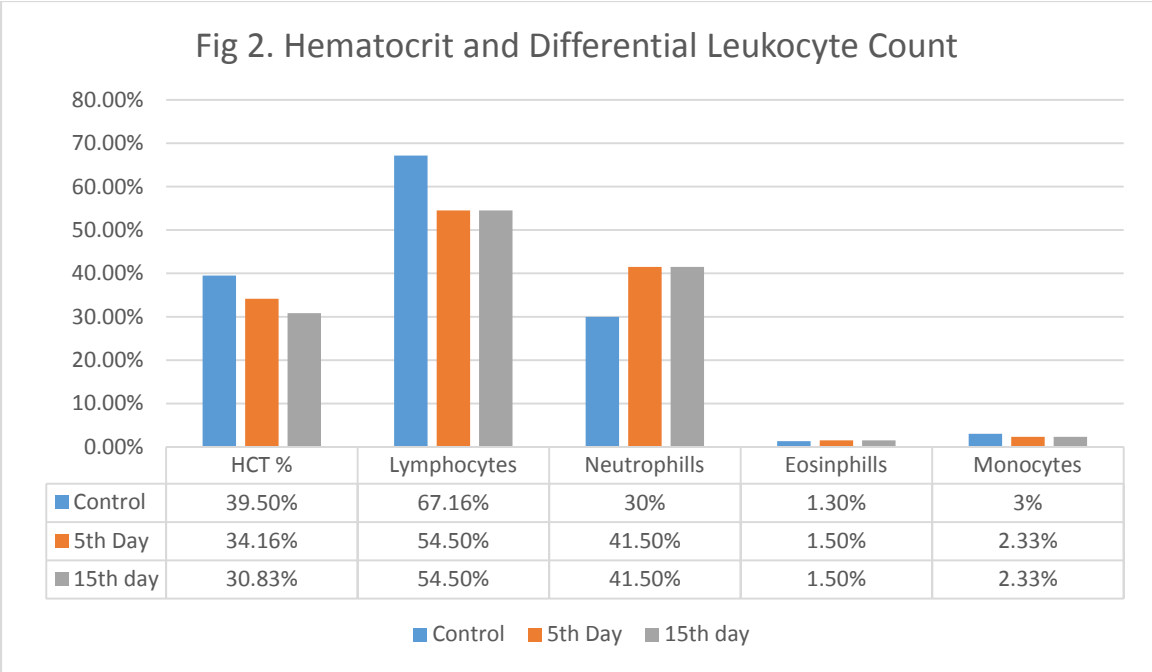


Figure 1. Hematocrit and differential leukocyte count of all the groups.



Discussion:

Glucose is a basic fuel and an essential nutrient required by almost all body cells, its abnormal concentrations may lead to change in biochemical and hematological parameters of individuals (24). Streptozotocin is highly recommended drug for induction of diabetes mellitus in animals (11) (13), after its intraperitoneal administration hyperglycemic profile was achieved in both the groups i.e. B and C at 60mg/kg dose. The results of our study (Table 1 group B) are parallel with the findings of many studies in which they have reported the decline of RBCs, WBCs and increase in platelet count after few days of administration of streptozotocin (25) (26) (27) but at the same time our study is showing variation in group C (Table 1 group C). The findings in group C (15 days) are somehow different from the previous researches as they have associated the recovery in cell count of blood parameters with different herbal and allopathic medications (28, 29) on the other hand we in our study have found that the recovery in blood parameters is a normal phenomenon that can happen with the advancement of time after administration of streptozotocin. However in accordance to other studies, no major changes in the number of lymphocytes, monocytes and neutrophils were displayed in our study results (26, 27). It was observed in a study that the change in hematological parameters is due to increase in blood viscosity that occur because of water deprivation before streptozotocin administration and change in glucose concentration after streptozotocin administration (30). It is seen that after administration of streptozotocin confirmation of diabetes mellitus is analyzed by glucometer (27, 29) and when the readings are found to be significant animal model is considered as a perfect model to carry out research, according to our study it is not true. Yeom et., al. in 2016 has highlighted that the change in hematological parameters specially in platelets after administration of streptozotocin is not a direct effect that is produced in response to its administration but this change is attributed to change in environment of body of animals due to induction of diabetes (31). According to our study it seems like that the recovery in blood parameters is a normal physiologic mechanism that is happening in the body of animals few days after the administration of streptozotocin and this reversal specially in hematological parameter should not be regarded as an attribution of any medication. This practice may give us biased results that can be a disaster in medical field because after animal based

experimental trials humans based trails are the next step. So if in animals we are having unclear and biased results how will we prove the efficacy and toxicity of medication in humans.

Conclusion:

It is concluded that change in hematological parameters of rats after administration of streptozotocin is reversible. The blood parameters may recover near to base line values without any intervention within two weeks. Therefore to get unbiased results after any intervention (drugs/herbs/alteration in diet etc.), the aforementioned should be administered at least two weeks after strepto administration in diabetic model.

Limitations:

In our study the major limitation was that the animals were observed only for 15 days and blood samples from three different animal groups (i.e. Control (A), Streptozotocin treated 5^{th} (B) and 15^{th}(C) days) were taken into consideration, rather than observing and following the same animal on various days

Suggestions:

Further studies should be performed in which animals should be observed for more than 15 days. There should be a follow-up of single group with more than 10 animals and analyzation of hematological parameters of same animals should be performed on different days. We suggest that while working on diabetic animal models there must be a gap of least 15 days after administration of streptozotocin to get unbiased results in further experiments. To rule out the mystery of this alteration we recommend animal based experimental trials to identify the molecular pathways responsible for decrease in hematological parameters after streptozotocin administration and their self-recovery from that declination period.

Declaration of conflict of Interest: There was no conflict of interest.

Ethical Approval: Animal ethics committee of Ziauddin University approved the study.
Patients consent form: Not applicable.

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