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Original Research

Clinic-Pathological Characterization of the Low Doses Alloxan-STZ Diabetic Rabbit Model

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ABSTRACT:
Purpose: Present investigation was aimed at clinic-pathological characterization of the low doses alloxan-STZ diabetic rabbit model. **Method:** New Zealand White rabbits, 1-1.5 kg body weight, were administered alloxan (@50mg/kg b.w.) and STZ (@35mg/kg b.w) cocktail, as single intravenous dose followed by glucose therapy after 9 hours. Haemato-biochemical investigations were carried out fortnightly from days 0 to 60 except for glucose which was recorded on weekly basis. **Results:** Alloxan (@50mg/kg b.w) streptozotocin (@35mg/kg b.w) cocktail induced mild to moderate hyperglycemia, with peak blood glucose levels at 1 week followed by progressive recovery with none of the rabbits diabetic by 6 weeks. Clinically rabbits showed polydypsia, polyurea, polyphagia, decreased physical activities, decreased body weight gain, and non-significant increase in heart rate. Haematology revealed significantly decreased Hb, PCV, and MCH; increased MCV and MCHC; while TEC was not altered, indicating normochromic microcytic anemia at day 15 followed by progressive recovery. Leukocytopenia without any changes in differential counts was noted. Serum biochemistry revealed mild decrease in plasma protein levels associated with mild increase in AST and ALT, indicating altered liver function. KFT showed significant increase in plasma creatinine and decrease in chloride, along with non-significant increase in BUN revealing altered kidney function. Also, Hypercholesterolemia and hypertriglyceridemia were observed. **Conclusion:** Alloxan-STZ cocktail shows synergistic effect for induction of diabetes in rabbits and maintains a moderate hyperglycemia for about 4 to 5 weeks, with subtle haemato-biochemical alterations. The model can be used for only subacute studies and long term investigations warrant induction using higher dosage with due consideration to direct toxic effects.
Keywords: diabetes, alloxan, rabbit, haematology, biochemical indices

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INTRODUCTION

Diabetes mellitus is one of the leading causes of morbidity and mortality in humans and poses staggering economic costs on account of management of the disease and its complications (Ettaro *et al.*, 2004). Investigations into the disease, its complications, or therapeutic and preventative strategies warrant use of experimental animal models (Srinivasan and Ramarao, 2007; Etuk, 2010). Over the years, besides identifying the spontaneous diabetic animal models, several experimental animal models have been developed. Most of the animal models that have been developed present similar characteristic properties as that for diabetes in humans, which otherwise would have made it impossible if proper experimentations were not conducted. However, not a single known reported species has been found to be comparatively equivalent to diabetes in humans (Cefalu, 2006). Due to the development of large animal models with different and improper characteristics has made it too much difficult for choosing the correct model under a given study and at instances leading to data misinterpretation (Thatte, 2009).

Experimental studies using Beta-cytotoxic drugs, streptozotocin (STZ) and alloxan induced diabetic animals models have been used extensively for screening of natural compounds (Srinivasan and Ramarao, 2007). Their effect is facilitated by GLUT-2 mediated uptake in pancreatic beta-cells (Elsner *et al.*, 2002). Alloxan, induces Type-I Diabetes Mellitus (T1DM) model following parental (intravenous, intraperitoneal) or subcutaneous administration (Szkudelski, 2001). The dose required depends on the species of animals, administration route and nutrition status (Lenzen, 2008). However, at higher doses wide spread toxic effects have been reported. Streptozotocin (STZ) is preferred over alloxan due to its specific action, wider dose range, long half-life (15 minutes), producing persistent hyperglycemia for longer period, increasing complications of diabetes with lower prevalence of ketosis and decreased mortality (Srinivasan and Ramarao, 2007). However, it has been reported that the sensitivity varies with sex, nutrition status, species, batch differences and strain (Srinivasan and Ramarao, 2007; Kramer et al., 2009).

As experimental animal models of diabetes, rabbits has been mostly used particularly in pharmacological studies (El-Said et al., 2010). For inducing diabetes in animals, a choice of chemical has been alloxan, as it is comparatively effective in inducing diabetes as well as defined disease complications (Mir *et al.*, 2017; Srinivasan and Ramarao, 2007). Combination of low doses of alloxan and streptozotocin in rabbits have effectively induced diabetes (Mir *et al.*, 2016). Although, the drug combinations markedly reduce the diabetogenic drug dose, they need to be carefully weighed for sustainability of hyperglycemia as models induced low drug doses show spontaneous recovery. The present study aimed at depicting the clinic-pathological characterization of the low doses alloxan-STZ diabetic rabbit animal model.

MATERIALS AND METHODS

EXPERIMENTAL MODELS

New Zealand white rabbits were procured from Laboratory Animal Resource and kept in cage system provided with standard conditions. In the present study, rabbits of age group (3 months) and weighing about 1-1.5 kg were used. In this study, the Institutional Animal Ethics Committee approved experimental protocols that were used and the guidelines for animal care and use of laboratory animals published were followed as per us National Institute of Health (NIH Publication No. 85-23, revised 1996). Prior to conduct of experiments, all the animals were acclimatized for 7 days. Equal random allocation was followed for constitution of experimental groups. Rabbits were offered feed and water *ad libtum*. Commercially procured rabbit feed and greens were given twice a day (morning and evening).

DEVELOPMENT OF DIABETIC MODEL

The beta-cytotoxic drugs, Alloxan monohydrate (Sigma-Aldrich), streptozotocin (Sigma-Aldrich) were administered as a cocktail for evaluating their diabetogenic effects in rabbits. Rabbits were fed in the morning and then fasted for 18 hours providing only water during the period. Their fasting blood glucose level was determined using glucometer (Accu-Chek, Roche diagnostics India Pvt. Ltd., Mumbai). Alloxan monohydrate @50mg/kg body weight in 1ml sterile water followed immediately by Streptozotocin @35mg/kg body weight in 1ml freshly prepared citrate buffer, pH 4.6 were given as slow intravenous injection through ear vein using insulin syringe. Freshly prepared solutions of the calculated dose of drugs were used. At 9 hours post alloxan administration rabbits were given 5ml of 25% dextrose intraperitoneally, and 10% glucose in drinking water up to 24 hours post-treatment, followed by normal management. Fasting blood glucose levels were recorded at days 3, 5 and 7 following administration of Alloxan,. Rabbits showing sustained or progressive hyperglycemia with blood glucose levels above 200 mg/dl at day 7 were considered diabetic (Mir *et al.*, 2016). A total of 12 hyperglycemic rabbits and 12 placebo treated normoglycemic rabbits were used for the investigations up to 60 days.

COLLECTION OF BLOOD AND PLASMA

Blood samples were collected from the auricular artery using standard techniques and heparin as anticoagulant. Samples (5 mL from each rabbit) were collected early in the morning prior to watering and feeding. 1 mL aliquots were taken for haematology. For biochemical investigations, plasma was collected following centrifugation at 5000 rpm for 5 min, and stored in multiple aliquots, at -40°C until used. The samples were divided into sufficient number of aliquots to avoid freezing and thawing effects during multiple analyses.

HAEMATOLOGY

In the present study, haematological indices that were studied include measurement of haemoglobin (Sahli's acid haematin method), packed cell volume (Microhaematocrit method), total leukocyte count (TLC) and total erythrocyte count (TEC) (Neubaur haemocytometer method). Freshly prepared smears of blood were stained properly with Wrights-Giemsa stain for differential leukocyte count (DLC). Apart from this, mean corpuscular haemoglobin (MCH), mean corpuscular volume (MCV) and mean corpuscular haemoglobin concentration (MCHC) were studied (Benjamin, 1985; Jain 1986).

BIOCHEMICAL STUDIES

The biochemical studies included blood glucose (Glucometer method); plasma proteins viz. total protein (Biuret method), albumin (BCG Dye binding

method), globulin (difference method) and albumin: globulin (A:G) ratio; plasma enzymology viz. aspartate transaminase (AST) and alanine transaminase (ALT) (IFCC method/ Reitman and Frankel's method); kidney function tests (KFT) viz. blood urea nitrogen (BUN) (Berthelot method), creatinine (modified Jaffe's method) and chloride (thiocyanate end point method); and plasma lipids viz. total cholesterol (CHOD-POD method) and triglycerides (GPO-POD method) using diagnostic kits (Aspen Laboratories Pvt. Ltd, Rapid Diagnostic Group of Companies, Karnal Road Industrial Area, Delhi, India) and semi-automatic blood chemistry analyzer (model ERBA CHEM-PRO).

STATISTICAL ANALYSIS

Results are expressed as Mean ± S.E. and data was analyzed by t-test, one-way ANOVA followed by Dunnet's test. A value of $P<0.05$ was considered to be statistically significant (Snedecor and Cochran, 1989).

RESULTS

GENERAL OBSERVATION

Polyuria and polydypsia were the most characteristic signs during the experimental period. Polydypsia was comparable to that of Alloxan treated rabbits. From 2nd week onwards rabbits were dull, lethargic and depressed with periodic increased activity. Increased feed intake was observed, with individual variations. One of the 10 rabbits succumbed on day 10 to severe hyperglycemia giving mortality rate of 10%. Besides the above signs and the rectal temperature of <90°F the dead rabbit showed wry-neck condition and underwent lateral recumbency with intermittent paddling of limbs and rolling on back.

BODY WEIGHT

The mean body weight of control as well as Alloxan-STZ diabetic rabbits revealed a consistent and significant ($P\leq0.05$) increase. However, the variations between the two groups was revealed at day 60 when the mean body weight of Alloxan-STZ diabetic rabbits was significantly ($P\leq0.05$) lower than that of the control (Table 1, Fig. 1(a).

TEMPERATURE

No significant variations were seen in body temperatures in control group, whereas, in Alloxan-STZ diabetic rabbits significantly ($P\leq0.05$) lower mean body temperatures were recorded from day 15 onwards. When compared with age matched control rabbit the mean body temperature was significantly ($P\leq0.05$) lower in Alloxan-STZ diabetic rabbits at day 60 ((Table 1, Fig. 1(b).

HEART RATE

A consistent and non-significant increase in the heart rate was observed in Alloxan-STZ diabetic rabbits, but the mean value differed significantly ($P\leq0.05$) from that of the baseline value and that of age matched control rabbits only at day 60 (Table 1, Fig. 1(c).

Table 1: Changes in body weight (kg), body temperature (°F) and heart rate (beats/min) in Alloxan (@ 50 mg/kg b.w) Streptozotocin (@ 35 mg/kg b.w) cocktail diabetic rabbits (Mean ± SE).

Parameter	Treatment	Days of treatment				
		0day	15day	30day	45day	60day
Number	ALL+STZ	10	10	8	6	4
	Control	6	6	6	6	6
Body weight (kg)	ALL+STZ	1.40^{aA} ± 0.043 (1.23 - 1.60)	1.56^{abA} ± 0.041 (1.29 - 1.72)	1.66^{bcA} ± 0.044 (1.43 - 1.82)	1.79^{cdA} ± 0.094 (1.55 - 2.19)	1.86^{dA} ± 0.105 (1.60 - 2.09)
	Control	1.415^{aA} ± 0.035 (1.33 – 1.52)	1.580^{bA} ± 0.041 (1.40 – 1.67)	1.737^{cA} ± 0.039 (1.57 – 1.82)	1.858^{dA} ± 0.04 (1.70 – 1.97)	2.030^{eB} ± 0.05 (1.85 – 2.15)
Body Temperature (°F)	ALL+STZ	101.66^{aA} ± 0.219 (100.90 - 103.10)	100.94^{bA} ± 0.123 (100.30 - 101.50)	100.63^{bcA} ± 0.271 (99.40 - 102.10)	100.48^{bcA} ± 0.322 (99.50 - 101.30)	100.13^{cA} ± 0.118 (99.80 - 100.30)
	Control	101.55^{aA} ± 0.29 (100.50 - 102.50)	101.40^{aA} ± 0.27 (100.40 - 102.30)	101.20^{aA} ± 0.33 (99.90 - 102.50)	101.15^{aA} ± 0.46 (99.90 - 103.10)	102.23^{aB} ± 0.32 (100.90 - 103.20)
Heart Rate (beats/min)	ALL+STZ	243.40^{aA} ± 3.045 (227.00 - 257.00)	245.10^{abA} ± 2.401 (233.00 - 255.00)	245.63^{abA} ± 2.449 (237.00 - 254.00)	247.33^{abA} ± 2.929 (241.00 - 258.00)	253.25^{bA} ± 2.136 (248.00 - 258.00)
	Control	247.17^{aA} ± 3.64 (235 – 258)	246.50^{aA} ± 2.92 (239 – 260)	248.50^{aA} ± 3.63 (233 – 259)	249.00^{aA} ± 2.68 (238 – 257)	240.17^{aB} ± 2.94 (231 – 249)

Means for a parameter in different columns bearing at least one common 'lowercase alphabet' superscript, and in different rows bearing at least one common 'uppercase alphabet'' superscript does not differ significantly (P≤ 0.05).

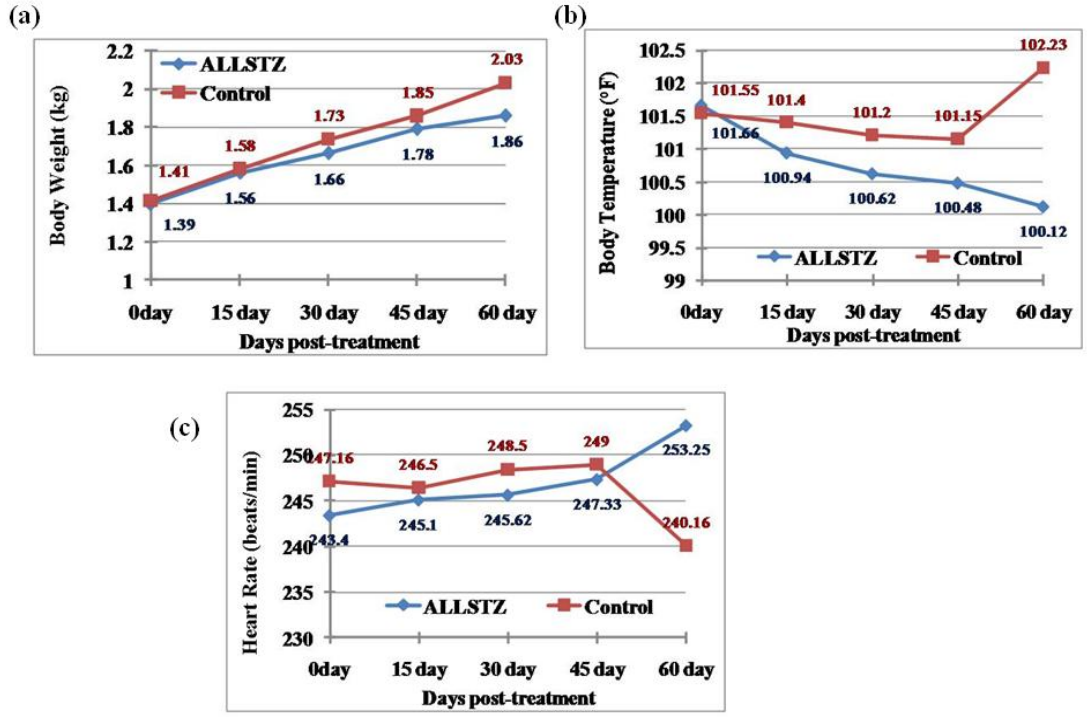

Fig. 1: Graphical representation of: (a) Body weight; (b) Body temperature; (c) Heart rate

HEMATOLOGY

Haemoglobin (Hb): A significant (P≤0.05) decrease in haemoglobin concentration was noted in Alloxan-STZ cocktail diabetic rabbits from day 30. Significant (P≤0.05) decreases with reference to age matched control rabbits were also observed at days 30 and 45 (Table 2, Fig. 2(a).

Packed cell volume (PCV): The mean haematocrit values were significantly(P≤0.05) lower from the baseline values or from that in age matched control only at day 15 (Table 2, Fig. 2(b).

Total erythrocyte count (TEC): Non-significant decrease in TEC was observed on day 15. (Table 2, Fig. 2(c).

MCV, MCH and MCHC: The mean MCV was significantly higher at day 30 compared to base level value but was comparable with age matched control. Significantly (P≤0.05) lower MCH values, when compared with base level value, were observed from day 30 and also on days 30 and 45 in reference to age matched control rabbits. MCHC showed a significant (P≤0.05) increase at day 15 followed by a significant (P≤0.05) decrease at day 30 and the value approached to normal level by day 60. The values were significantly (P≤0.05) lower when compared with age matched control rabbits from day 30 onwards (Table 2, Fig. 2(d-f).

Table 2: Changes in erythrocytic attributes in Alloxan (@ 50 mg/kg b.w) Streptozotocin (@ 35 mg/kg b.w) cocktail diabetic rabbits (Mean ± SE).

Parameter	Treatment	Days of treatment				
		0day	15day	30day	45day	60day
Number	ALL+STZ	10	10	8	6	4
	Control	6	6	6	6	6
Haemoglobin (gm/dl)	ALL+STZ	12.05^{aA} ± 0.317 (10.20 - 13.40)	11.90^{aA} ± 0.300 (10.00 - 13.00)	9.33^{bA} ± 0.516 (7.50 - 12.10)	10.28^{bcA} ± 0.386 (9.20 - 11.50)	10.60^{cA} ± 0.258 (10.00 - 11.20)
	Control	12.00^{aA} ± .339	12.10^{aA} ± .282 (11.20 -	11.77^{aB} ± .201	11.77^{aB} ± .267	11.30^{aA} ± .375

		(10.80 - 13.20)	13.20)	(11.00 - 12.50)	(10.80 - 12.60)	(9.90 - 12.50)
PCV (%)	**ALL+STZ**	41.40^{aA} ± 0.921 (36.00 - 45.00)	36.80^{bA} ± 1.402 (31.25 - 43.70)	43.68^{aA} ± 1.202 (39.20 - 48.57)	41.85^{aA} ± 0.507 (40.00 - 43.40)	41.03^{aA} ± 0.541 (39.90 - 42.40)
	Control	41.87^{aA} ± 1.031 (38.00 - 44.80)	41.63^{aB} ± .545 (40.00 - 43.00)	41.23^{aA} ± .561 (39.40 - 43.00)	41.28^{aA} ± .620 (38.80 - 43.20)	40.25^{aA} ± .670 (37.90 - 42.40)
TEC (10^6/cmm)	**ALL+STZ**	6.47^{aA} ± 0.243 (4.69 - 7.40)	5.92^{aA} ± 0.075 (5.40 - 6.20)	6.03^{aA} ± 0.100 (5.60 - 6.40)	6.22^{aA} ± 0.105 (5.90 - 6.50)	6.13^{aA} ± 0.118 (5.80 - 6.30)
	Control	6.41^{aA} ± .308 (5.20 - 7.20)	6.28^{aA} ± .215 (5.60 - 7.00)	6.25^{aA} ± .138 (5.80 - 6.60)	6.13^{aA} ± .186 (5.40 - 6.60)	6.02^{aA} ± .210 (5.30 - 6.80)
MCV (fL)	**ALL+STZ**	64.67^{aA} ± 2.367 (55.23 - 76.76)	62.14^{aA} ± 2.268 (52.35 - 73.68)	72.65^{bA} ± 2.477 (66.67 - 85.21)	67.43^{abA} ± 1.585 (62.77 - 72.33)	67.10^{abA} ± 2.104 (63.33 - 73.10)
	Control	65.82^{aA} ± 2.026 (58.33 - 73.08)	66.69^{aA} ± 2.423 (57.71 - 73.10)	66.06^{aA} ± .966 (63.49 - 69.32)	67.50^{aA} ± 1.321 (63.18 - 71.85)	67.15^{aA} ± 1.587 (62.35 - 71.51)
MCH (pg)	**ALL+STZ**	18.82^{acA} ± 0.727 (16.22 - 21.78)	20.10^{aA} ± 0.476 (16.67 - 21.59)	15.55^{bA} ± 1.027 (11.72 - 21.61)	16.58^{bcA} ± 0.753 (14.15 - 19.17)	17.34^{bcA} ± 0.716 (15.87 - 19.31)
	Control	18.86^{aA} ± .609 (16.67 - 20.77)	19.35^{aA} ± .635 (16.57 - 20.69)	18.85^{aB} ± .262 (18.18 - 20.00)	19.22^{aB} ± .289 (18.33 - 20.00)	18.81^{aA} ± 457 (17.54 - 20.88)
MCHC (%)	**ALL+STZ**	29.10^{aA} ± 0.346 (27.91 - 31.90)	32.79^{bA} ± 1.576 (26.32 - 40.96)	21.38^{cA} ± 1.067 (16.67 - 25.36)	24.57^{cdA} ± 0.836 (21.90 - 27.21)	25.84^{adA} ± 0.613 (24.21 - 27.07)
	Control	28.65^{aA} ± .225 (27.83 - 29.46)	29.05^{aA} ± .410 (28.00 - 30.70)	28.54^{aB} ± .358 (27.55 - 29.76)	28.49^{aB} ± .319 (27.74 - 29.79)	28.04^{aB} ± .527 (26.12 - 29.48)

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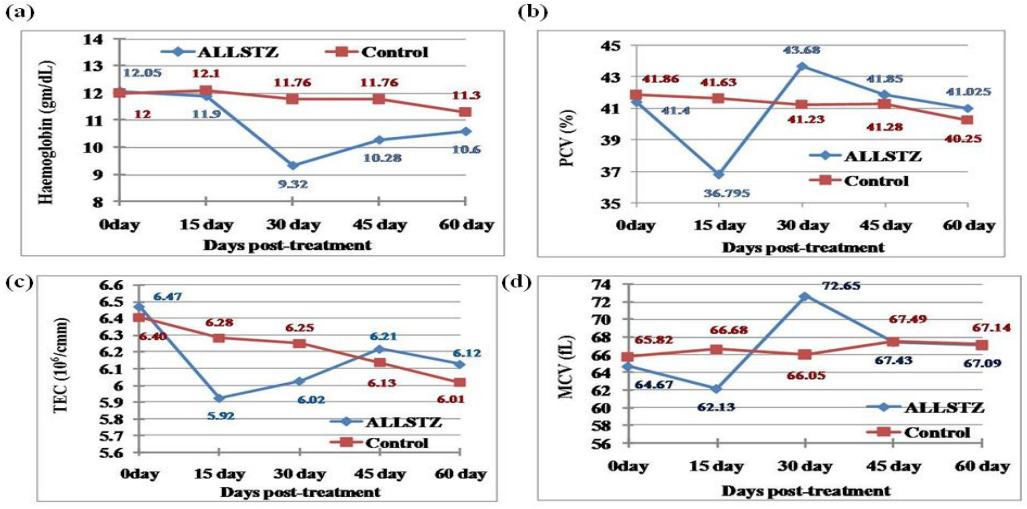

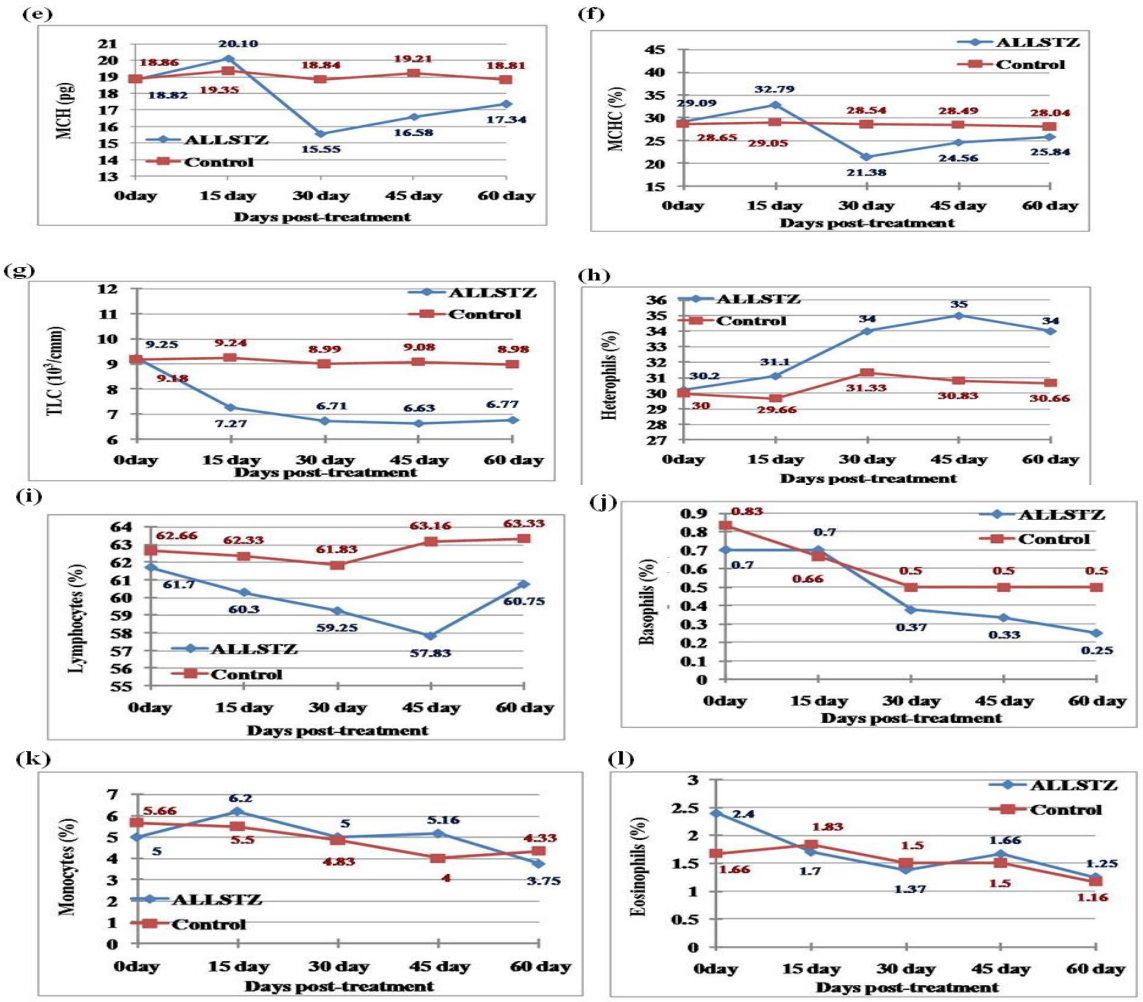

Fig. 2: Haematological indices in Alloxan-STZ Diabetic Rabbit Model

TOTAL LEUKOCYTE COUNT (TLC)

TLC was significantly ($P \leq 0.05$) lower than the values of baseline and that of age matched control rabbits from day 15 (Table 3, Fig. 2 (g).

DIFFERENTIAL LEUKOCYTE COUNT (DLC)

Compared to base level values heterophil, lymphocytes and basophil showed a non-significant ($P \geq 0.05$) variation while the monocytes decreased at day 60 and eosinophils at days 30 and 60, significantly ($P \leq 0.05$). No significant variation was observed when compared with age-matched control (Table 3, Fig. 2(h-l).

Table 3: Changes in leukocytic attributes in Alloxan (@ 50mg/kg b.w) streptozotocin (@35 mg/kg b.w) cocktail diabetic rabbits (Mean ± SE).

Parameter	Treatment	Days of treatment				
		0day	15day	30day	45day	60day
Number	ALL+STZ	10	10	8	6	4
	Control	6	6	6	6	6
TLC (10^3/cmm)	ALL+STZ	9.26^{aA} ± 0.315 (8.30 - 11.90)	7.28^{bA} ± 0.140 (6.90 - 8.50)	6.72^{bA} ± 0.134 (6.20 - 7.30)	6.63^{bA} ± 0.240 (5.90 - 7.40)	6.78^{bA} ± 0.170 (6.30 - 7.10)
	Control	9.18^{aA} ± .453 (8.30 - 11.30)	9.24^{aB} ± .241 (8.35 - 9.90)	8.99^{aB} ± .107 (8.70 - 9.40)	9.08^{aB} ± .113 (8.75 - 9.55)	8.98^{aB} ± .092 (8.70 - 9.30)
Heterophils (%)	ALL+STZ	30.20^{aA} ±	31.10^{aA} ±	34.00^{aA} ±	35.00^{aA} ±	34.00^{aA} ±

		1.497 (23.00 - 38.00)	1.233 (25.00 - 36.00)	1.535 (27.00 - 40.00)	1.592 (30.00 - 40.00)	0.913 (32.00 - 36.00)
	Control	30.00^{aA} ± 1.713 (25.00 - 35.00)	29.67^{aA} ± 2.362 (23.00 - 37.00)	31.33^{aA} ± 2.140 (25.00 - 37.00)	30.83^{aA} ± 1.957 (26.00 - 37.00)	30.67^{aA} ± 1.961 (23.00 - 35.00)
Lymphocytes (%)	**ALL+STZ**	61.70^{aA} ± 1.230 (56.00 - 69.00)	60.30^{aA} ± 1.317 (55.00 - 68.00)	59.25^{aA} ± 1.306 (55.00 - 66.00)	57.83^{aA} ± 1.424 (54.00 - 63.00)	60.75^{aA} ± 0.250 (60.00 - 61.00)
	Control	62.67^{aA} ± 1.606 (58.00 - 68.00)	62.33^{aA} ± 1.764 (56.00 - 68.00)	61.83^{aA} ± 2.482 (56.00 - 71.00)	63.17^{aB} ± 2.272 (55.00 - 70.00)	63.33^{aA} ± 2.261 (56.00 - 72.00)
Monocytes (%)	**ALL+STZ**	5.00^{abA} ± 0.298 (4.00 - 7.00)	6.20^{aA} ± 0.742 (3.00 - 10.00)	5.00^{abA} ± 0.463 (3.00 - 7.00)	5.17^{abA} ± 0.793 (3.00 - 8.00)	3.75^{bA} ± 0.750 (2.00 - 5.00)
	Control	5.67^{aA} ± 0.715 (3.00 - 8.00)	5.50^{aA} ± 0.922 (3.00 - 9.00)	4.83^{aA} ± 0.401 (3.00 - 6.00)	4.00^{aA} ± 0.365 (3.00 - 5.00)	4.33^{aA} ± 0.494 (3.00 - 6.00)
Eosinophils (%)	**ALL+STZ**	2.40^{aA} ± 0.306 (1.00 - 4.00)	1.70^{abA} ± 0.260 (1.00 - 3.00)	1.38^{bA} ± 0.263 (0.00 - 2.00	1.67^{abA} ± 0.333 (1.00 - 3.00)	1.25^{bA} ± 0.250 (1.00 - 2.00)
	Control	1.67^{aA} ± 0.211 (1.00 - 2.00)	1.83^{aA} ± 0.307 (1.00 - 3.00)	1.50^{aA} ± 0.224 (1.00 - 2.00)	1.50^{aA} ± 0.224 (1.00 - 2.00)	1.17^{aA} ± 0.307 (0.00 - 2.00)
Basophils (%)	**ALL+STZ**	0.70^{aA} ± 0.213 (0.00 - 2.00)	0.70^{aA} ± 0.153 (0.00 - 1.00)	0.38^{aA} ± 0.183 (0.00 - 1.00)	0.33^{aA} ± 0.211 (0.00 - 1.00)	0.25^{aA} ± 0.250 (0.00 - 1.00)
	Control	0.83^{aA} ± 0.167 (0.00 - 1.00)	0.67^{aA} ± 0.333 (0.00 - 2.00)	0.50^{aA} ± 0.224 (0.00 - 1.00)	0.50^{aA} ± 0.224 (0.00 - 1.00)	0.50^{aA} ± 0.224 (0.00 - 1.00)

Means for a parameter in different columns bearing at least one common 'lowercase alphabet' superscript, and in different rows bearing at least one common 'uppercase alphabet' superscript does not differ significantly (P≤ 0.05).

BLOOD BIOCHEMICAL INDICES

BLOOD GLUCOSE

The mean blood glucose level was highly significantly (P≤0.05) at 1 week post Alloxan-STZ administration. Thereafter, the glucose levels revealed a progressive drop up to 8 weeks, but the values were still significantly higher from the values at base level as well as from the control group. At 6 weeks the mean values were less than 200gm/dl and none of the rabbits had blood glucose levels more than 200gm/dl from 7 weeks (Table 4, Fig. 3(a)

Table 4: Changes in blood glucose levels (mg/dL) in Alloxan (@ 50 mg/kg b.w) Streptozotocin (@ 35 mg/kg b.w) cocktail diabetic rabbits (Mean ± SE).

	ALLOXAN + STZ			**CONTROL**		
	N	**Mean ± S.E.**	**Range**	**N**	**Mean ± S.E.**	**Range**
0 day	10	111.50^{aA} ± 4.942	97.00 - 146.00	6	113.00^{aA} ± 7.165	98.00 - 145.00
1 week	10	270.10^{bA} ± 22.570	220.00 - 467.00	6	111.50^{aB} ± 5.271	98.00 - 135.00
2 week	10	264.70^{bA} ± 10.129	235.00 - 350.00	6	117.17^{aB} ± 6.882	102.00 - 140.00
3 week	8	261.88^{bA} ± 11.925	232.00 - 335.00	6	113.83^{aB} ± 3.400	99.00 - 122.00
4 week	8	239.38^{bcA} ± 7.035	210.00 - 270.00	6	114.67^{aB} ± 6.637	100.00 - 145.00
5 week	6	212.00^{cdA} ± 12.604	180.00 - 263.00	6	118.17^{aB} ± 6.348	100.00 - 143.00
6 week	6	184.50^{deA} ± 9.804	150.00 - 210.00	6	117.83^{aB} ± 6.332	97.00 - 138.00
7 week	4	160.25^{eA} ± 15.532	130.00 - 189.00	6	111.00^{aB} ± 4.524	101.00 - 125.00

8 week	4	$154.25^{eA} \pm 12.065$	137.00 - 190.00	6	$120.67^{aB} \pm 6.020$	108.00 - 148.00

Means in different rows bearing at least one common 'lowercase alphabet' superscript, and in different columns bearing at least one common 'uppercase alphabet' superscript does not differ significantly (P≤ 0.05).

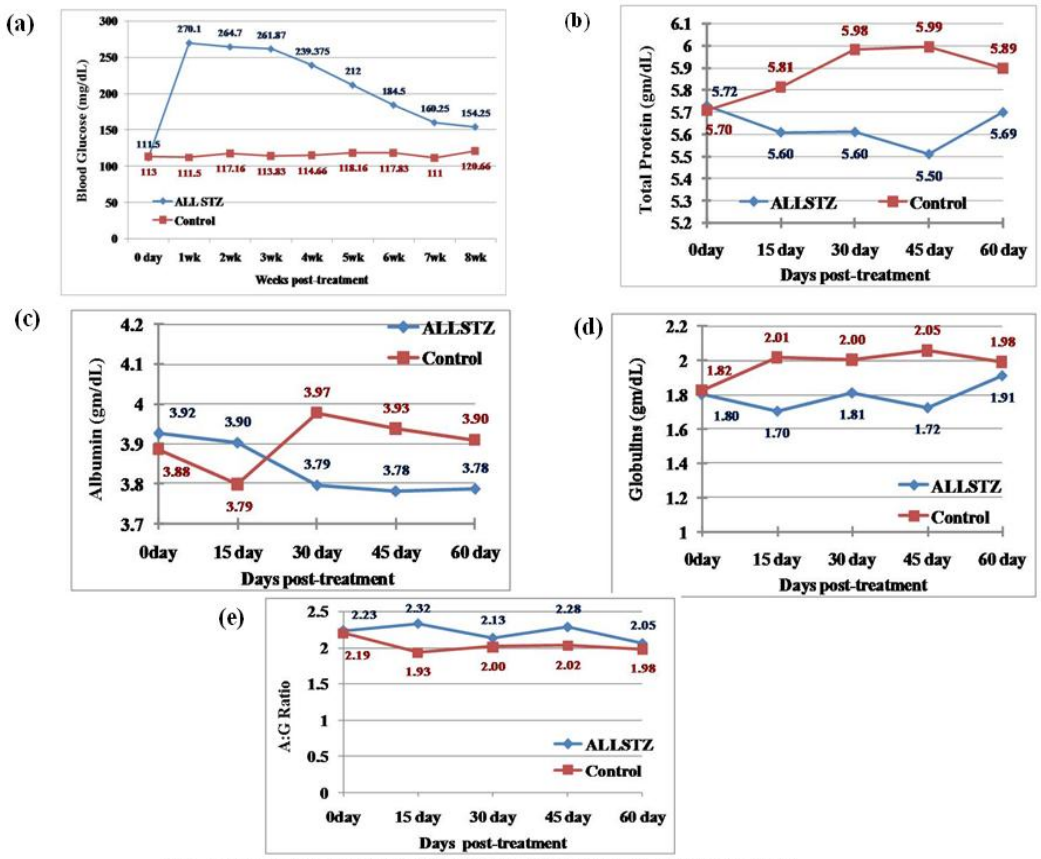

Fig. 3: Biochemical indices in Alloxan-STZ Diabetic Rabbit Model

PLASMA PROTEINS

The variations in the plasma levels of total protein, albumin, globulin and albumin:globulin (A:G) ratio were statistically non-significant (P≥0.05) (Table 5, Fig. 3(b-e).

Table 5: Changes in plasma protein levels in Alloxan (@ 50 mg/kg b.w) Streptozotocin (@ 35 mg/kg b.w) cocktail diabetic rabbits (Mean ± SE).

Parameter	Treatment	Days of treatment				
		0day	15day	30day	45day	60day
Number	ALL+STZ	10	10	8	6	4
	Control	6	6	6	6	6
Total Protein (gm/dL)	ALL+STZ	$5.73^{aA} \pm 0.281$ (4.79 - 7.56)	$5.61^{aA} \pm 0.261$ (4.53 - 7.01)	$5.61^{aA} \pm 0.302$ (4.35 - 6.90)	$5.51^{aA} \pm 0.405$ (4.47 - 7.00)	$5.70^{aA} \pm 0.487$ (4.87 - 6.87)
	Control	$5.71^{aA} \pm 0.348$ (4.62 - 7.05)	$5.81^{aA} \pm 0.400$ (4.53 - 7.34)	$5.98^{aA} \pm 0.287$ (4.99 - 6.74)	$5.99^{aA} \pm 0.310$ (5.25 - 6.99)	$5.90^{aA} \pm 0.214$ (5.32 - 6.69)
Albumin (gm/dL)	ALL+STZ	$3.93^{aA} \pm 0.239$ (3.18 - 5.34)	$3.90^{aA} \pm 0.248$ (2.98 - 5.41)	$3.80^{aA} \pm 0.271$ (2.93 - 5.15)	$3.78^{aA} \pm 0.333$ (3.11 - 5.37)	$3.79^{aA} \pm 0.378$ (3.12 - 4.87)
	Control	$3.89^{aA} \pm 0.385$ (2.68 - 5.34)	$3.80^{aA} \pm 0.387$ (2.82 - 5.25)	$3.98^{aA} \pm 0.186$ (3.26 - 4.47)	$3.94^{aA} \pm 0.108$ (3.54 - 4.35)	$3.91^{aA} \pm 0.128$ (3.58 - 4.45)
Globulin	ALL+STZ	$1.80^{aA} \pm$	$1.70^{aA} \pm$	$1.81^{aA} \pm$	$1.73^{aA} \pm$	$1.91^{aA} \pm$

(gm/dL)		0.112 (1.34 - 2.40)	0.077 (1.38 - 2.15)	0.107 (1.40 - 2.38)	0.197 (1.36 - 2.69)	0.237 (1.35 - 2.50)
	Control	1.82^{aA} ± 0.118 (1.56 - 2.36)	2.02^{aA} ± 0.144 (1.64 - 2.64)	2.00^{aA} ± 0.132 (1.57 - 2.37)	2.06^{aA} ± 0.238 (1.36 - 2.96)	1.99^{aA} ± 0.105 (1.70 - 2.37)
Albumin:Globulin Ratio	**ALL+STZ**	2.23^{aA} ± 0.158 (1.47 - 2.81)	2.33^{aA} ± 0.174 (1.69 - 3.38)	2.13^{aA} ± 0.171 (1.45 - 2.95)	2.28^{aA} ± 0.249 (1.40 - 3.29)	2.06^{aA} ± 0.276 (1.46 - 2.61)
	Control	2.20^{aA} ± 0.291 (1.38 - 3.13)	1.93^{aA} ± 0.243 (1.27 - 2.79)	2.01^{aA} ± 0.106 (1.64 - 2.42)	2.03^{aA} ± 0.210 (1.36 - 2.91)	1.98^{aA} ± 0.071 (1.68 - 2.16)

Means for a parameter in different columns bearing at least one common 'lowercase alphabet' superscript, and in different rows bearing at least one common 'uppercase alphabet' superscript does not differ significantly (P≤ 0.05).

PLASMA ENZYMOLOGY

The mean ALT and AST levels increased non-significantly (P≤0.05). ALT:AST ratio, also, showed a non-significant(P≤0.05) variation during the experimental period. (Table 6, Fig. 4(a-c).

Table 6: Changes in plasma enzyme activity in Alloxan (@ 50 mg/kg b.w) Streptozotocin (@ 35 mg/kg b.w) cocktail diabetic rabbits (Mean ± SE).

Parameter	**Treatment**	**Days of treatment**				
		0day	**15day**	**30day**	**45day**	**60day**
Number	**ALL+STZ**	10	10	8	6	4
	Control	6	6	6	6	6
SGOT /AST (IU/L)	**ALL+STZ**	12.52^{aA} ± 2.408 (3.75 - 30.32)	16.41^{aA} ± 2.690 (6.45 - 35.54)	19.48^{aA} ± 2.639 (10.23 - 35.10)	19.55^{aA} ± 2.536 (12.64 - 30.89)	17.46^{aA} ± 1.910 (12.35 - 20.53)
	Control	14.85^{aA} ± 3.416 (4.05 - 28.25)	$15.\ 50^{aA}$ ± 3.966 (7.40 - 33.30)	14.71^{aA} ± 3.536 (5.98 - 30.35)	14.79^{aA} ± 3.210 (7.32 - 27.85)	15.17^{aA} ± 2.794 (6.45 - 23.34)
SGPT/ ALT (IU/L)	**ALL+STZ**	15.22^{aA} ± 2.568 (5.88 - 33.56)	18.98^{aA} ± 2.488 (9.98 - 36.67)	21.91^{aA} ± 2.319 (15.48 - 36.73)	21.05^{aA} ± 2.660 (15.47 - 33.21)	21.44^{aA} ± 1.263 (19.28 - 25.03)
	Control	17.98^{aA} ± 3.839 (5.91 - 32.80)	16.55^{aA} ± 4.246 (8.35 - 35.56)	17.18^{aA} ± 4.233 (7.54 - 36.32)	16.66^{aA} ± 3.527 (9.20 - 30.43)	15.63^{aA} ± 2.952 (6.87 - 25.47)
OT:PT Ratio	**ALL+STZ**	0.80^{aA} ± 0.026 (0.64 - 0.90)	0.84^{aA} ± 0.052 (0.53 - 1.08)	0.88^{aA} ± 0.051 (0.56 - 1.01)	0.93^{aA} ± 0.048 (0.79 - 1.08)	0.82^{aA} ± 0.090 (0.61 - 1.05)
	Control	0.81^{aA} ± 0.031 (0.69 - 0.90)	0.94^{bcA} ± 0.020 (0.89 - 1.03)	0.86^{abA} ± 0.026 (0.79 - 0.98)	0.89^{abA} ± 0.027 (0.79 - 0.96)	0.97^{cA} ± 0.037 (0.92 - 1.16)

Means for a parameter in different columns bearing at least one common 'lowercase alphabet' superscript, and in different rows bearing at least one common 'uppercase alphabet' superscript does not differ significantly (P≤ 0.05).

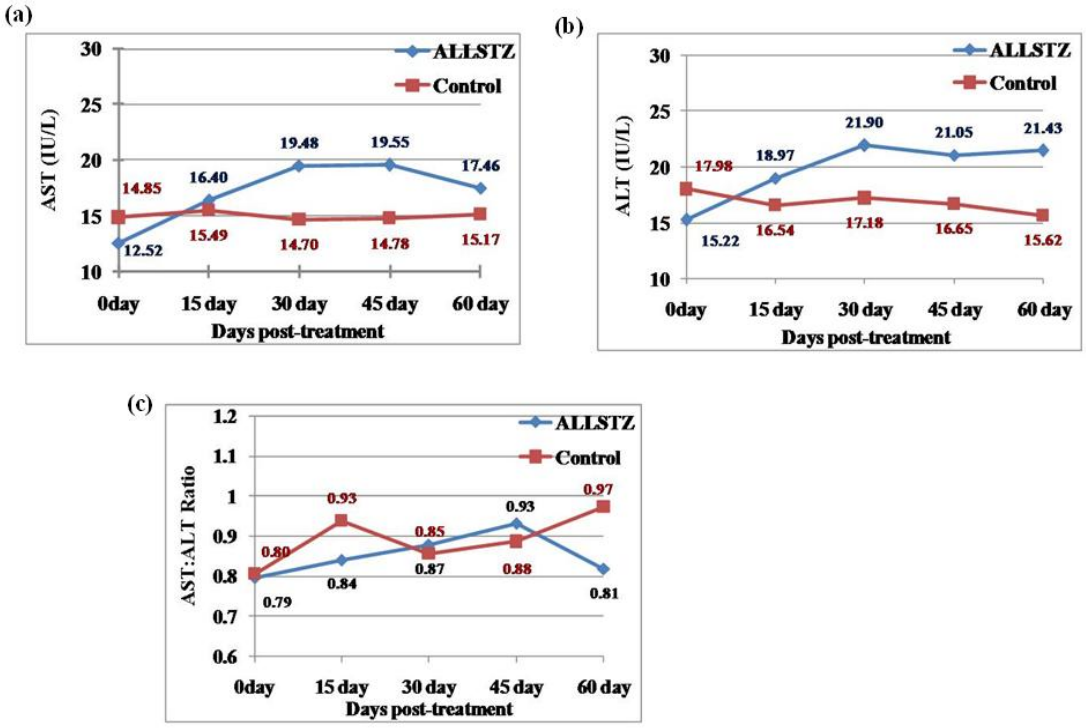

Fig. 4: Estimation of plasma liver enzymes in Alloxan-STZ Diabetic Rabbit Model

KIDNEY FUNCTION TEST (KFT)

The mean values of BUN levels did not differ significantly (P≥0.05). When compared with the base level value a progressive and significant (P≤0.05) increase in plasma creatinine was found from day 15. Though the control rabbits also showed a significant increase in creatinine values yet they were significantly (P≤0.05) lower than the treated rabbits at day 60. Plasma chloride values showed a progressive and significant (P≤0.05) decrease from day 15 (Table 7, Fig. 5(a-c).

Table 7: Changes in kidney function test attributes in Alloxan (@ 50mg/kg b.w) Streptozotocin (@ 35mg/kg b.w) cocktail diabetic rabbits (Mean ± SE).

Parameter	Treatment	Days of treatment				
		0day	15day	30day	45day	60day
Number	ALL+STZ	10	10	8	6	4
	Control	6	6	6	6	6
Blood Urea Nitrogen (mg/dL)	ALL+STZ	31.46^{aA} ± 3.030 (17.47 - 42.20)	33.68^{aA} ± 2.756 (22.57 - 45.13)	34.16^{aA} ± 3.299 (24.97 - 48.88)	38.31^{aA} ± 3.996 (27.95 - 51.09)	39.87^{aA} ± 6.225 (30.12 - 56.33)
	Control	29.97^{aA} ± 3.064 (21.10 - 42.56)	30.79^{aA} ± 3.416 (19.37 - 38.96)	28.68^{aA} ± 2.654 (22.47 - 40.54)	30.23^{aA} ± 2.785 (23.78 - 41.17)	30.01^{aA} ± 2.489 (22.32 - 38.82)
Creatinine (mg/dL)	ALL+STZ	0.87^{aA} ± 0.058 (0.58 - 1.24)	1.08^{bA} ± 0.050 (0.88 - 1.33)	1.23^{bcA} ± 0.054 (1.00 - 1.45)	1.37^{cdA} ± 0.052 (1.22 - 1.54)	1.51^{dA} ± 0.053 (1.40 - 1.65)
	Control	0.89^{aA} ± 0.050 (0.67 - 0.99)	0.98^{abA} ± 0.050 (0.76 - 1.12	1.04^{bcA} ± 0.048 (0.88 - 1.21	1.14^{cdA} ± 0.030 (1.03 - 1.23	1.20^{dB} ± 0.034 (1.12 - 1.35
Chloride (mmol/L)	ALL+STZ	114.99^{aA} ± 1.679 (107.60 - 123.10)	94.17^{bA} ± 0.801 (91.10 - 99.70)	89.09^{cA} ± 1.274 (83.60 - 94.10)	84.38^{dA} ± 1.036 (79.90 - 87.40)	84.15^{dA} ± 2.075 (78.40 - 88.30)
	Control	116.03^{aA} ± 3.309	114.90^{aB} ± 1.353	116.27^{aB} ± 2.843	114.13^{aB} ± 2.774	113.75^{aB} ± 2.531

		(102.70 - 123.30	(109.80 - 118.50	(107.50 - 124.50	(105.90 - 122.40	(105.80 - 122.50

Means for a parameter in different columns bearing at least one common 'lowercase alphabet' superscript, and in different rows bearing at least one common 'uppercase alphabet' superscript does not differ significantly (P≤ 0.05).

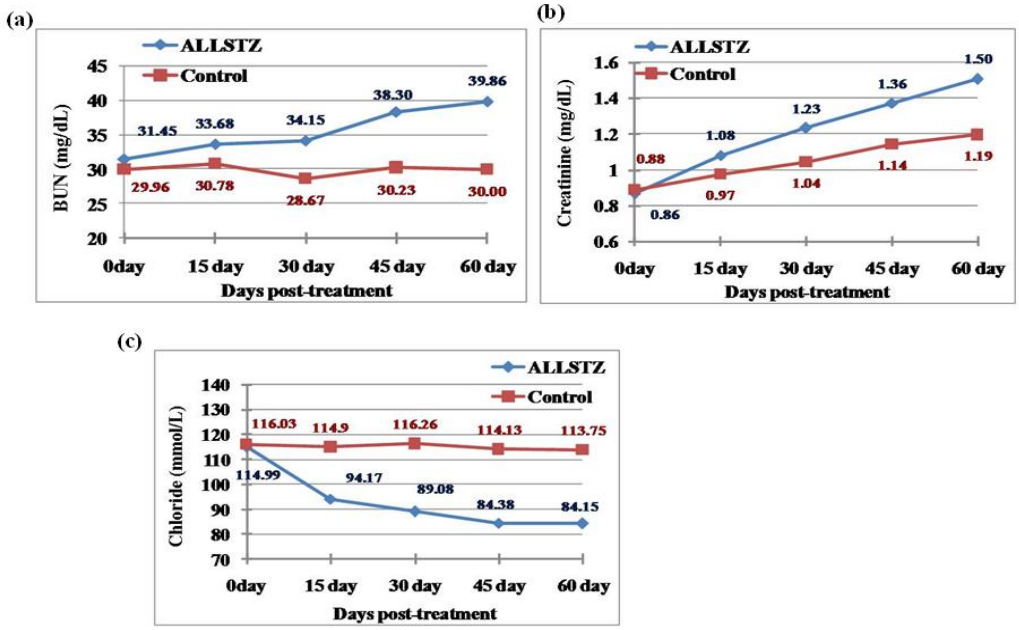

Fig. 5: Estimation of Kidney function test in Alloxan-STZ Diabetic Rabbit Model

PLASMA LIPIDS

Total plasma cholesterol was significantly (P≤0.05) higher than base values on days 45 and 60. When compared with control rabbits, the mean values were significantly (P≤0.05) higher from day 15. Plasma triglyceride levels were significantly (P≤0.05) higher than baseline values and that of control rabbits from day 30 (Table 8, Fig. 6(a-b)

Table 8: Changes in plasma lipid levels in Alloxan (@ 50mg/kg b.w) Streptozotocin (@ 35mg/kg b.w) cocktail diabetic rabbits (Mean ± SE).

Parameter	Treatment	Days of treatment				
		0day	15day	30day	45day	60day
Number	ALL+STZ	10	10	8	6	4
	Control	6	6	6	6	6
Total Cholesterol (mg/dL)	ALL+STZ	42.55^{aA} ± 1.618 (33.10 - 47.20)	43.34^{aA} ± 1.267 (37.50 - 49.30)	45.86^{aA} ± 1.209 (40.50 - 50.40)	54.85^{bA} ± 1.266 (51.50 - 60.30)	66.58^{cA} ± 2.463 (60.40 - 72.10)
	Control	39.30^{aA} ± 2.053 (31.90 - 46.20	39.28^{aB} ± 1.705 (33.80 - 43.80)	39.38^{aB} ± 1.244 (34.50 - 42.80)	41.08^{aB} ± 2.224 (33.80 - 48.10)	41.33^{aB} ± 1.377 (37.50 - 46.30)
Triglyceride (mg/dL)	ALL+STZ	58.53^{aA} ± 3.832 (42.36 - 76.36)	64.53^{aA} ± 3.253 (49.85 - 80.76)	81.09^{bA} ± 4.541 (63.89 - 105.33)	95.91^{cA} ± 3.773 (87.45 - 108.45)	108.74^{dA} ± 5.901 (95.45 - 121.34)
	Control	56.52^{aA} ± 8.919 (25.70 - 83.09)	60.35^{aA} ± 6.791 (33.65 - 78.23)	65.46^{aB} ± 5.498 (46.72 - 82.57)	57.80^{aB} ± 4.059 (43.67 - 70.33)	64.03^{aB} ± 4.266 (50.43 - 76.23)

Means for a parameter in different columns bearing at least one common 'lowercase alphabet' superscript, and in different rows bearing at least one common 'uppercase alphabet' superscript does not differ significantly (P≤ 0.05).

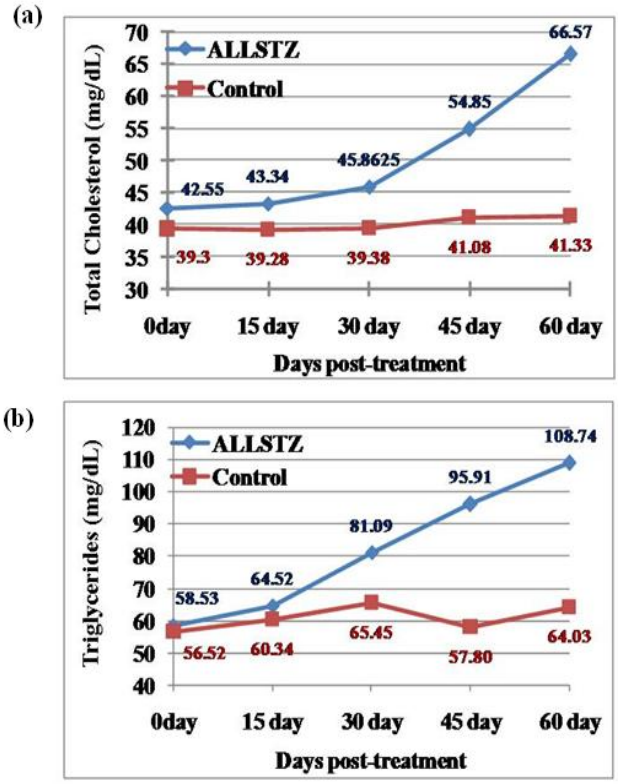

Fig. 6: Estimation of plasma lipids in Alloxan-STZ Diabetic Rabbit Model

DISCUSSION

The behavioral alterations including polydypsia, polyurea, polyphagia and decreased physical activities were characteristic of either Alloxan or STZ induced diabetes mellitus in rabbits and other animals (Mir, 2007; Wang *et al.*, 2010; Azeez *et al.*, 2010; Oyedemi *et al.* 2011). The observed mortality of 10% over a period of 60 days was lower than 20% observed in Alloxan diabetes and may be attributed to milder hyperglycemia and early onset of recovery.

Although the diabetic rabbits gained weight, it was much less than the age-matched nondiabetic rabbits and differed significantly at the end of the experiment. Loss of body weight has been reported in diabetic humans as well as animal models and attributed to metabolic disturbances associated with insulin insufficiency and hyperglycemia related oxidative stress (Wang *et al.*, 2010; Ghaisas *et al.*, 2010; Azeez *et al.*, 2010). The significantly lower body temperature may be attributed to glucotoxic effects in CNS leading to altered thermoregulation. Disturbances in heart rate may be attributed to development of cardiovascular lesions observed histopathologically. Howarth *et al.* (2011a,b) reported decreased body temperature and heart rate in Alloxan-diabetic rats.

Haematological evaluations revealed normochromic microcytic anemia at day 15 followed by progressive recovery. Haematopoietic parameters have been found to be variably affected in induced diabetic models (Azeez *et al.*, 2010; Tanko *et al.*, 2011; Valilou *et al.*, 2011; Oyedemi *et al.*, 2011). The observed changes correlated well with the glycemic changes. However, the direct effects of the toxins on erythrocytic fragility and interference with iron utilization due to redox disturbance (oxidative stress) need to be evaluated (Rao *et al.*, 2003).

Leukocytopenia without any changes in differential counts may be attributed to direct effects on myelopoiesis (Nichols *et al.*, 1981).

Mild to moderate diabetes was observed up to 6 weeks. However, a progressive decline was noted from 2nd week. This may be attributed to low dose of individual drugs which probably showed a synergistic effect for induction of diabetes with mean blood glucose level peaking at 1 week post drug-cocktail administration. Thus while the combination favours induction of diabetes by using lower doses avoiding direct toxic effects of the higher doses of individual drugs (Anderson *et al.* 1993), the short term maintenance of the condition due to spontaneous recovery remains a limiting factor (Etuk, 2010). Signs of islet regeneration were evident histopathologically.

Liver function test (LFT) was not significantly altered. The mild decrease in plasma protein levels associated with mild increase in AST and ALT, indicated altered liver function (Garella, 1997). Altered LFT have been reported in diabetes in humans (Elizabeth and Harris, 2005; Leeds *et al.* 2009) as well as in short and long term animal models (Wang *et al.*, 2010; Iweala and Oludare, 2011; Shanmugasundaram *et al.*, 2011; Erejuwa, 2012). The severity of altered LFT has been directly related to changes glycemic status and oxidative stress (Imaeda *et al.*, 2002). The

slight recovery observed at end of the experiment paralled the change in blood glucose levels.

The non-significant increase in BUN and significant changes in plasma creatinine and chloride levels revealed altered kidney function. Diabetes associated nephropathy has been considered as critical complication and directly related to glucolipotoxicity (Khushk *et al.*, 2010; Ghaisas *et al.*, 2010; Tavafi *et al.*, 2011; Ibrahim and Abdelatif, 2011; Sayed *et al.*, 2012).

Hypercholesterolemia and hypertriglyceridemia has been reported in both Alloxan and STZ induced diabetic models (Sharma *et al.*, 2010; Ghaisas *et al.*, 2010; Iweala and Oludare, 2011; Ayinla *et al.*, 2011; Ibrahim and Abdelatif, 2011) and has been associated with impaired insulin action (Kedar and Chakrabarti, 1983; Gibbons, 1988) and abnormalities in cellular cholesterol metabolism (O'Meara *et al.*, 1990).

CONCLUSION

It could be concluded that Alloxan-STZ cocktail shows synergistic effect for induction of diabetes in rabbits and maintains a moderate hyperglycemia for about 4 to 5 weeks, with subtle haemato-biochemical alterations. The model can be used for only subacute studies and long term investigations warrant induction using higher dosage with due consideration to direct toxic effects.

ETHICAL STATEMENT

All the relevant procedures related to animal health in this study were performed in compliance with aforementioned laws and institutional guidelines

CONFLICT OF INTEREST

The authors declare that they have no competing interests

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