CHAPTER – 11

NEONATAL FUNCTIONAL MELATONIN DEFICIENCY PREDISPOSES ADULT RATS TO B CELL LOSS AND DIABETOGENIC CHANGES IN RESPONSE TO WEANING ALLOXAN TREATMENT.

INTRODUCTION:

Diabetes mellitus is a metabolic disorder involving altered metabolic profile involving carbohydrates, lipids and proteins with a predeliction/predisposition towards vascular disorders (Keen and Tang Fui, 1982; Pickup and Williams, 2003). Persistent hyperglycemia is a characteristic mark for the disease (Keen and Tang Fui, 1982; Ziv *et al.*, 1999). Diabetes mellitus may present as relatively sudden, potentially lethal metabolic derangement or it can be associated few if any, symptoms or signs and may escape detection for many years. These extremes of clinical manifestations constitute the basis for sub dividing diabetes mellitus into the insulin dependent (IDDM) and the non-insulin dependent (NIDDM) types. These have been renamed as type I and type II diabetes mellitus respectively with the former characterized by B cell deficiency and the latter characterized by insulin resistance which might ultimately lead to insulin secretary defect (Keen and Tang Fui, 1982). In recent times relationship between

production of activated oxygen species and diabetes has been gaining attention. Activated oxygen species such as hydrogen peroxide, superoxide anions, singlet oxygen and hydroxyl radicals can be formed in cell not only by ionizing radiation, but also during aerobic metabolism of either endogenous or exogenous substances. Cells have enzymatic and non-enzymatic scavenger systems against these free radicals. However, if free radical production and scavenger systems become imbalanced, cells would be exposed to oxidative damage resulting in cell death (Parinandi *et al.*, 1990; Griesmacher *et al.*, 1995). Diabetes has now been shown to be a result of increased free radical production (Baynes, 1991). Mechanisms that contribute to the formation of free radicals in diabetes may include not only increased non-enzymatic and auto-oxidative glycosylation, but also metabolic stress resulting from changes in energy metabolism, levels of inflammatory mediators and the status of antioxidant defense (Grieshmacher *et al.*, 1995).

Since experimental animal models of diabetes are used conveniently for studying various diabetes associated changes/dysregulation. Streptozotocin an antibiotic and alloxan a tetra oxy pyrimidine, are the commonly used agents in experimental diabetes (Rakieten *et al.*, 1963; Szkudelski, 2001). Both these agents cause degeneration and necrosis of pancreatic B cells (Merzouk *et al.*, 2000) and evidences accumulating to suggest the crucial role of free radicals in streptozotocin and alloxan induced diabetes (Bromme *et al.*, 1999; Szkudelski, 2001; Damasceno *et al.*, 2002; Gul *et al.*, 2002). Recently neonatal models of diabetes are being generated by treating neonatal

rats in the 1st week with streptozotocin, these rats are now shown to have type II diabetic syndromes like hyperglycemia, abnormal glucose tolerance and hyperinsulinemia (Arulmozi *et al.*, 2004). Recent studies from this laboratory have shown significant age dependent chronological alterations in metabolic features in rats subjected to neonatal functional deficiency of melatonin by treating them with the melatonin receptor antagonist luzindole (nL rats) (Chapter 9). In this context another study was attempted to understand the consequences of experimental induction of diabetes at the weaning age (22 days) which were pretreated with luzindole in pre-weaning period. This study showed increased B cell proliferation and neogenesis with hyperinsulinemia, hypoglycemia and hypolipidemia about 3 weeks after experimental induction of diabetes by alloxan treatment on day 22 (Chapter 10). Since the neonatal streptozotocin diabetic models of rats have shown hyperglycemia and type II diabetic symptoms in the adult stage as long term consequences (Arulmozi *et al.*, 2004), it was thought pertinent to study the long term effect of weaning alloxan treatment on carbohydrate, lipid and protein profiles of tissues and serum along with serum insulin levels and histoarchitectural patterns of pancreas for possible long term effects about 6 weeks after alloxan treatment (60 days). Alloxan was used instead of streptozotocin as the latter is known to show differential sensitivity depending on species, strain, sex and nutrition state (Okamato, 1981).

MATERIAL AND METHODS: See page numbers 18-38.

RESULTS:

Body and Organs weights: The body weight of all the alloxanised rats decreased significantly as compared to control and nLT rats. The relative weight of pancreas of LA(100) and LA(75) rats decreased significantly as compared to CA(100) and the age matched controls. The relative weight of liver of all the alloxanised rats showed no significant alterations as compared to age matched controls. The relative weight of spleen of LA(75) rats increased significantly as compared to all the other groups. The relative weight of kidney of all the alloxanised rats decreased significantly as compared to age matched control rats. The relative weight of testes of LA(100) rats decreased significantly as compared to all other groups. The relative weight of adrenals of LA(100) increased significantly as compared to all the other groups of same age.

Serum glucose and insulin levels: The serum glucose level of all the alloxanised groups decreased significantly as compared to nLT rats. While the, serum insulin levels of LA(100) and LA(75) rats decreased significantly as compared to CA(100) and age matched controls. (Figure and Table; 11.16, 11.35).

Hepatic glycogen content and the activities of glycogen synthetase, glycogen phosphorylase and glucose-6-phosphatase: The hepatic glycogen content of LA(100) and LA(75) rats increased significantly as compared to the CA(100) rats. The hepatic glycogen synthetase activity of all the alloxanised rats decreased significantly as compared to nLT rats. The hepatic glycogen phosphorylase activity of

LA(100) rats increased significantly as compared to all other groups of same age. The glucose-6-phosphatase activity of all the alloxanised rats decreased significantly as compared to both nLT and age matched control rats. (Figure and Table; 11.17, 11.19, 11.21).

Muscle glycogen content and the activities of glycogen synthetase and glycogen phosphorylase: The muscle glycogen content of LA(100) rats increased significantly while that of LA(75) rats decreased significantly as compared to CA(100) rats. The muscle glycogen synthetase activity decreased significantly in all the alloxanised rats as compared nLT rats. The muscle glycogen phosphorylase activity decreased in LA(100) and LA(75) rats as compared to CA(100) all the alloxanised groups as compared to the nLT rats (Figure and Table; 11.15, 11.18).

`Hepatic and muscle protein content: The muscle protein content of LA(100), CA(100) and LA(75) rats increased significantly as compared to control rats. The liver protein content showed no significant alteration in alloxanised rats as compared to controls (Figure and Table; 11.22, 11.23).

Hepatic total lipid and cholesterol contents: The hepatic total lipid content of LA(75) rats increased significantly as compared to LA(100), CA(100) and age matched control and nLT rats. The hepatic cholesterol content of the alloxanised rats decreased significantly as compared to nLT rats (Figure and Table; 11.24, 11.25).

Muscle total lipid and cholesterol contents: The muscle total lipid content of the LA(100) and LA(75) rats decreased significantly as

compared to the CA(100) and age matched controls. The muscle cholesterol content of LA(75) rats increased significantly as compared to age matched control but decreased significantly as compared to nLT rats (Figure and Table; 11.26, 11.27).

Adipose tissue total lipid and cholesterol contents: The adipose tissue total lipid content of all the alloxanised rats decreased significantly as compared to age matched control rats while, the adipose tissue cholesterol content of LA(100) and LA(75) rats decreased significantly as compared to CA(100) and nLT and age matched controls (Figure and Table; 11.28, 11.29).

Serum lipid fractions: The serum triglyceride, total lipid, phospholipid and free fatty acid levels of all the alloxanised groups decreased significantly as compared to control rats. However the serum cholesterol level of LA(100) rats decreased significantly as compared to CA(100), nLT and control rats. (Figure and Table; 11.30, 11.31, 11.32, 11.33 and 11.34).

Histological observations: The pancreatic islets show significant reduction in B cell number with loss of B cells and poor recovery/regeneration in the luzindole treated animals. The pancreatic islets of experimental rats show loosely packed cells with a reduced B:A cell ratio.

Figure 11.1: Body weight of adult rats on 60th day subjected to neonatal luzindole treatment and weaning alloxanisation on 22nd day:

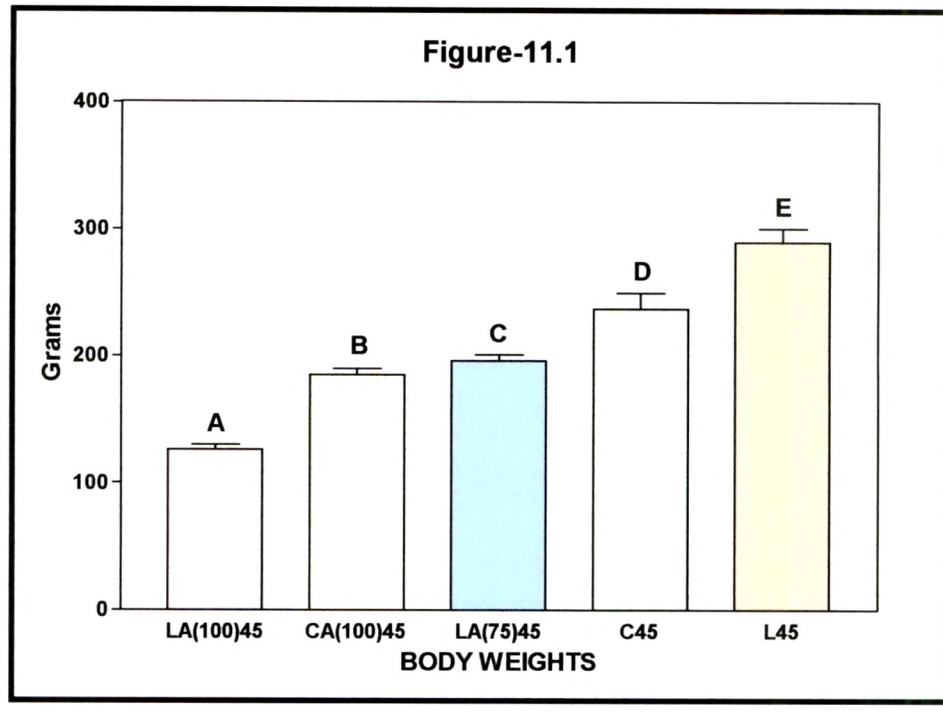


Table 11.1: Body weight of adult rats on 60th day subjected to neonatal luzindole treatment and weaning alloxanisation on 22nd day:

	LA(100) (A)	CA(100) (B)	LA(75) (C)	C (D)	L (E)
BODY WEIGHT	126.50 ±3.50	185.00 ±5.01	196.00 ±5.01	237.25 ±12.38	290.00 ±10.28

Bonferroni's Multiple Comparison Test

	AvsB	AvsC	AvsD	AvsE	BvsC	BvsD	BvsE	CvsD	CvsE	DvsE
p	■	◆	◆	◆	NS	■	◆	●	◆	■

Values are expressed as mean ± SEM, ◆p<0.001; ■P<0.01; ●P<0.05; NS Non Significant

Figure 11.2: Absolute pancreas weight of adult rats on 60th day subjected to neonatal luzindole treatment and weaning alloxanisation on 22nd day:

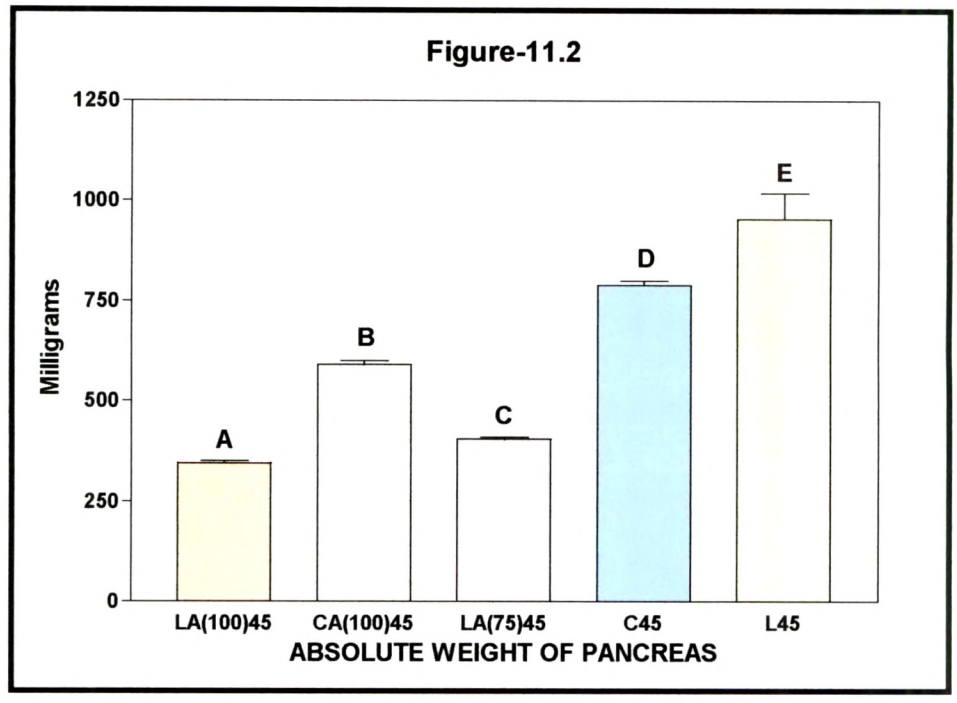


Table 11.2: Absolute pancreas weight of adult rats on 60th day subjected to neonatal luzindole treatment and weaning alloxanisation on 22nd day:

	LA(100) (A)	CA(100)(B)	LA(75)(C)	C(D)	L(E)
PANCREAS	345.00 ±5.01	590.00 ±10.02	405.00 ±5.01	790.00 ±10.02	955.00 ±64.00

Bonferroni's Multiple Comparison Test

	AvsB	AvsC	AvsD	AvsE	BvsC	BvsD	BvsE	CvsD	CvsE	DvsE
p	◆	NS	◆	◆	■	■	◆	◆	◆	●

Values are expressed as mean ± SEM, ◆p<0.001; ■P<0.01; ●P<0.05; NSNon Significant

Figure 11.3: Absolute liver weight of adult rats on 60th day subjected to neonatal luzindole treatment and weaning alloxanisation on 22nd day:

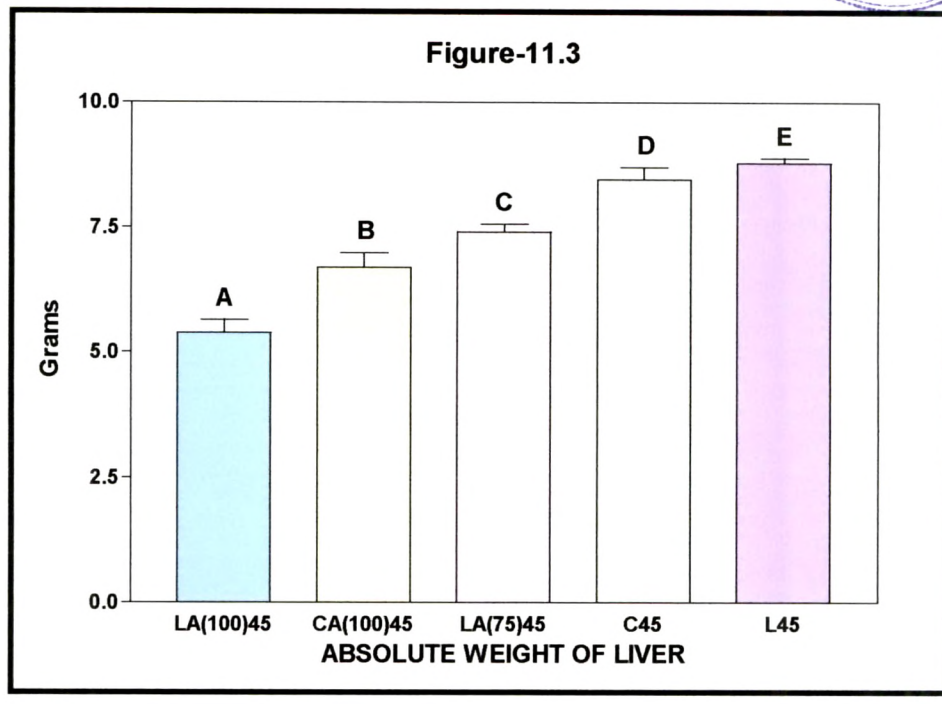


Table 11.3: Absolute liver weight of adult rats on 60th day subjected to neonatal luzindole treatment and weaning alloxanisation on 22nd day:

	LA(100) (A)	CA(100)(B)	LA(75)(C)	C(D)	L(E)
LIVER	5.39 ±0.26	6.70 ±0.29	7.41 ±0.15	8.46 ±0.24	8.79 ±0.099

Bonferroni's Multiple Comparison Test

	AvsB	AvsC	AvsD	AvsE	BvsC	BvsD	BvsE	CvsD	CvsE	DvsE
p	■	◆	◆	◆	NS	◆	◆	●	■	NS

Values are expressed as mean ± SEM, ◆p<0.001; ■P<0.01; ●P<0.05; NSNon Significant

Figure 11.4: Absolute spleen weight of adult rats on 60th day subjected to neonatal luzindole treatment and weaning alloxanisation on 22nd day:

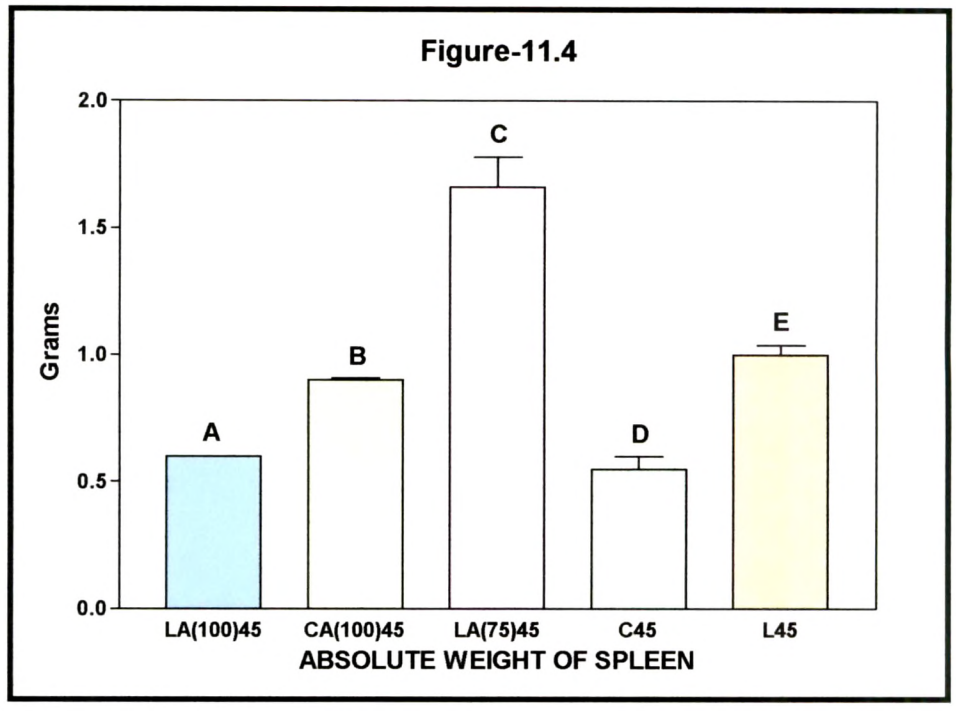


Table 11.4: Absolute spleen weight of adult rats on 60th day subjected to neonatal luzindole treatment and weaning alloxanisation on 22nd day:

	LA(100) (A)	CA(100) (B)	LA(75) (C)	C (D)	L (E)
SPLEEN	0.60 ±0.001	0.90 ±0.009	1.66 ±0.12	0.55 ±0.005	1.00 ±0.039

Bonferroni's Multiple Comparison Test

	AvsB	AvsC	AvsD	AvsE	BvsC	BvsD	BvsE	CvsD	CvsE	DvsE
p	●	◆	NS	■	◆	●	NS	◆	◆	■

Values are expressed as mean ± SEM, ◆p<0.001; ■P<0.01; ●P<0.05; NSNon Significant

Figure 11.5: Absolute kidney weight of adult rats on 60th day subjected to neonatal luzindole treatment and weaning alloxanisation on 22nd day:

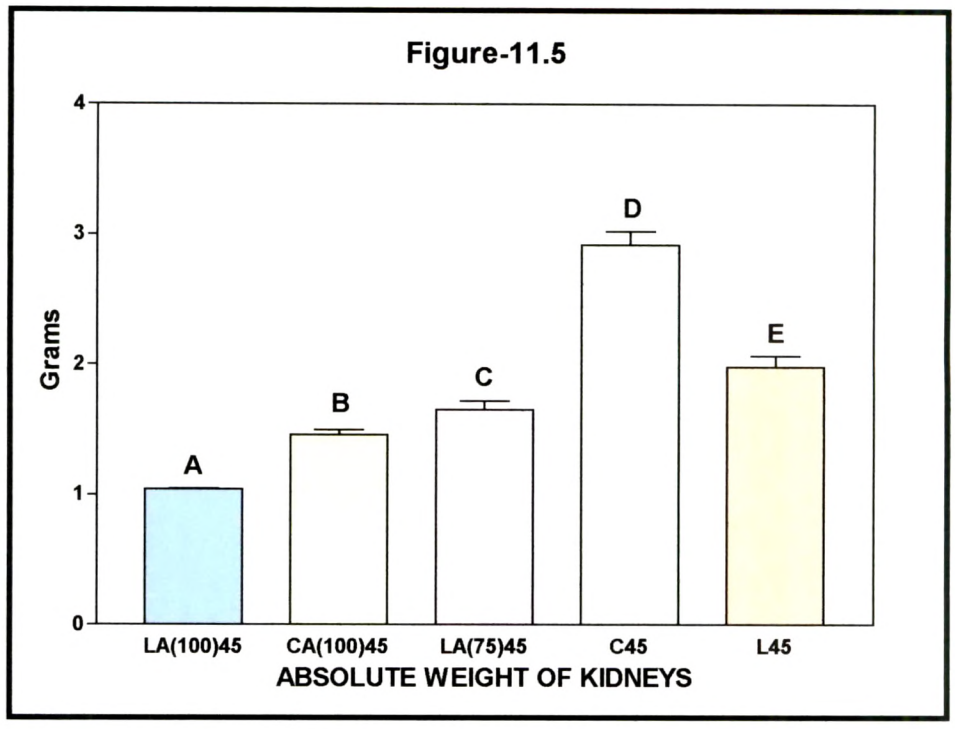


Table 11.5: Absolute kidney weight of adult rats on 60th day subjected to neonatal luzindole treatment and weaning alloxanisation on 22nd day:

	LA(100) (A)	CA(100) (B)	LA(75) (C)	C (D)	L (E)
KIDNEY	1.04 ±0.005	1.46 ±0.039	1.65 ±0.070	2.92 ±0.10	1.98 ±0.087

Bonferroni's Multiple Comparison Test

	AvsB	AvsC	AvsD	AvsE	BvsC	BvsD	BvsE	CvsD	CvsE	DvsE
p	■	◆	◆	◆	NS	◆	◆	◆	●	◆

Values are expressed as mean ± SEM, ◆p<0.001; ■P<0.01; ●P<0.05; NS Non Significant

Figure 11.6: Absolute testes weight of adult rats on 60th day subjected to neonatal luzindole treatment and weaning alloxanisation on 22nd day:

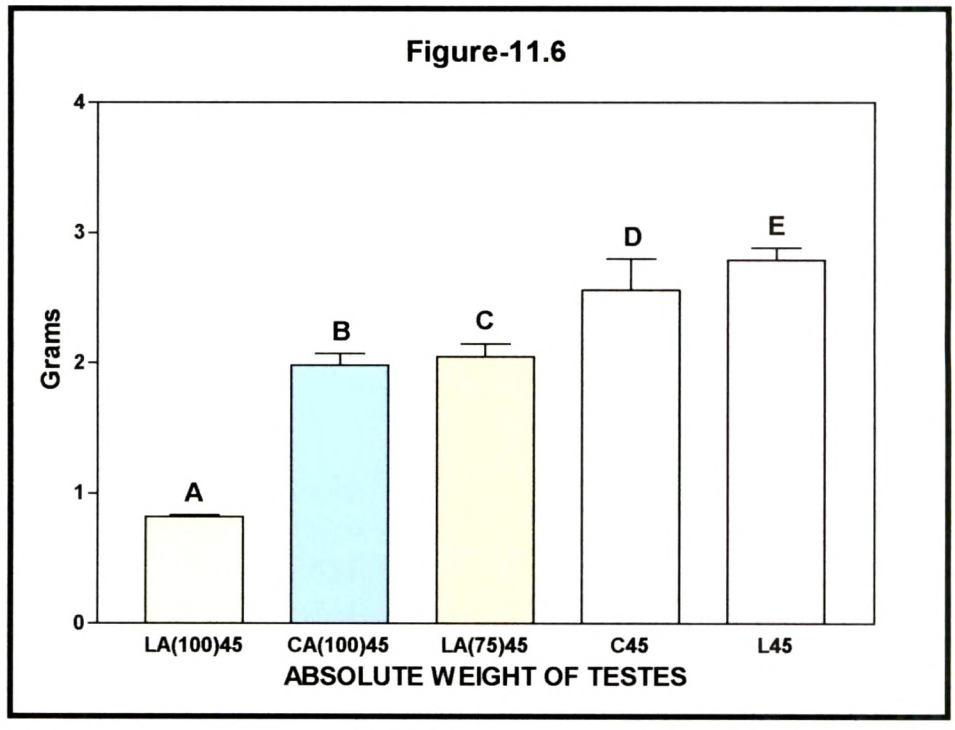


Table 11.6: Absolute testes weight of adult rats on 60th day subjected to neonatal luzindole treatment and weaning alloxanisation on 22nd day:

	LA(100) (A)	CA(100)(B)	LA(75)(C)	C(D)	L(E)
TESTES	0.82 ±0.015	1.98 ±0.092	2.05 ±0.10	2.56 ±0.24	2.79 ±0.095

Bonferroni's Multiple Comparison Test

	AvsB	AvsC	AvsD	AvsE	BvsC	BvsD	BvsE	CvsD	CvsE	DvsE
p	◆	◆	◆	◆	NS	NS	■	NS	●	NS

Values are expressed as mean ± SEM, ◆p<0.001; ■P<0.01; ●P<0.05; NS Non Significant

Figure 11.7: Absolute adrenal weight of adult rats on 60th day subjected to neonatal luzindole treatment and weaning alloxanisation on 22nd day:

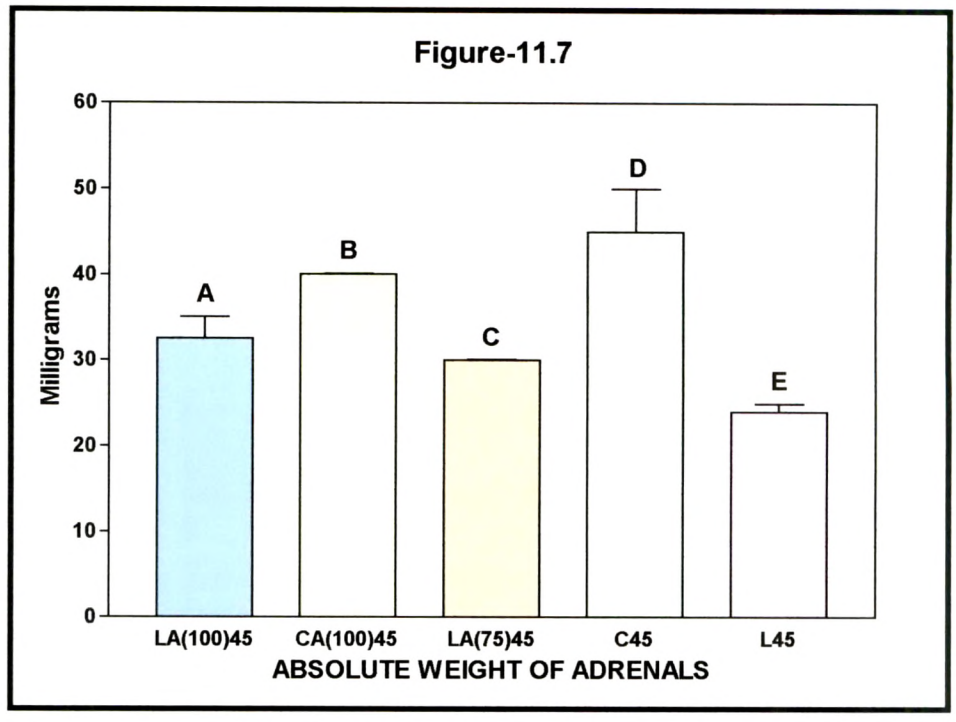


Table 11.7: Absolute adrenal weight of adult rats on 60th day subjected to neonatal luzindole treatment and weaning alloxanisation on 22nd day:

	LA(100) (A)	CA(100) (B)	LA(75) (C)	C (D)	L (E)
ADRENAL	32.50 ±2.50	40.00 ±0.10	30.00 ±0.09	45.00 ±5.01	24.00 ±0.90

Bonferroni's Multiple Comparison Test

	AvsB	AvsC	AvsD	AvsE	BvsC	BvsD	BvsE	CvsD	CvsE	DvsE
p	NS	NS	●	NS	NS	NS	■	■	NS	◆

Values are expressed as mean ± SEM, ◆p<0.001; ■P<0.01; ●P<0.05; NSNon Significant

Figure 11.8: Relative ancreas weight of adult rats on 60th day subjected to neonatal luzindole treatment and weaning alloxanisation on 22nd day:

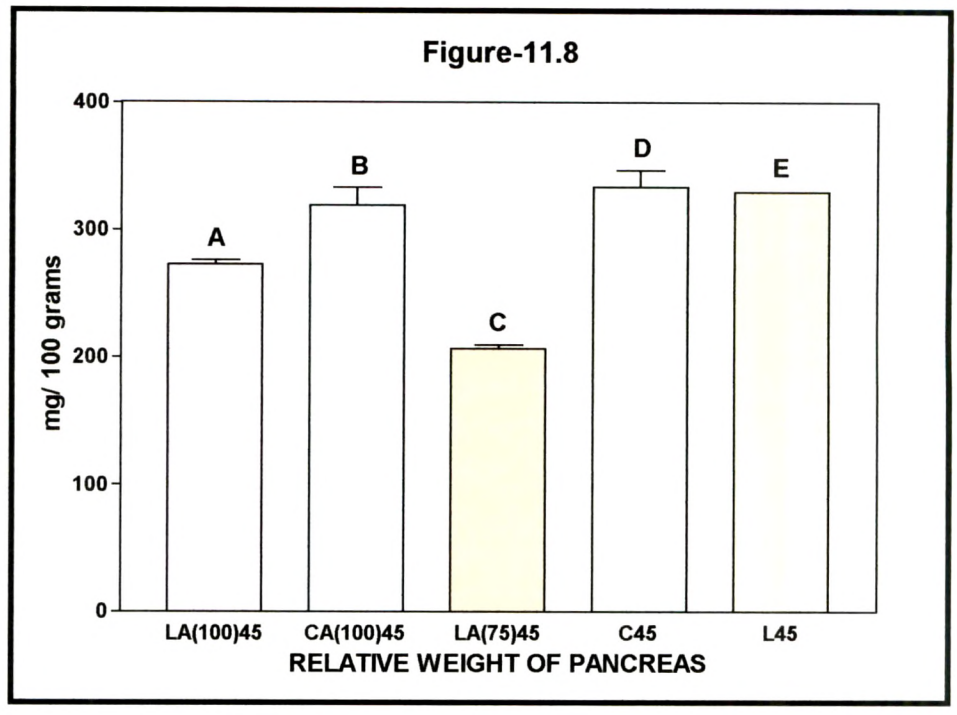


Table 11.8: Relative pancreas weight of adult rats on 60th day subjected to neonatal luzindole treatment and weaning alloxanisation on 22nd day:

	LA(100) (A)	CA(100) (B)	LA(75) (C)	C (D)	L (E)
PANCREAS	272.82 ±3.59	319.29 ±2.78	206.70 ±2.72	333.66 ±13.19	330.00 ±0.014

Bonferroni's Multiple Comparison Test

	AvsB	AvsC	AvsD	AvsE	BvsC	BvsD	BvsE	CvsD	CvsE	DvsE
p	●	◆	■	■	◆	NS	NS	◆	◆	NS

Values are expressed as mean ± SEM, ◆p<0.001; ■P<0.01; ●P<0.05; NS Non Significant

Figure 11.9: Relative liver weight of adult rats on 60th day subjected to neonatal luzindole treatment and weaning alloxanisation on 22nd day:

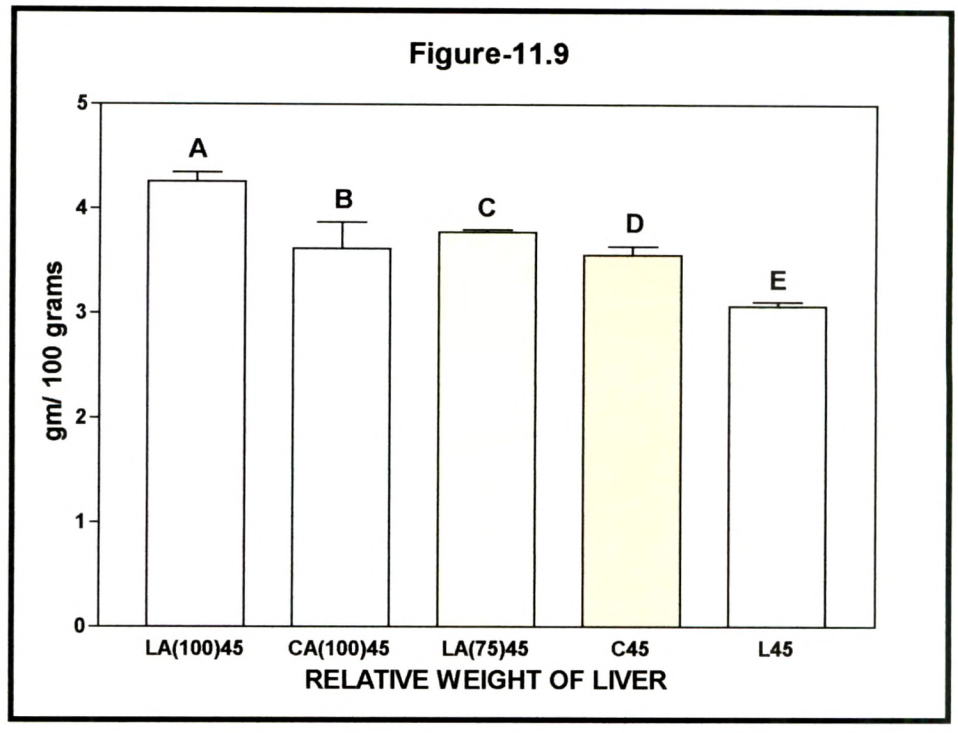


Table 11.9: Relative liver weight of adult rats on 60th day subjected to neonatal luzindole treatment and weaning alloxanisation on 22nd day:

	LA(100) (A)	CA(100) (B)	LA(75) (C)	C (D)	L (E)
LIVER	4.26 ±0.09	3.62 ±0.25	3.78 ±0.02	3.56 ±0.085	3.07 ±0.044

Bonferroni's Multiple Comparison Test

	AvsB	AvsC	AvsD	AvsE	BvsC	BvsD	BvsE	CvsD	CvsE	DvsE
p	●	NS	●	◆	NS	NS	NS	NS	●	NS

Values are expressed as mean ± SEM, ◆p<0.001; ●P<0.05;
NS Non Significant

Figure 11.10: Relative spleen weight of adult rats on 60th day subjected to neonatal luzindole treatment and weaning alloxanisation on 22nd day:

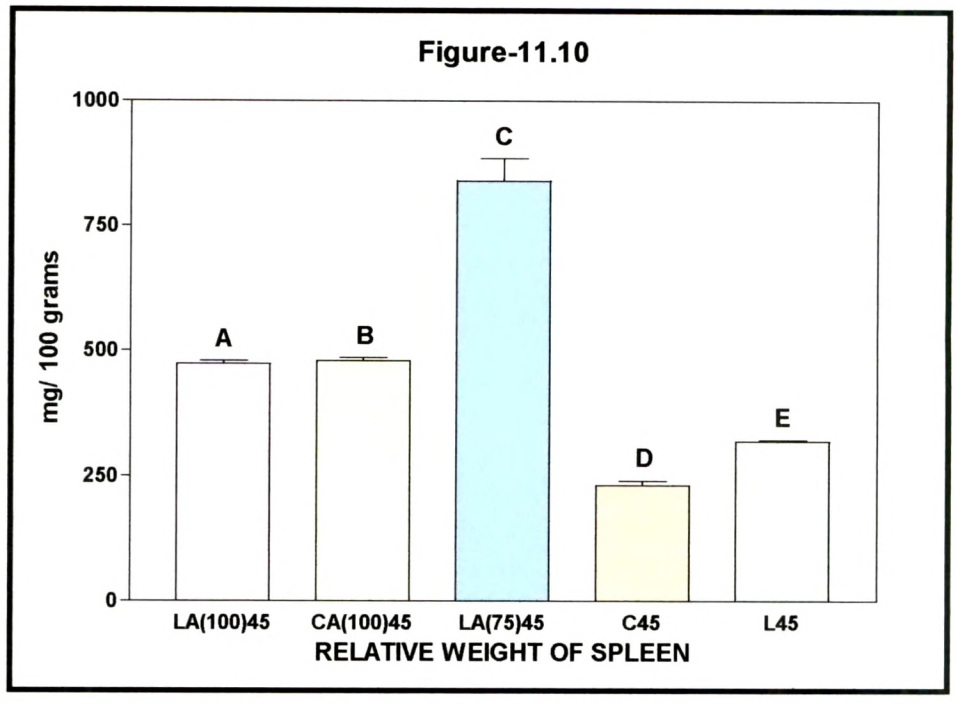


Table 11.10: Relative spleen weight of adult rats on 60th day subjected to neonatal luzindole treatment and weaning alloxanisation on 22nd day:

	LA(100) (A)	CA(100)(B)	LA(75)(C)	C(D)	L(E)
SPLEEN	474.45 ±5.22	480.00 ±6.40	840.00 ±45.00	231.35 ±9.05	320.00 ±1.90

Bonferroni's Multiple Comparison Test

	AvsB	AvsC	AvsD	AvsE	BvsC	BvsD	BvsE	CvsD	CvsE	DvsE
p	NS	◆	◆	■	◆	◆	◆	◆	◆	NS

Values are expressed as mean ± SEM, ◆p<0.001; ■P<0.01; NSNon Significant

Figure 11.11: Relative kidney weight of adult rats on 60th day subjected to neonatal luzindole treatment and weaning alloxanisation on 22nd day:

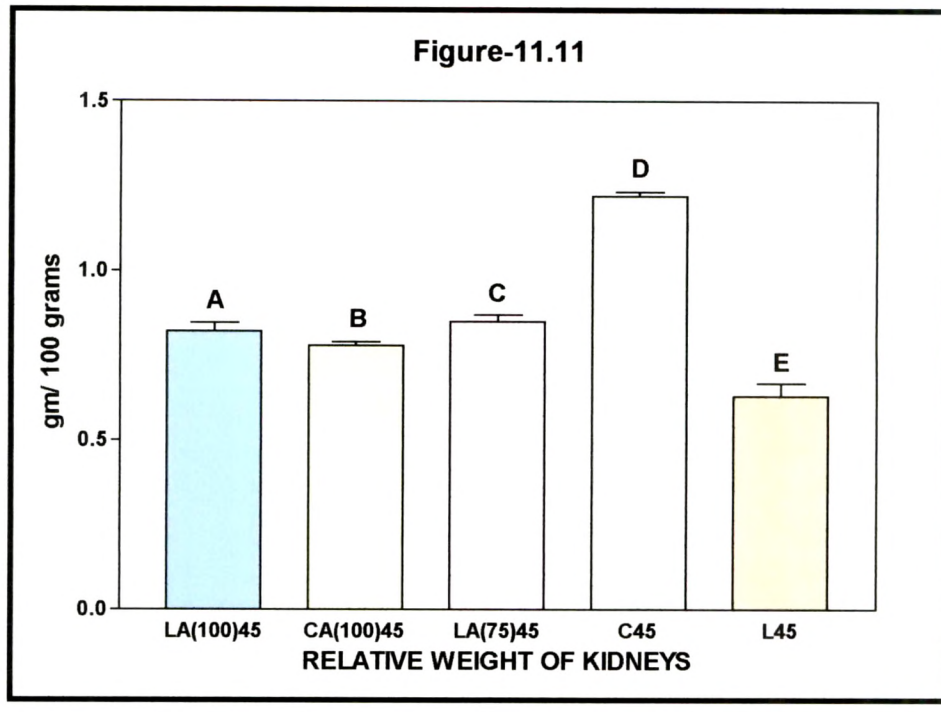


Table 11.11: Relative kidney weight of adult rats on 60th day subjected to neonatal luzindole treatment and weaning alloxanisation on 22nd day:

	LA(100) (A)	CA(100)(B)	LA(75)(C)	C(D)	L(E)
KIDNEY	0.82 ±0.026	0.78 ±0.001	0.85 ±0.02	1.22 ±0.014	0.63 ±0.039

Bonferroni's Multiple Comparison Test

	AvsB	AvsC	AvsD	AvsE	BvsC	BvsD	BvsE	CvsD	CvsE	DvsE
p	NS	NS	◆	◆	NS	◆	■	◆	◆	◆

Values are expressed as mean ± SEM, ◆p<0.001; ■P<0.01;
NSNon Significant

Figure 11.12: Relative testes weight of adult rats on 60th day subjected to neonatal luzindole treatment and weaning alloxanisation on 22nd day:

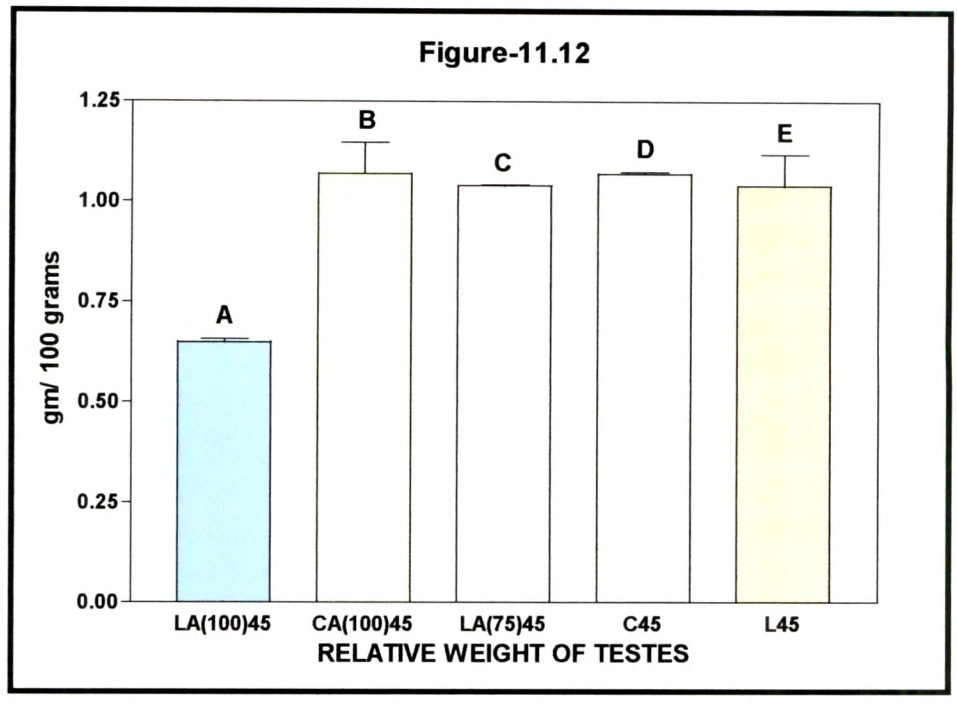


Table 11.12: Relative testes weight of adult rats on 60th day subjected to neonatal luzindole treatment and weaning alloxanisation on 22nd day:

	LA(100) (A)	CA(100)(B)	LA(75)(C)	C(D)	L(E)
TESTES	0.65 ±0.006	1.07 ±0.078	1.04 ±0.002	1.07 ±0.042	1.04 ±0.078

Bonferroni's Multiple Comparison Test

	AvsB	AvsC	AvsD	AvsE	BvsC	BvsD	BvsE	CvsD	CvsE	DvsE
p	◆	◆	◆	◆	NS	NS	NS	NS	NS	NS

Values are expressed as mean ± SEM, ◆p<0.001; NSNon Significant

Figure 11.13: Relative adrenal weight of adult rats on 60th day subjected to neonatal luzindole treatment and weaning alloxanisation on 22nd day:

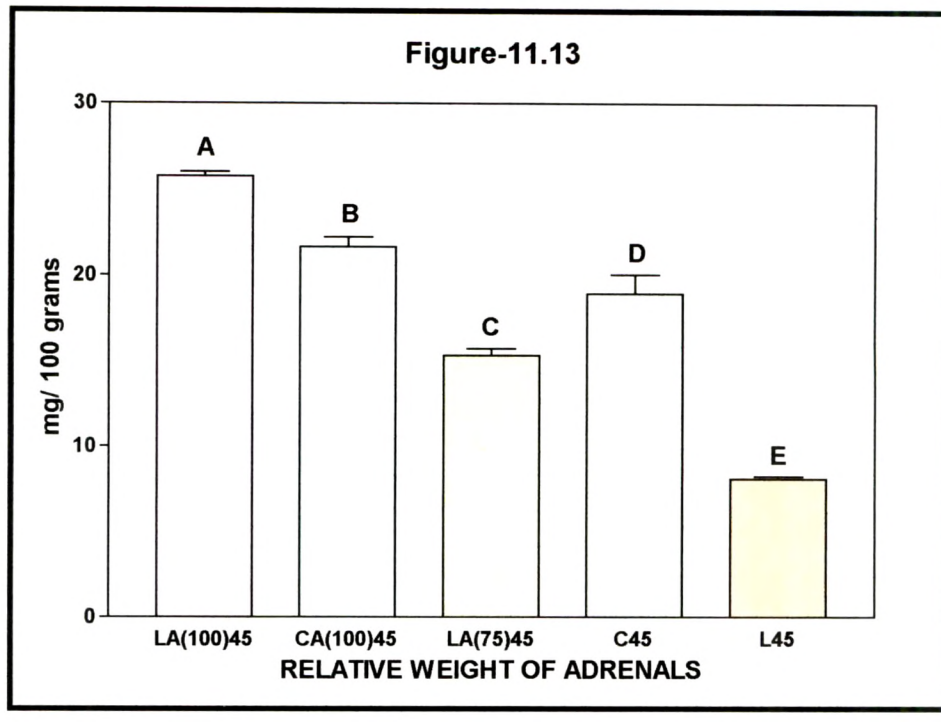


Table 11.13: Relative adrenal weight of adult rats on 60th day subjected to neonatal luzindole treatment and weaning alloxanisation on 22nd day:

	LA(100) (A)	CA(100) (B)	LA(75) (C)	C (D)	L (E)
ADRENAL	25.76 ±0.26	21.63 ±0.58	15.31 ±0.39	18.90 ±1.12	8.11 ±0.14

Bonferroni's Multiple Comparison Test

	AvsB	AvsC	AvsD	AvsE	BvsC	BvsD	BvsE	CvsD	CvsE	DvsE
p	■	◆	◆	◆	◆	NS	◆	■	◆	◆

Values are expressed as mean ± SEM, ◆p<0.001; ■P<0.01; NSNon Significant

Figure 11.14: Hepatic glycogen content of adult rats on 60th day subjected to neonatal luzindole treatment and weaning alloxanisation on 22nd day:

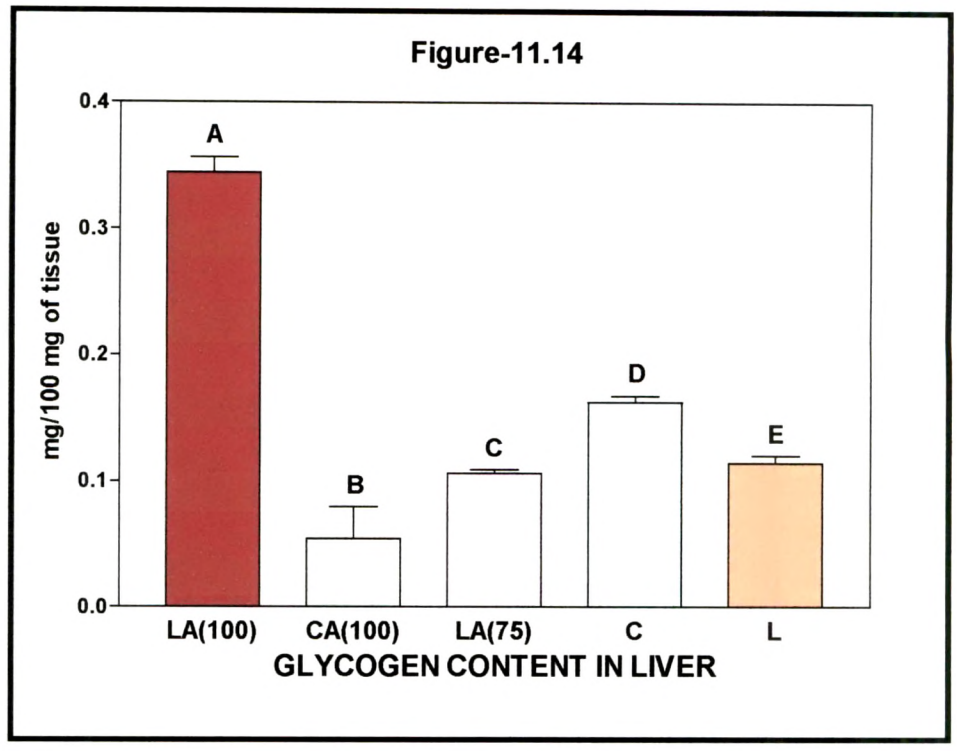


Table 11.14: Hepatic glycogen content of adult rats on 60th day subjected to neonatal luzindole treatment and weaning alloxanisation on 22nd day:

	LA(100)(A)	CA(100)(B)	LA(75)(C)	C(D)	L(E)
GLYCOGEN	0.3442 ±0.0125	0.0541 ±0.0254	0.1064 ±0.00275	0.1672 ±0.0048	0.1147 ±0.005778

Bonferroni's Multiple Comparison Test

	AvsB	AvsC	AvsD	AvsE	BvsC	BvsD	BvsE	CvsD	CvsE	DvsE
p	◆	◆	◆	◆	NS	◆	NS	NS	NS	NS

Values are expressed as mean ± SEM, ◆p<0.001; NSNon Significant

Figure 11.15: Muscle glycogen content of adult rats on 60th day subjected to neonatal luzindole treatment and weaning alloxanisation on 22nd day:

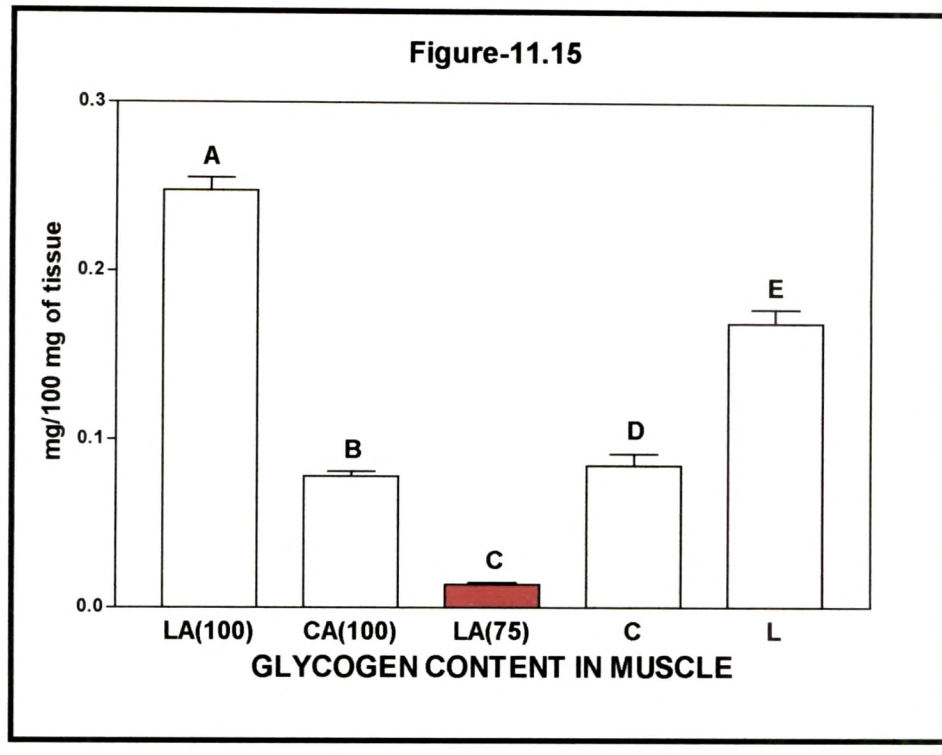


Table 11.15: Muscle glycogen content of adult rats on 60th day subjected to neonatal luzindole treatment and weaning alloxanisation on 22nd day:

	LA(100) (A)	CA(100) (B)	LA(75) (C)	C (D)	L (E)
GLYCOGEN	0.2476 ±0.0077	0.0783 ±0.00264	0.0137 ±0.0013	0.0848 ±0.0069	0.17 ±0.0085

Bonferroni's Multiple Comparison Test

	AvsB	AvsC	AvsD	AvsE	BvsC	BvsD	BvsE	CvsD	CvsE	DvsE
p	◆	◆	◆	◆	◆	NS	◆	◆	◆	◆

Values are expressed as mean ± SEM, ◆p<0.001; NSNon Significant

Figure 11.16: Serum glucose level of adult rats on 60th day subjected to neonatal luzindole treatment and weaning alloxanisation on 22nd day:

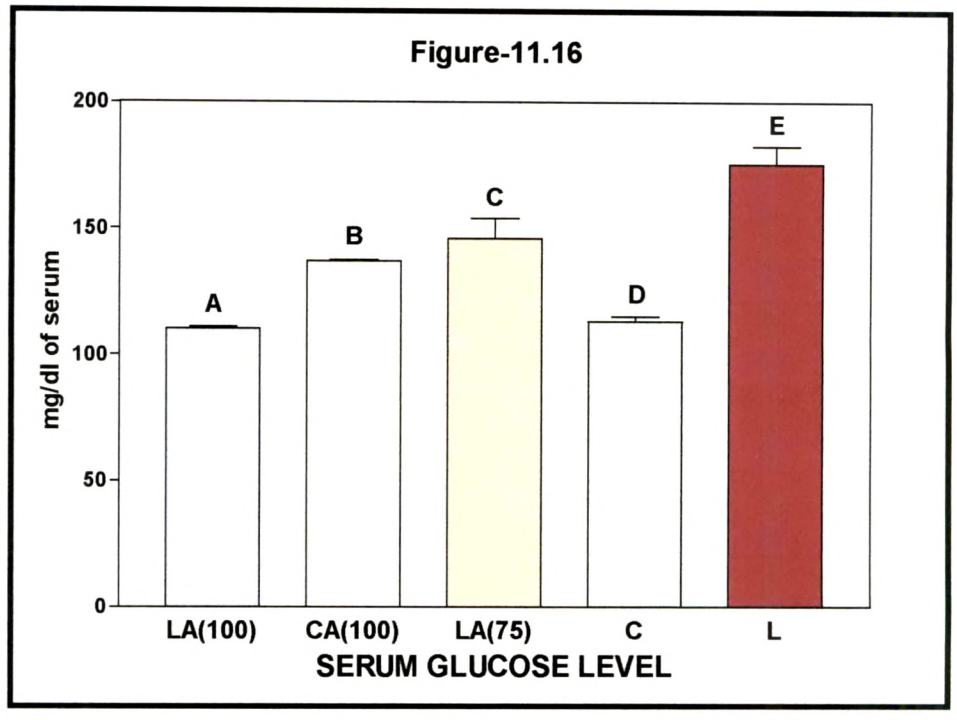


Table 11.16: Serum glucose level of adult rats on 60th day subjected to neonatal luzindole treatment and weaning alloxanisation on 22nd day:

	LA(100) (A)	CA(100) (B)	LA(75) (C)	C (D)	L (E)
GLUCOSE	110.20 ±0.9035	137.07 ±0.4956	145.87 ±8.2265	113.15 ±1.9210	175.53 ±7.225

Bonferroni's Multiple Comparison Test

	AvsB	AvsC	AvsD	AvsE	BvsC	BvsD	BvsE	CvsD	CvsE	DvsE
p	●	■	NS	◆	NS	●	◆	■	■	◆

Values are expressed as mean ± SEM, ◆p<0.001; ■P<0.01; ●P<0.05; NS Non Significant

Figure 11.17: Hepatic protein content of adult rats on 60th day subjected to neonatal luzindole treatment and weaning alloxanisation on 22nd day:

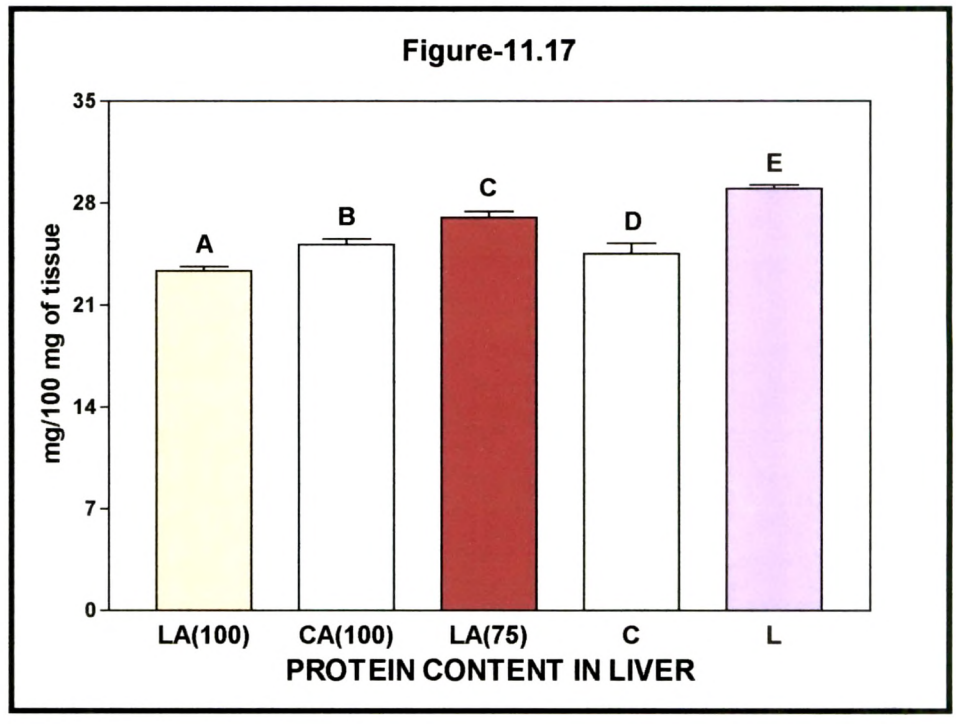


Table 11.17: Hepatic protein content of adult rats on 60th day subjected to neonatal luzindole treatment and weaning alloxanisation on 22nd day:

	LA(100) (A)	CA(100) (B)	LA(75) (C)	C (D)	L (E)
PROTEIN	23.33 ±0.3045	25.16 ±0.347	26.99 ±0.4305	24.49 ±0.7485	28.99 ±0.2355

Bonferroni's Multiple Comparison Test

	AvsB	AvsC	AvsD	AvsE	BvsC	BvsD	BvsE	CvsD	CvsE	DvsE
p	NS	◆	NS	◆	NS	NS	◆	●	NS	◆

Values are expressed as mean ± SEM, ◆p<0.001; ●P<0.05;
NS Non Significant

Figure 11.18: Muscle protein content of adult rats on 60th day subjected to neonatal luzindole treatment and weaning alloxanisation on 22nd day:

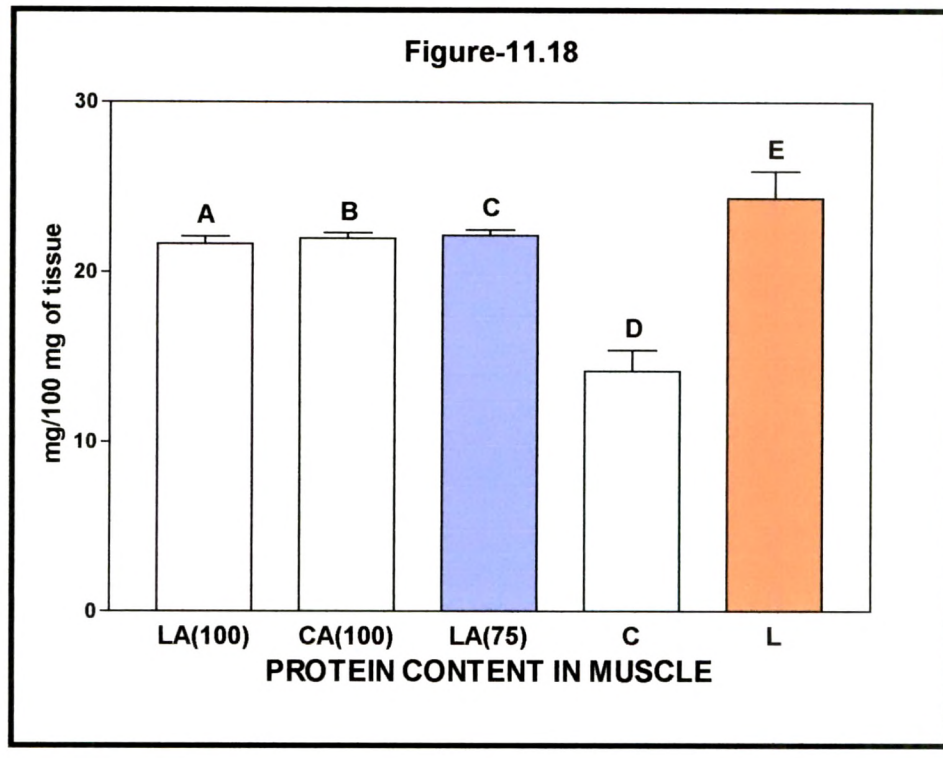


Table 11.18: Muscle protein content of adult rats on 60th day subjected to neonatal luzindole treatment and weaning alloxanisation on 22nd day:

	LA(100) (A)	CA(100) (B)	LA(75) (C)	C (D)	L (E)
PROTEIN	21.66 ±0.4305	21.99 ±0.303	22.16 ±0.347	14.16 ±1.2595	24.38 ±1.5563

Bonferroni's Multiple Comparison Test

	AvsB	AvsC	AvsD	AvsE	BvsC	BvsD	BvsE	CvsD	CvsE	DvsE
p	NS	NS	◆	NS	NS	◆	NS	◆	NS	◆

Values are expressed as mean ± SEM, ◆p<0.001; NSNon Significant

Figure 11.19: Hepatic glycogen synthetase activity in adult rats on 60th day subjected to neonatal luzindole treatment and weaning alloxanisation on 22nd day:

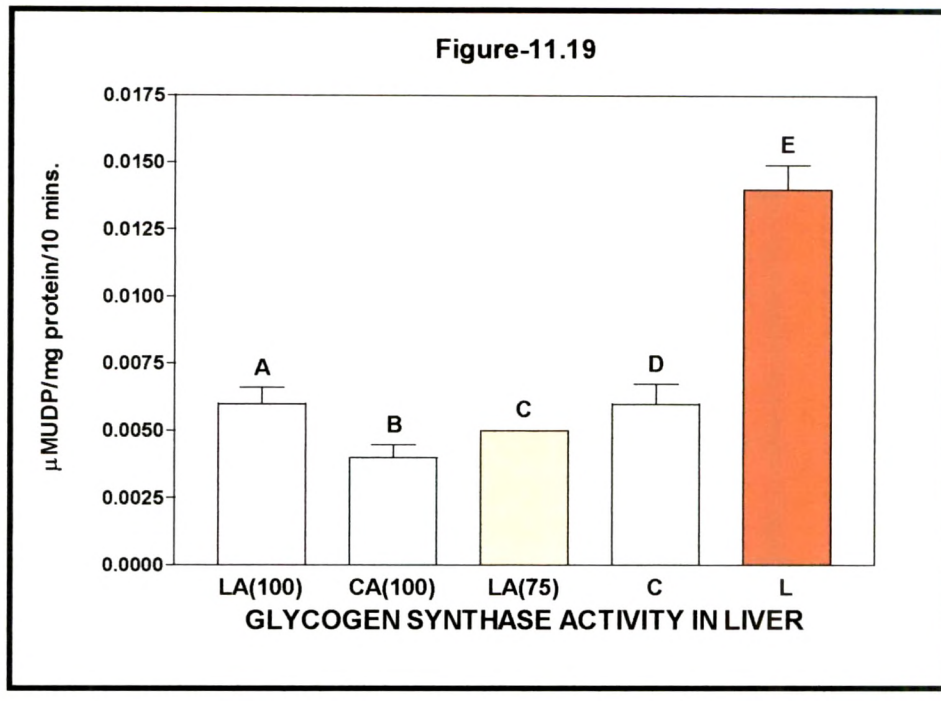


Table 11.19: Hepatic glycogen synthetase activity in adult rats on 60th day subjected to neonatal luzindole treatment and weaning alloxanisation on 22nd day:

	LA(100)(A)	CA(100)(B)	LA(75)(C)	C(D)	L(E)
GLYCOGEN SYNTHETASE	0.006 ±0.0006	0.004 ±0.000475	0.005 ±0.00	0.006 ±0.00075	0.014 ±0.00091

Bonferroni's Multiple Comparison Test

	AvsB	AvsC	AvsD	AvsE	BvsC	BvsD	BvsE	CvsD	CvsE	DvsE
p	NS	NS	NS	◆	NS	NS	◆	NS	◆	◆

Values are expressed as mean ± SEM, ◆p<0.001; NSNon Significant

Figure 11.20: Muscle glycogen synthetase activity in adult rats on 60th day subjected to neonatal luzindole treatment and weaning alloxanisation on 22nd day:

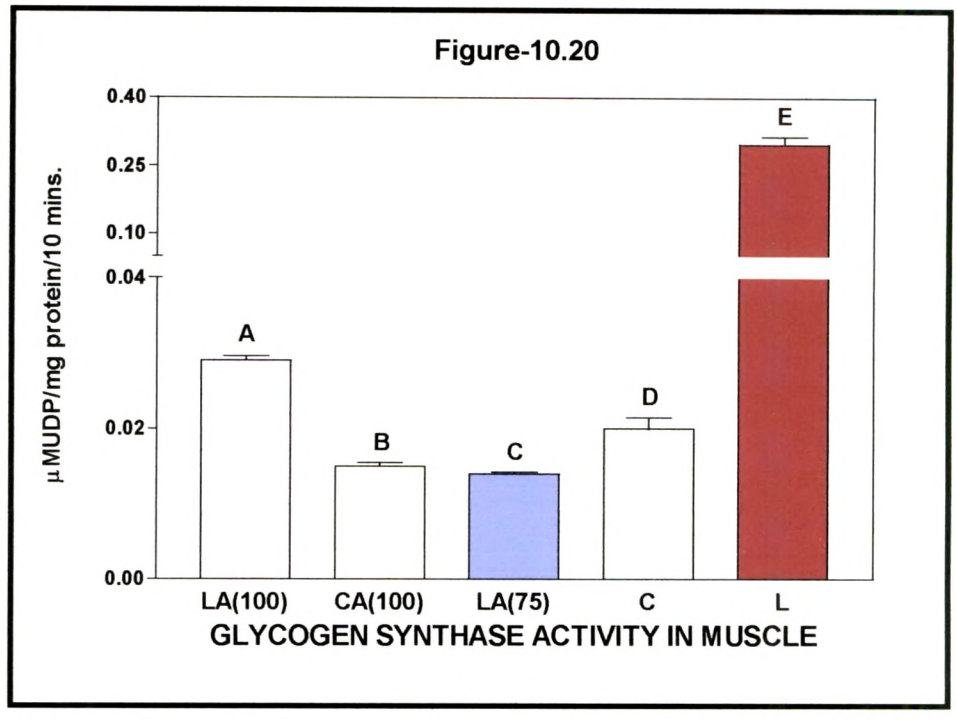


Table 11.20: Muscle glycogen synthetase activity in adult rats on 60th day subjected to neonatal luzindole treatment and weaning alloxanisation on 22nd day:

	LA(100)[A]	CA(100)[B]	LA(75)[C]	C[D]	L[E]
GLYCOGEN SYNTHETASE	0.029 ±0.0006	0.015 ±0.0005	0.014 ±0.00025	0.02 ±0.0015	0.298 ±0.01675

Bonferroni's Multiple Comparison Test

	AvsB	AvsC	AvsD	AvsE	BvsC	BvsD	BvsE	CvsD	CvsE	DvsE
p	NS	NS	NS	◆	NS	NS	◆	NS	◆	◆

Values are expressed as mean ± SEM, ◆p<0.001; [NS]Non Significant

Figure 11.21: Hepatic glycogen phosphorylase activity in adult rats on 60th day subjected to neonatal luzindole treatment and weaning alloxanisation on 22nd day:

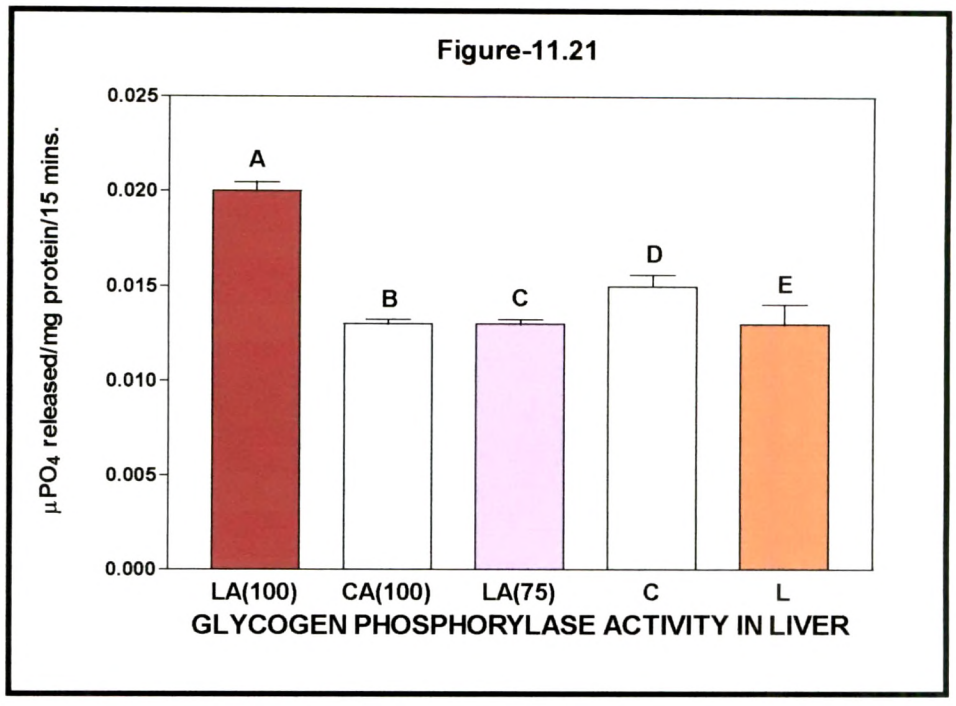


Table 11.21: Hepatic glycogen phosphorylase activity in adult rats on 60th day subjected to neonatal luzindole treatment and weaning alloxanisation on 22nd day:

	LA(100)(A)	CA(100)(B)	LA(75)(C)	C(D)	L(E)
GLYCOGEN PHOSPHORYLASE	0.02 ±0.000475	0.013 ±0.00025	0.013 ±0.00025	0.015 ±0.0006	0.013 ±0.00105

Bonferroni's Multiple Comparison Test

	AvsB	AvsC	AvsD	AvsE	BvsC	BvsD	BvsE	CvsD	CvsE	DvsE
p	◆	◆	◆	◆	NS	NS	NS	NS	NS	NS

Values are expressed as mean ± SEM, ◆p<0.001; NSNon Significant

Figure 11.22: Muscle glycogen phosphorylase activity in adult rats on 60th day subjected to neonatal luzindole treatment and weaning alloxanisation on 22nd day:

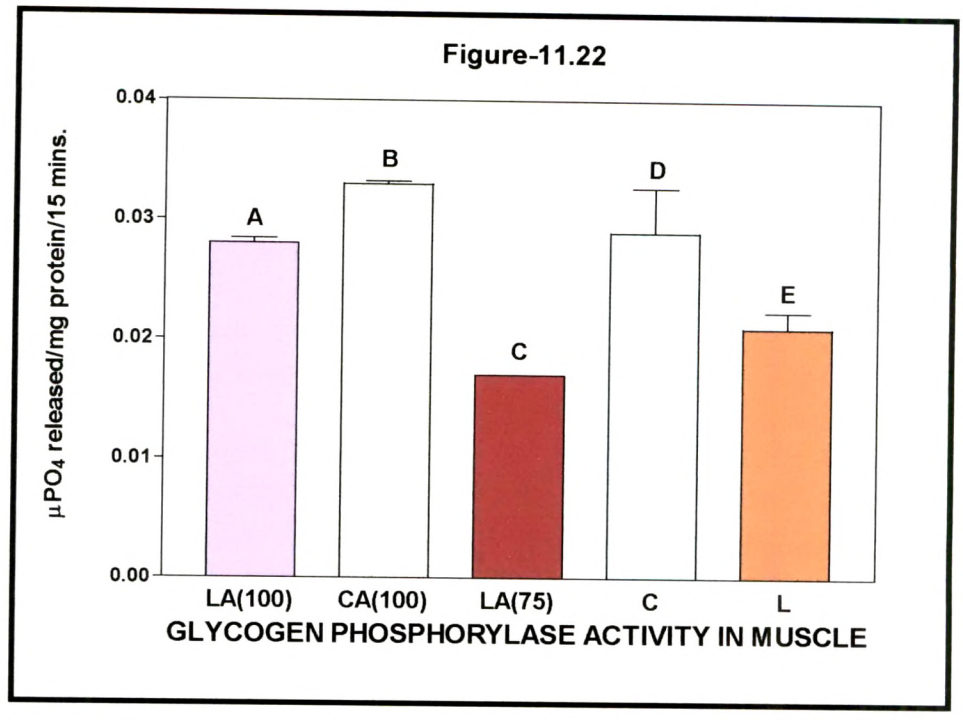


Table 11.22: Muscle glycogen phosphorylase activity in adult rats on 60th day subjected to neonatal luzindole treatment and weaning alloxanisation on 22nd day:

	LA(100)(A)	CA(100)(B)	LA(75)(C)	C(D)	L(E)
GLYCOGEN PHOSPHORYLASE	0.028 ±0.0004	0.033 ±0.00025	0.017 ±0.00	0.029 ±0.00375	0.021 0.0013

Bonferroni's Multiple Comparison Test

	AvsB	AvsC	AvsD	AvsE	BvsC	BvsD	BvsE	CvsD	CvsE	DvsE
p	NS	■	NS	NS	◆	NS	■	■	NS	NS

Values are expressed as mean ± SEM, ◆p<0.001; ■P<0.01;
NS Non Significant

Figure 11.23: Hepatic glucose-6-phosphatase activity in adult rats on 60th day subjected to neonatal luzindole treatment and weaning alloxanisation on 22nd day:

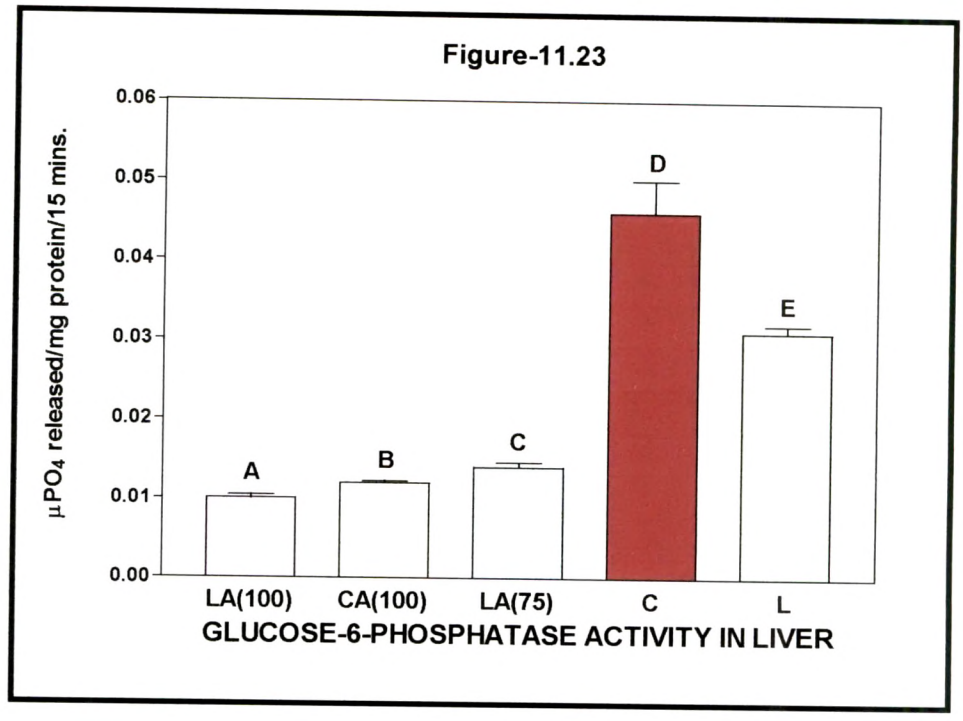


Table 11.23: Hepatic glucose-6-phosphatase activity in adult rats on 60th day subjected to neonatal luzindole treatment and weaning alloxanisation on 22nd day:

	LA(100)(A)	CA(100)(B)	LA(75)(C)	C(D)	L(E)
GLUCOSE-6-PHOSPHATASE	0.01 ±0.000475	0.012 ±0.00025	0.014 ±0.0006	0.046 ±0.0041	0.031 ±0.0009

Bonferroni's Multiple Comparison Test

	AvsB	AvsC	AvsD	AvsE	BvsC	BvsD	BvsE	CvsD	CvsE	DvsE
p	NS	NS	◆	◆	NS	◆	◆	◆	◆	◆

Values are expressed as mean ± SEM, ◆p<0.001; NSNon Significant

Figure 11.24: Hepatic total lipid content of adult rats on 60th day subjected to neonatal luzindole treatment and weaning alloxanisation on 22nd day:

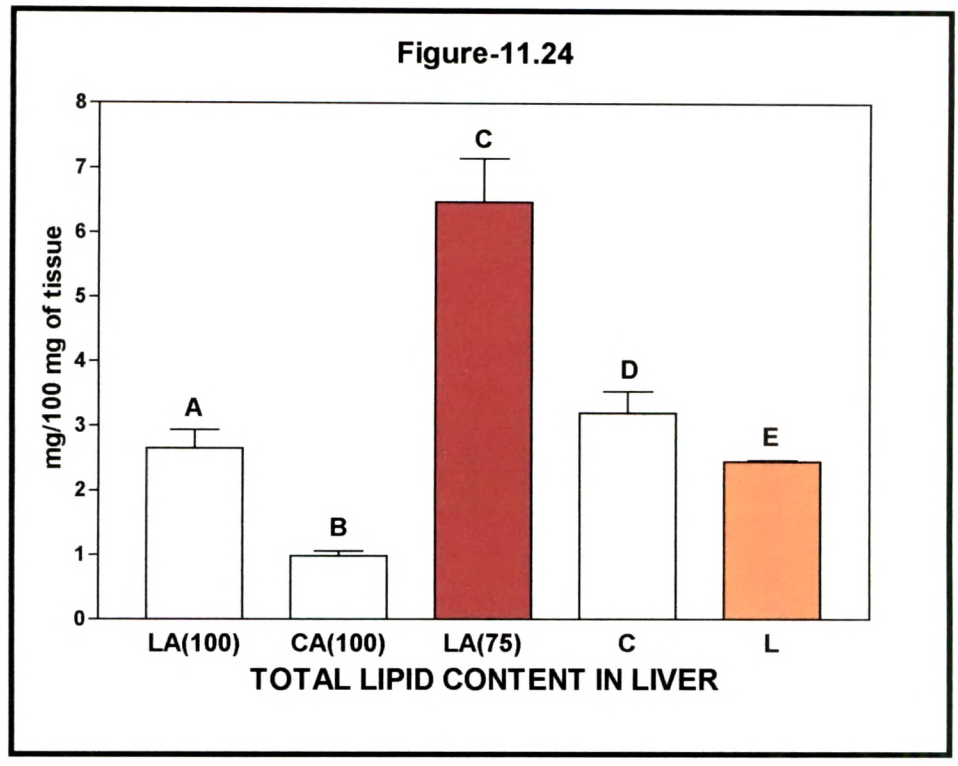


Table 11.24: Hepatic total lipid content of adult rats on 60th day subjected to neonatal luzindole treatment and weaning alloxanisation on 22nd day:

	LA(100) (A)	CA(100) (B)	LA(75) (C)	C (D)	L (E)
TOTAL LIPIDS	2.65 ±0.2872	0.975 ±0.0853	6.475 ±0.6725	3.2 ±0.33415	2.45 ±0.0206

Bonferroni's Multiple Comparison Test

	AvsB	AvsC	AvsD	AvsE	BvsC	BvsD	BvsE	CvsD	CvsE	DvsE
p	NS	◆	NS	NS	◆	■	NS	◆	◆	NS

Values are expressed as mean ± SEM, ◆p<0.001; ■P<0.01; NSNon Significant

Figure 11.25: Hepatic cholesterol content of adult rats on 60th day subjected to neonatal luzindole treatment and weaning alloxanisation on 22nd day:

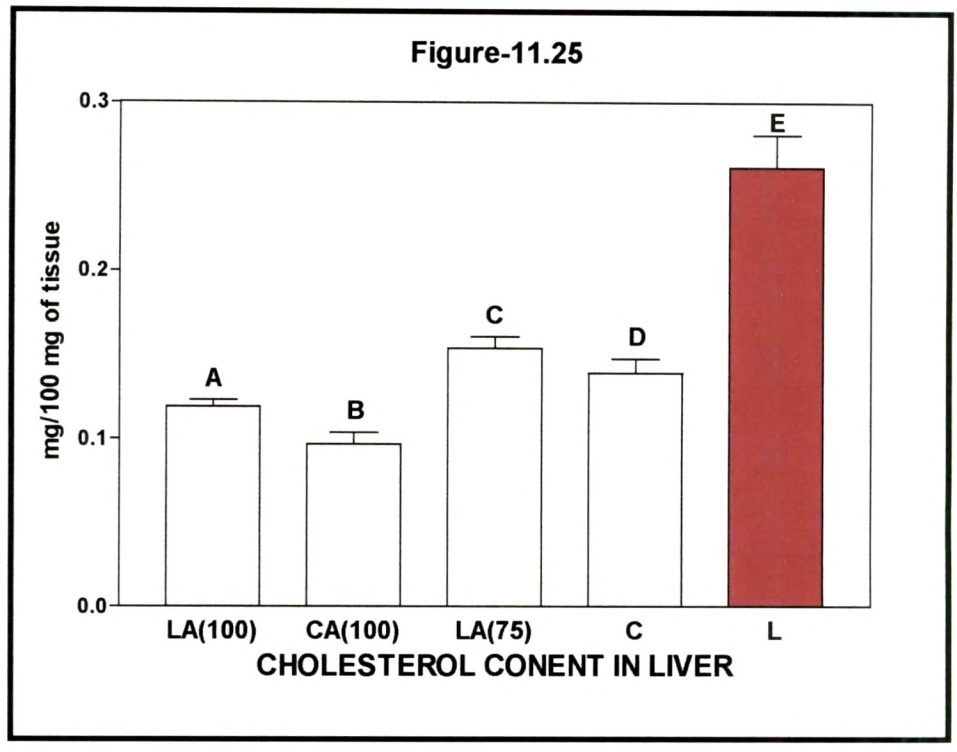


Table 11.25: Hepatic cholesterol content of adult rats on 60th day subjected to neonatal luzindole treatment and weaning alloxanisation on 22nd day:

	LA(100)(A)	CA(100)(B)	LA(75)(C)	C(D)	L(E)
CHOLESTEROL	0.119 ±0.00385	0.0969 ±0.00685	0.1536 ±0.00685	0.1391 ±0.0084	0.2616 ±0.01905

Bonferroni's Multiple Comparison Test

	AvsB	AvsC	AvsD	AvsE	BvsC	BvsD	BvsE	CvsD	CvsE	DvsE
p	NS	NS	NS	◆	●	NS	◆	NS	◆	◆

Values are expressed as mean ± SEM, ◆p<0.001; ●P<0.05;
NSNon Significant

Figure 11.26: Muscle total lipid content of adult rats on 60th day subjected to neonatal luzindole treatment and weaning alloxanisation on 22nd day:

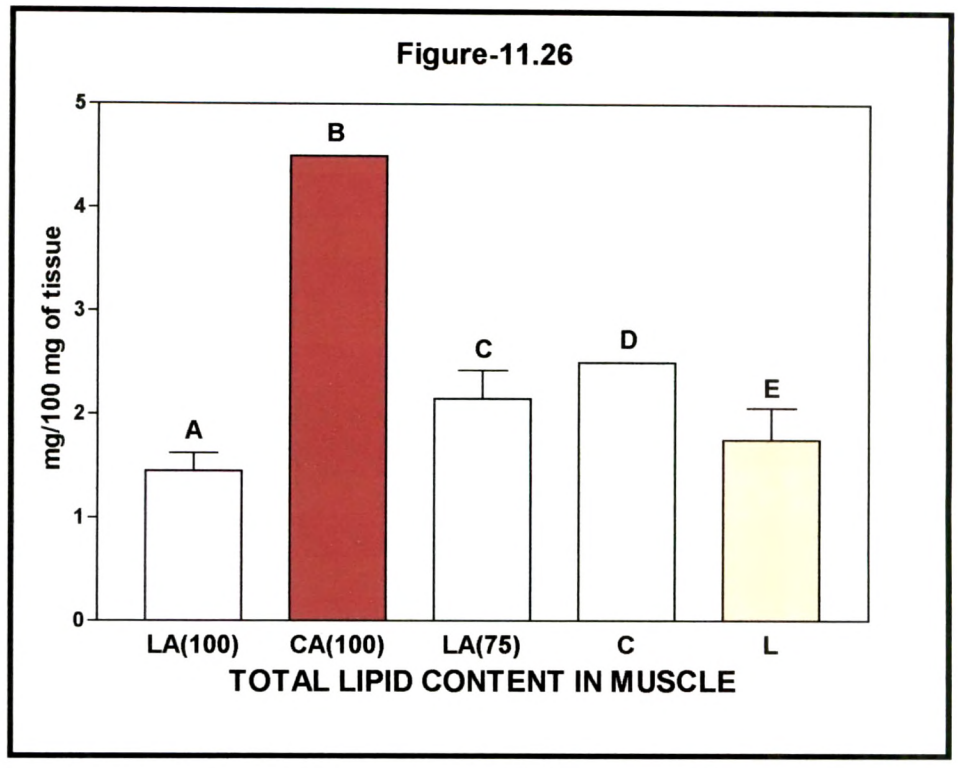


Table 11.26: Muscle total lipid content of adult rats on 60th day subjected to neonatal luzindole treatment and weaning alloxanisation on 22nd day:

	LA(100) (A)	CA(100) (B)	LA(75) (C)	C (D)	L (E)
TOTAL LIPIDS	1.45 ±0.1707	4.5 ±0.00006	2.15 ±0.2753	2.5 ±0.00006	1.75 ±0.03122

Bonferroni's Multiple Comparison Test

	AvsB	AvsC	AvsD	AvsE	BvsC	BvsD	BvsE	CvsD	CvsE	DvsE
p	◆	NS	●	NS	◆	◆	◆	NS	NS	NS

Values are expressed as mean ± SEM, ◆p<0.001; ●P<0.05;
NS Non Significant

Figure 11.27: Muscle cholesterol content of adult rats on 60th day subjected to neonatal luzindole treatment and weaning alloxanisation on 22nd day:

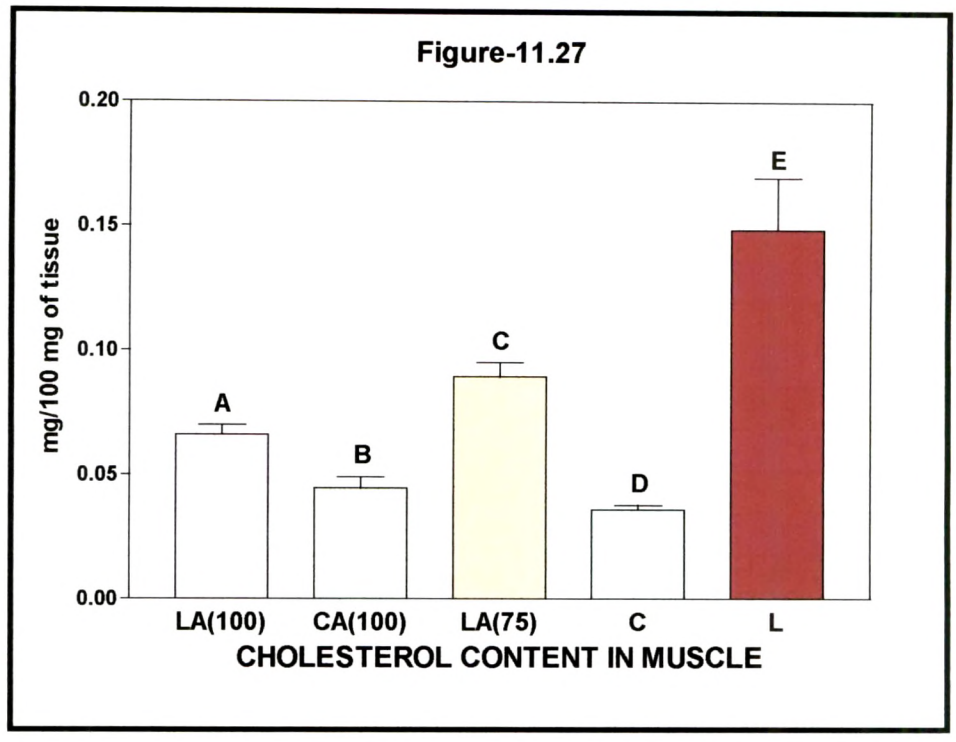


Table 11.27: Muscle cholesterol content of adult rats on 60th day subjected to neonatal luzindole treatment and weaning alloxanisation on 22nd day:

	LA(100)[A]	CA(100)[B]	LA(75)[C]	C[D]	L[E]
CHOLESTEROL	0.066 ±0.004	0.0445 ±0.0045	0.0893 ±0.00575	0.036 ±0.0018	0.1486 ±0.0208

Bonferroni's Multiple Comparison Test

	AvsB	AvsC	AvsD	AvsE	BvsC	BvsD	BvsE	CvsD	CvsE	DvsE
p	NS	NS	NS	◆	NS	NS	◆	●	■	◆

Values are expressed as mean ± SEM, ◆p<0.001; ■P<0.01; ●P<0.05; NSNon Significant

Figure 11.28: Adipose tissue total lipid content of adult rats on 60th day subjected to neonatal luzindole treatment and weaning alloxanisation on 22nd day:

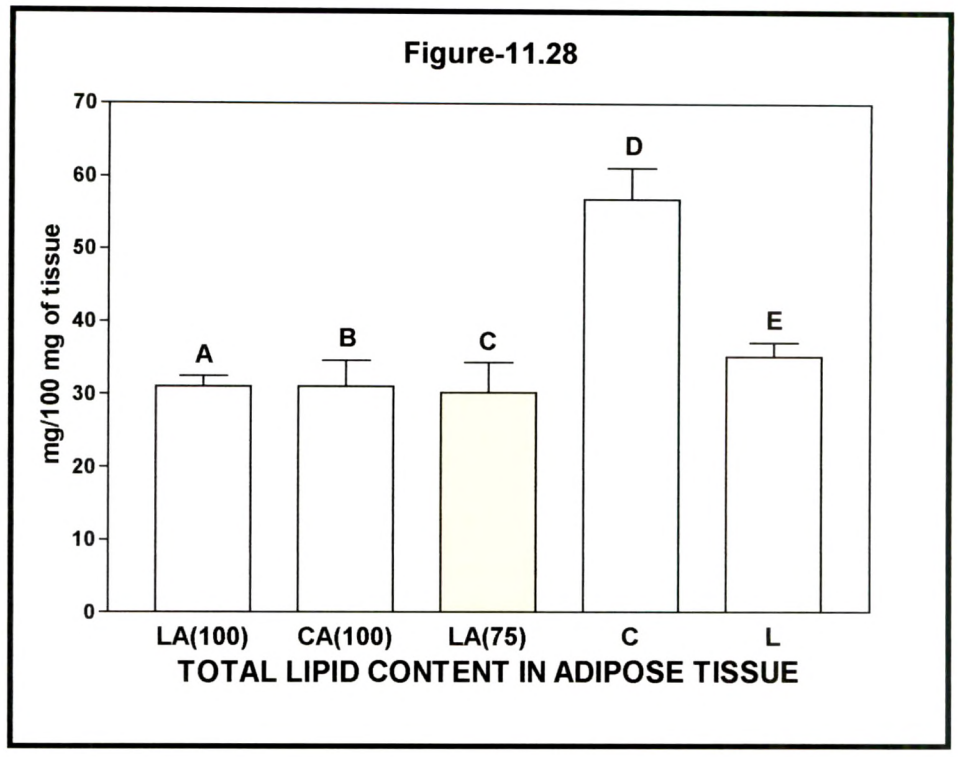


Table 11.28: Adipose tissue total lipid content of adult rats on 60th day subjected to neonatal luzindole treatment and weaning alloxanisation on 22nd day:

	LA(100) (A)	CA(100) (B)	LA(75) (C)	C (D)	L (E)
TOTAL LIPIDS	31.075 ±1.3912	31.1 ±3.54	30.233 ±4.1516	56.8 ±4.3395	35.15 ±2.0176

Bonferroni's Multiple Comparison Test

	AvsB	AvsC	AvsD	AvsE	BvsC	BvsD	BvsE	CvsD	CvsE	DvsE
p	NS	NS	◆	NS	NS	◆	NS	◆	NS	■

Values are expressed as mean ± SEM, ◆p<0.001; •P<0.05; NSNon Significant

Figure 11.29: Adipoe tissue cholesterol content of adult rats on 60th day subjected to neonatal luzindole treatment and weaning alloxanisation on 22nd day:

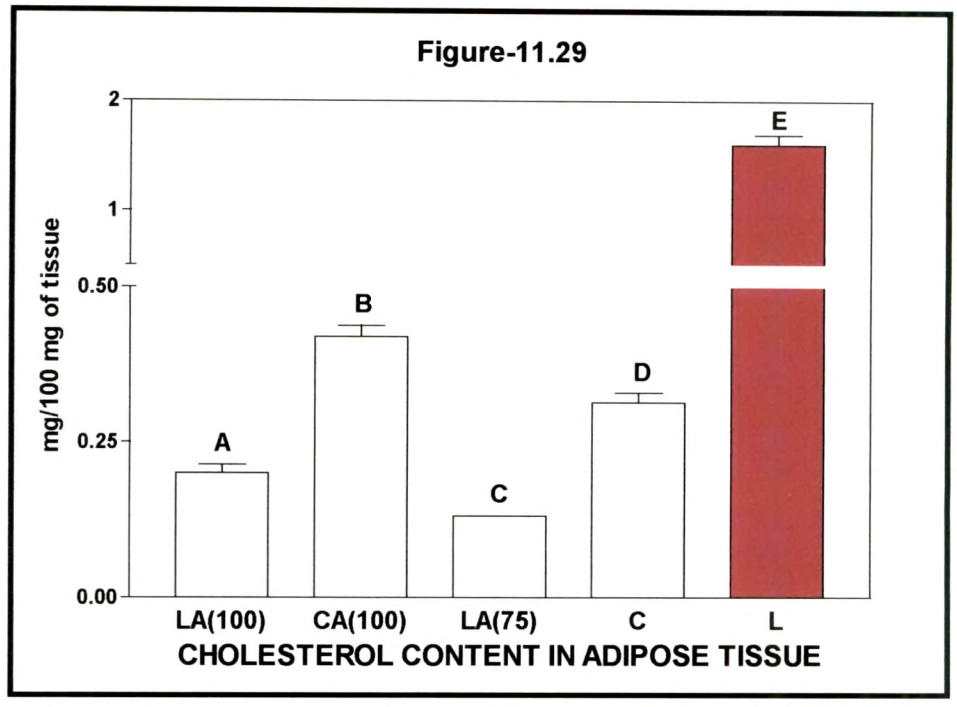


Table 11.29: Adipose tissue cholesterol content of adult rats on 60th day subjected to neonatal luzindole treatment and weaning alloxanisation on 22nd day:

	LA(100)(A)	CA(100)(B)	LA(75)(C)	C(D)	L(E)
CHOLESTEROL	0.2 ±0.0138	0.4205 ±0.0184	0.1307 ±0.00	0.3148 ±0.016	1.6039 ±0.0896

Bonferroni's Multiple Comparison Test

	AvsB	AvsC	AvsD	AvsE	BvsC	BvsD	BvsE	CvsD	CvsE	DvsE
p	●	NS	NS	◆	■	NS	◆	NS	◆	◆

Values are expressed as mean ± SEM, ◆p<0.001; ■P<0.01; ●P<0.05; NS Non Significant

Figure 11.30: Serum triglyceride level of adult rats on 60th day subjected to neonatal luzindole treatment and weaning alloxanisation on 22nd day:

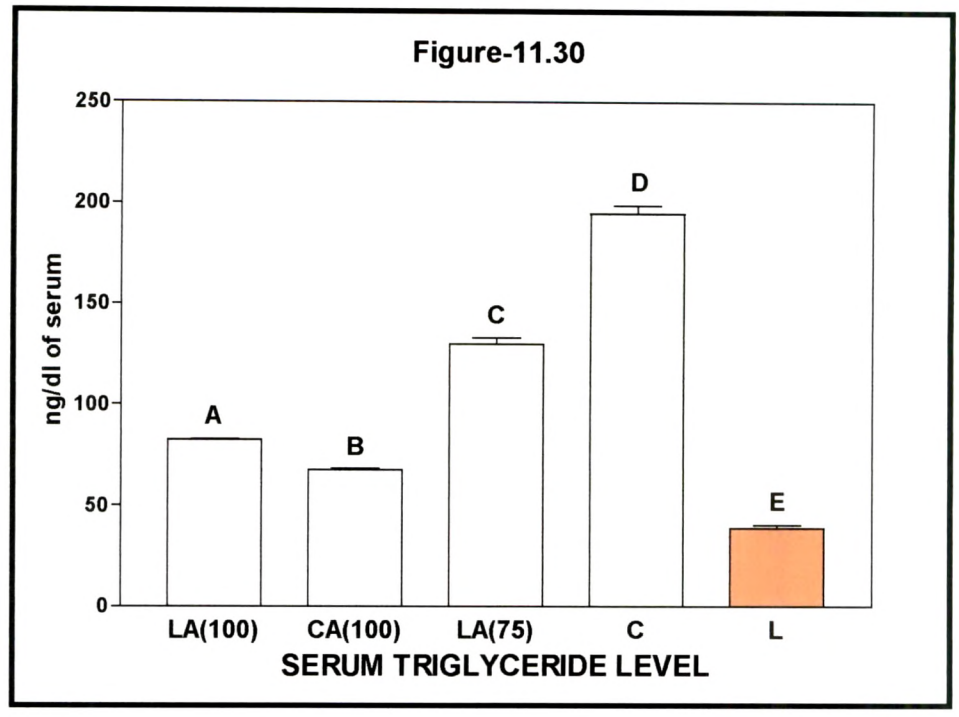


Table 11.30: Serum triglyceride level of adult rats on 60th day subjected to neonatal luzindole treatment and weaning alloxanisation on 22nd day:

	LA(100)(A)	CA(100)(B)	LA(75)(C)	C(D)	L(E)
TRIGLYCERIDE	82.59 ±0.3845	67.63 ±0.728	130.10 ±2.883	194.94 ±3.8433	38.88 ±1.6465

Bonferroni's Multiple Comparison Test

	AvsB	AvsC	AvsD	AvsE	BvsC	BvsD	BvsE	CvsD	CvsE	DvsE
p	■	◆	◆	◆	◆	◆	◆	◆	◆	◆

Values are expressed as mean ± SEM, ◆p<0.001; ■P<0.01;
NS Non Significant

Figure 11.31: Serum cholesterol level of adult rats on 60th day subjected to neonatal luzindole treatment and weaning alloxanisation on 22nd day:

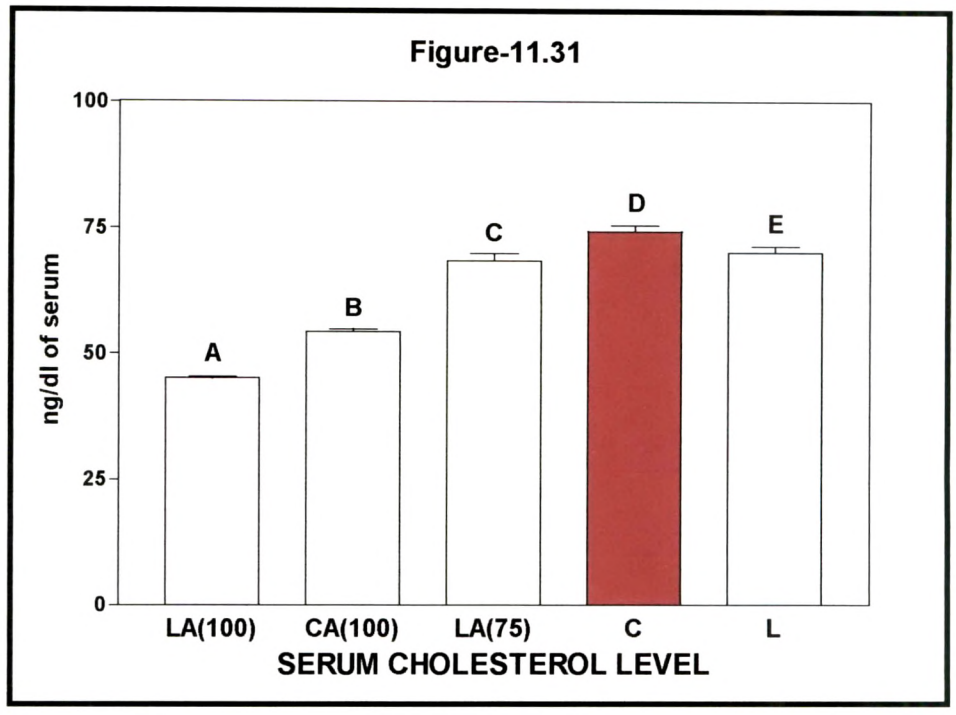


Table 11.31: Serum cholesterol level of adult rats on 60th day subjected to neonatal luzindole treatment and weaning alloxanisation on 22nd day:

	LA(100)(A)	CA(100)(B)	LA(75)(C)	C(D)	L(E)
CHOLESTEROL	45.13 ±0.2916	59.29 ±0.556	68.402 ±1.4318	74.31 ±1.2021	70.13 ±1.1555

Bonferroni's Multiple Comparison Test

	AvsB	AvsC	AvsD	AvsE	BvsC	BvsD	BvsE	CvsD	CvsE	DvsE
p	◆	◆	◆	◆	◆	◆	◆	■	NS	NS

Values are expressed as mean ± SEM, ◆p<0.001; ■P<0.01;
NSNon Significant

Figure 11.32: Serum total lipid level of adult rats on 60th day subjected to neonatal luzindole treatment and weaning alloxanisation on 22nd day:

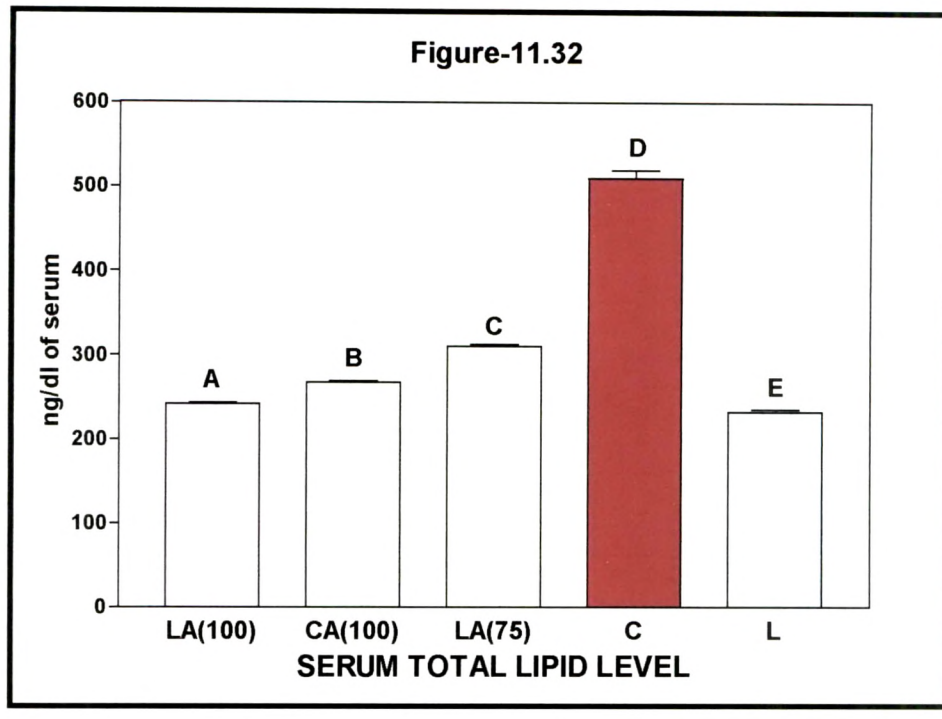


Table 11.32: Serum total lipid level of adult rats on 60th day subjected to neonatal luzindole treatment and weaning alloxanisation on 22nd day:

	LA(100) (A)	CA(100) (B)	LA(75) (C)	C (D)	L (E)
TOTAL LIPIDS	241.47 ±2.3295	267.66 ±1.2815	310.72 ±1.6115	509.75 ±9.78	232.54 ±2.4345

Bonferroni's Multiple Comparison Test

	AvsB	AvsC	AvsD	AvsE	BvsC	BvsD	BvsE	CvsD	CvsE	DvsE
p	●	◆	◆	NS	◆	◆	◆	◆	◆	◆

Values are expressed as mean ± SEM, ◆p<0.001; ●P<0.05; NS Non Significant

Figure 11.33: Serum phospholipid level of adult rats on 60th day subjected to neonatal luzindole treatment and weaning alloxanisation on 22nd day:

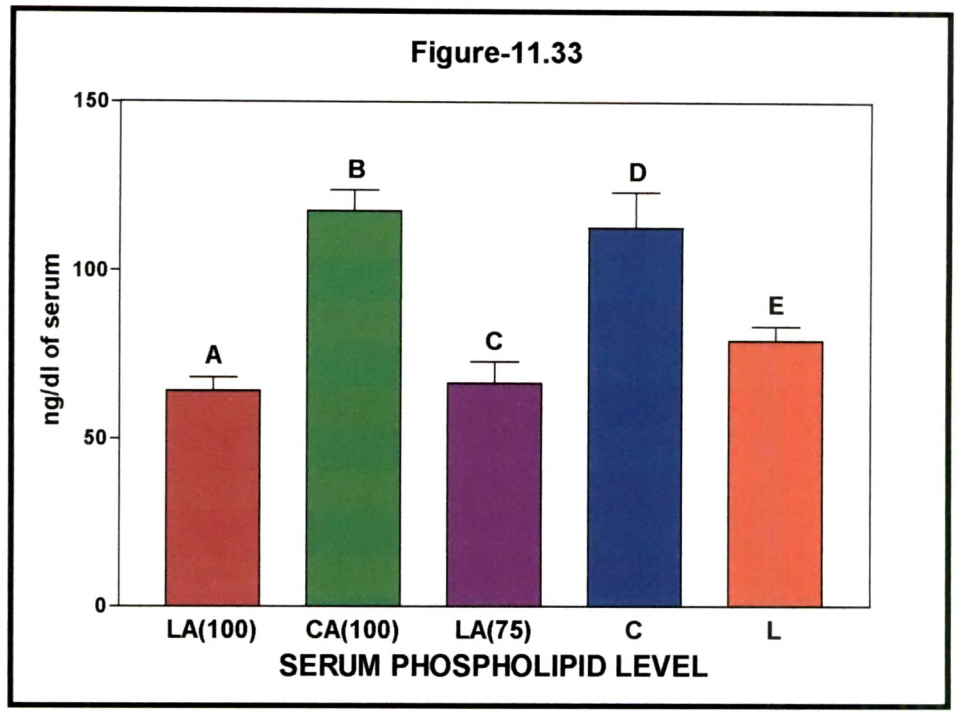


Table 11.33: Serum phospholipid level of adult rats on 60th day subjected to neonatal luzindole treatment and weaning alloxanisation on 22nd day:

	LA(100)(A)	CA(100)(B)	LA(75)(C)	C(D)	L(E)
PHOSPHOLIPID	64.04 ±3.9855	117.62 ±6.265	66.405 ±6.5459	112.76 ±10.4413	79.34 ±4.115

Bonferroni's Multiple Comparison Test

	AvsB	AvsC	AvsD	AvsE	BvsC	BvsD	BvsE	CvsD	CvsE	DvsE
p	◆	NS	■	NS	◆	NS	●	■	NS	●

Values are expressed as mean ± SEM, ◆p<0.001; ■P<0.01; ●P<0.05; NSNon Significant

Figure 11.34: Serum free fatty acid level of adult rats on 60th day subjected to neonatal luzindole treatment and weaning alloxanisation on 22nd day:

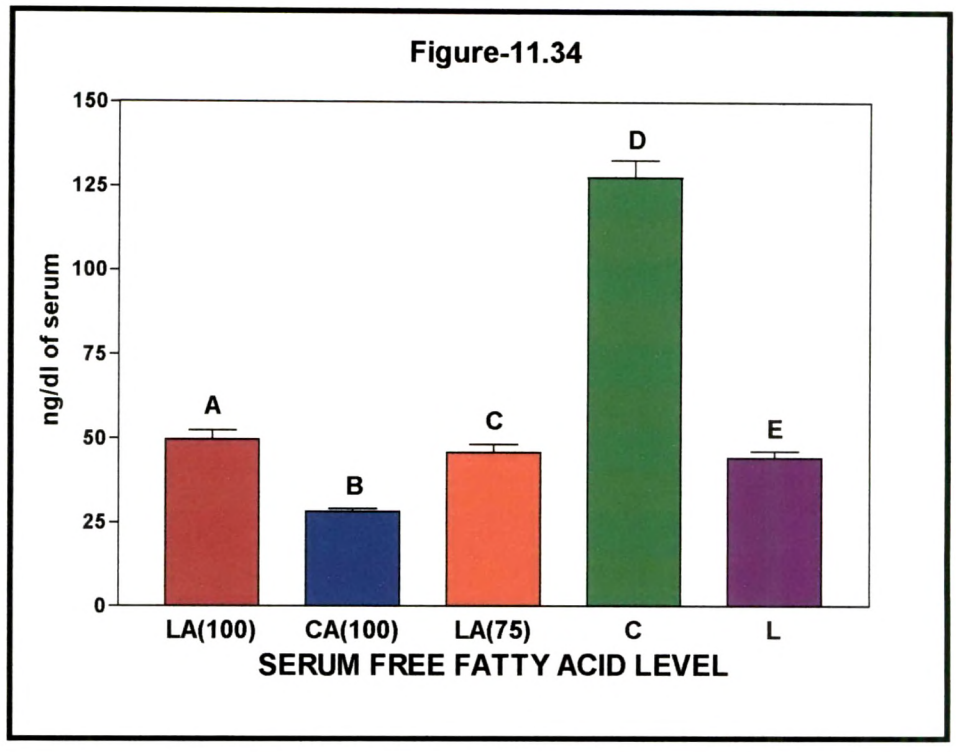


Table 11.34: Serum free fatty acid (FFA) level of adult rats on 60th day subjected to neonatal luzindole treatment and weaning alloxanisation on 22nd day:

	LA(100) (A)	CA(100) (B)	LA(75) (C)	C (D)	L (E)
SERUM FFA	49.71 ±2.70	28.12 ±1.07	45.82 2.36	127.74 ±4.96	44.19 ±2.13

Bonferroni's Multiple Comparison Test

	AvsB	AvsC	AvsD	AvsE	BvsC	BvsD	BvsE	CvsD	CvsE	DvsE
p	■	NS	◆	NS	■	◆	●	◆	NS	◆

Values are expressed as mean ± SEM, ◆p<0.001; ■P<0.01; ●P<0.05; NSNon Significant

Figure11.35: Serum insulin level of adult rats on 60th day subjected to neonatal luzindole treatment and weaning alloxanisation on 22nd day:

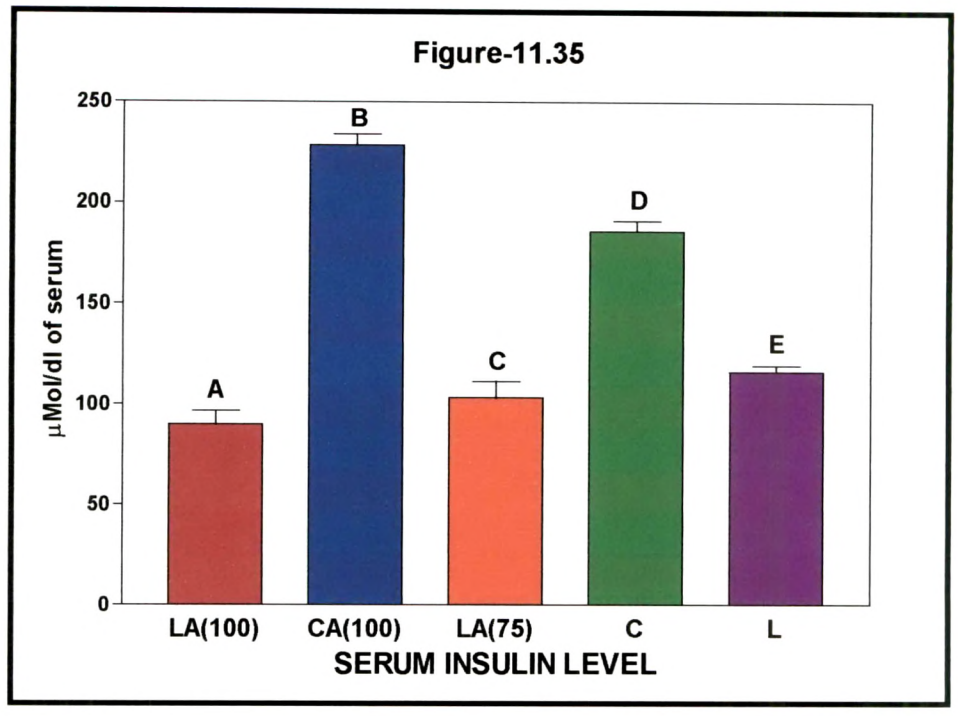


Table11.35: Serum insulin level of adult rats on 60th day subjected to neonatal luzindole treatment and weaning alloxanisation on 22nd day:

	LA(100) (A)	CA(100) (B)	LA(75) (C)	C (D)	L (E)
INSULIN	89.74 ±6.75	228.41 ±5.623	103.13 ±8.11	185.82 ±5.259	116.09 ±2.9235

Bonferroni's Multiple Comparison Test

	AvsB	AvsC	AvsD	AvsE	BvsC	BvsD	BvsE	CvsD	CvsE	DvsE
p	◆	NS	◆	NS	◆	■	◆	◆	NS	◆

Values are expressed as mean ± SEM, ◆p<0.001; ■P<0.01;
NS Non Significant

PLATE – 15

Photomicrographs of sections of pancreas – 1000 X

FIGURE (A): Transverse section of the pancreas of male luzindole alloxanised (100) treated rats on the 60th day showing decreased B cell population with an increased effect of allxoan induced damage. Note the clear areas (double headed dotted arrow) formed due to loss of cells.

FIGURE (B): Transverse section of the pancreas of male control alloxanised (100) adult (60th day) rats showing islet and pancreatic acini. Note the increased B as well as A cell population. Also the islet and acinar cells show reduced clear areas due to recovery of cells.

FIGURE (C): Transverse section of the pancreas of male luzindole alloxanised (75) treated rats on the 60th day showing islet and pancreatic acini. There is an decrease in the islet size and islet cell number, with an decreased B:A cell ratio. Note the clear areas (double headed dotted arrow) formed due to loss of cells.

PLATE - 15

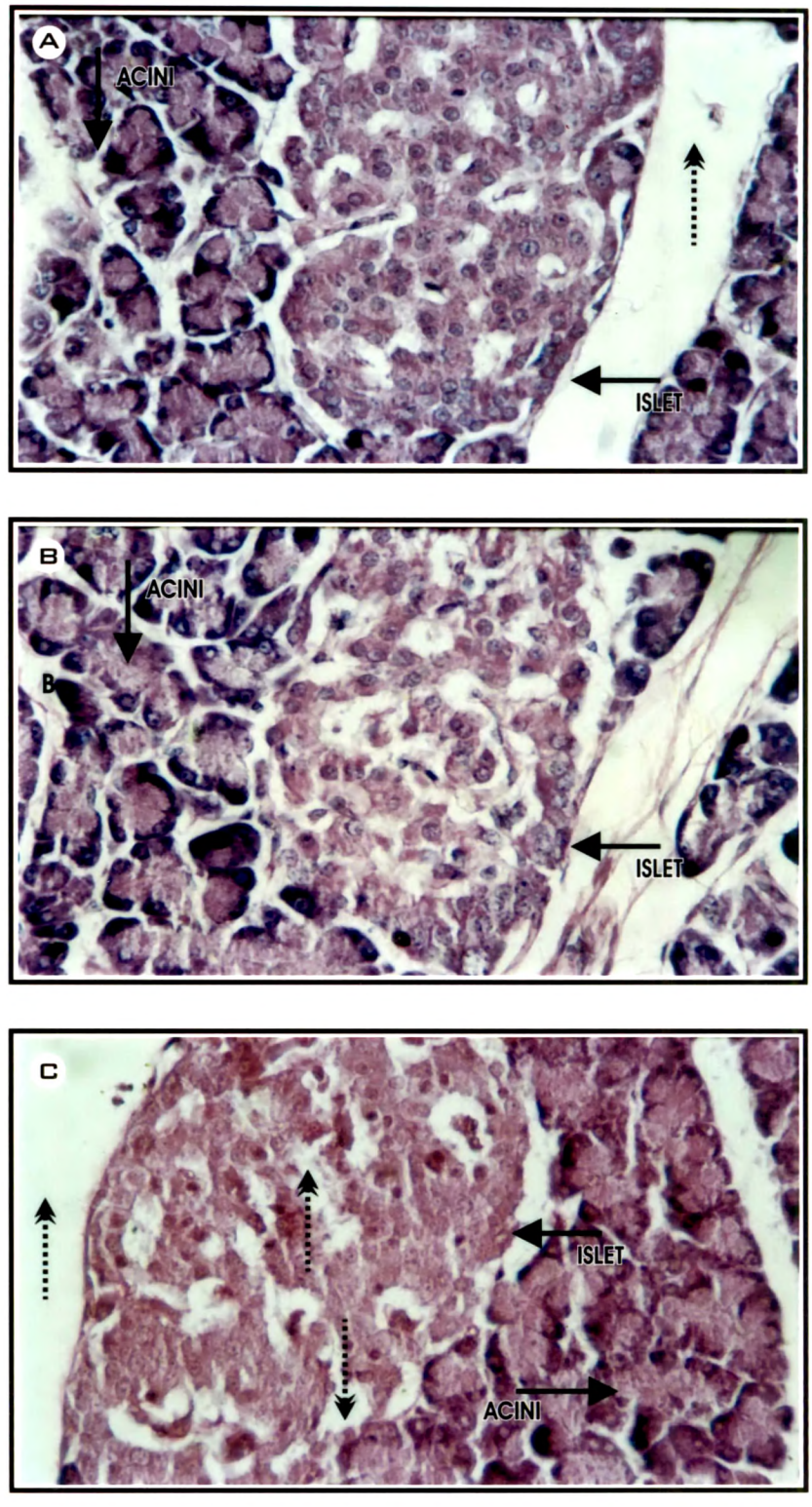


PLATE – 16

Photomicrographs of sections of pancreas – 1000 X

FIGURE (A): Transverse section of the pancreas of male luzindole alloxanised (100) treated rats on the 60^{th} day showing decreased B cell population with an increased effect of allxoan induced damage. Note the clear areas (double headed dotted arrow) formed due to loss of cells.

FIGURE (B): Transverse section of the pancreas of male control alloxanised (100) adult (60^{th} day) rats showing islet and pancreatic acini. Note the increased B as well as A cell population. Also the islet and acinar cells show reduced clear areas (double headed dotted arrow) due to recovery of cells.

FIGURE (C): Transverse section of the pancreas of male luzindole alloxanised (75) treated rats on the 60^{th} day showing islet and pancreatic acini. There is an decrease in the islet size and islet cell number, with an decreased B:A cell ratio. Note the clear areas (double headed dotted arrow) formed due to loss of cells.

PLATE - 16

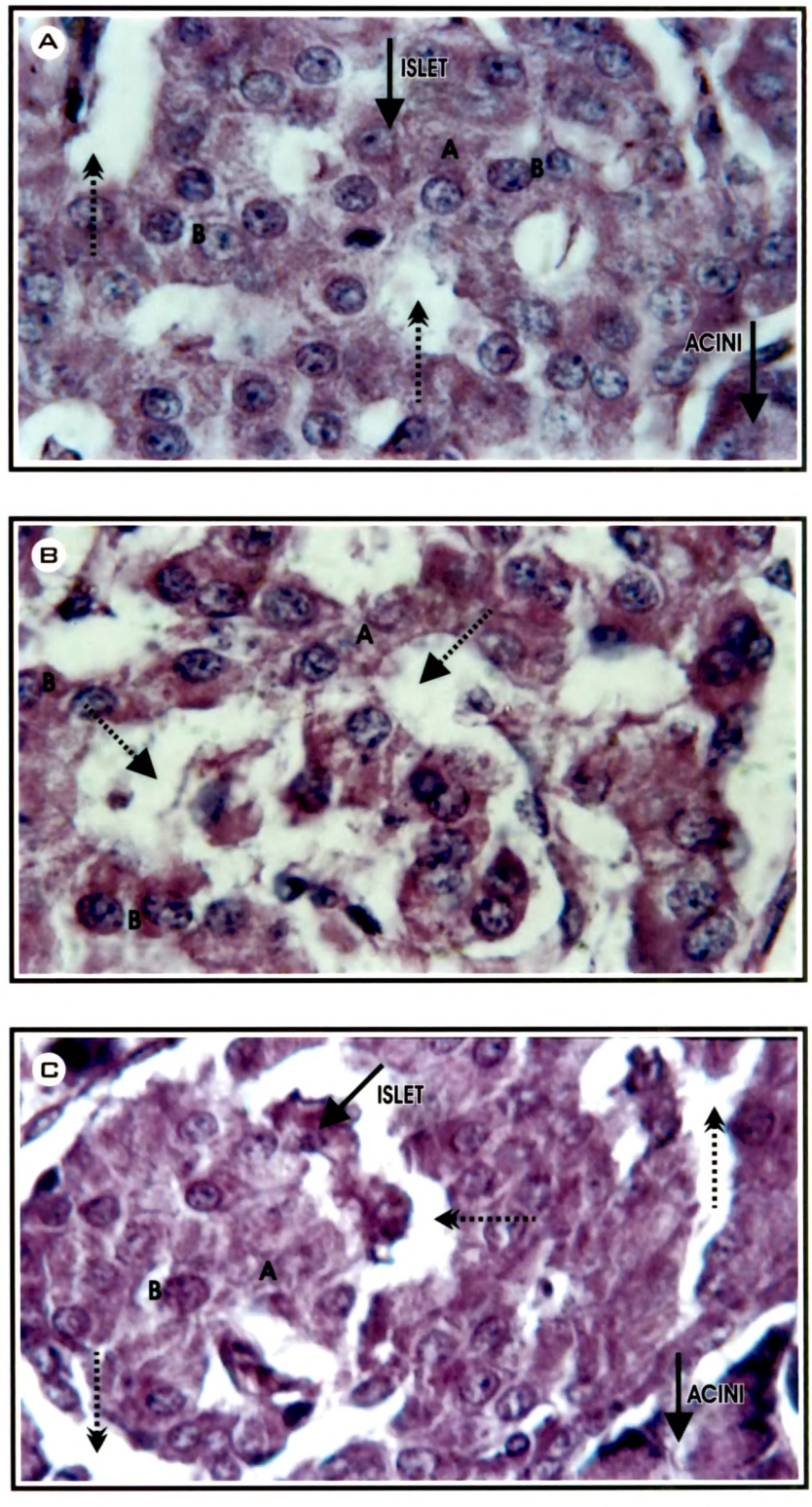


PLATE – 17

Photomicrographs of sections of pancreas – 1000 X

FIGURE (A): Transverse section of the pancreas of male luzindole alloxanised (100) treated rats on the 60th day showing decreased B cell population with an increased effect of allxoan induced damage. Note the clear areas (double headed dotted arrow) formed due to loss of cells also some degree of transdifferentiation (double headed arrow) is visible.

FIGURE (B): Transverse section of the pancreas of male control alloxanised (100) adult (60th day) rats showing islet and pancreatic acini. Note the increased B as well as A cell population. Also the islet and acinar cells show reduced clear areas (double headed dotted arrow) due to recovery of cells. Transdifferentiation (double headed arrow) of acinar cells is also visible.

FIGURE (C): Transverse section of the pancreas of male luzindole alloxanised (75) treated rats on the 60th day showing islet and pancreatic acini. There is an decrease in the islet size and islet cell number, with an decreased B:A cell ratio. Note the clear areas (double headed dotted arrow) formed due to loss of cells. Also some degree of transdifferentiation (double headed arrow) is visible.

PLATE - 17

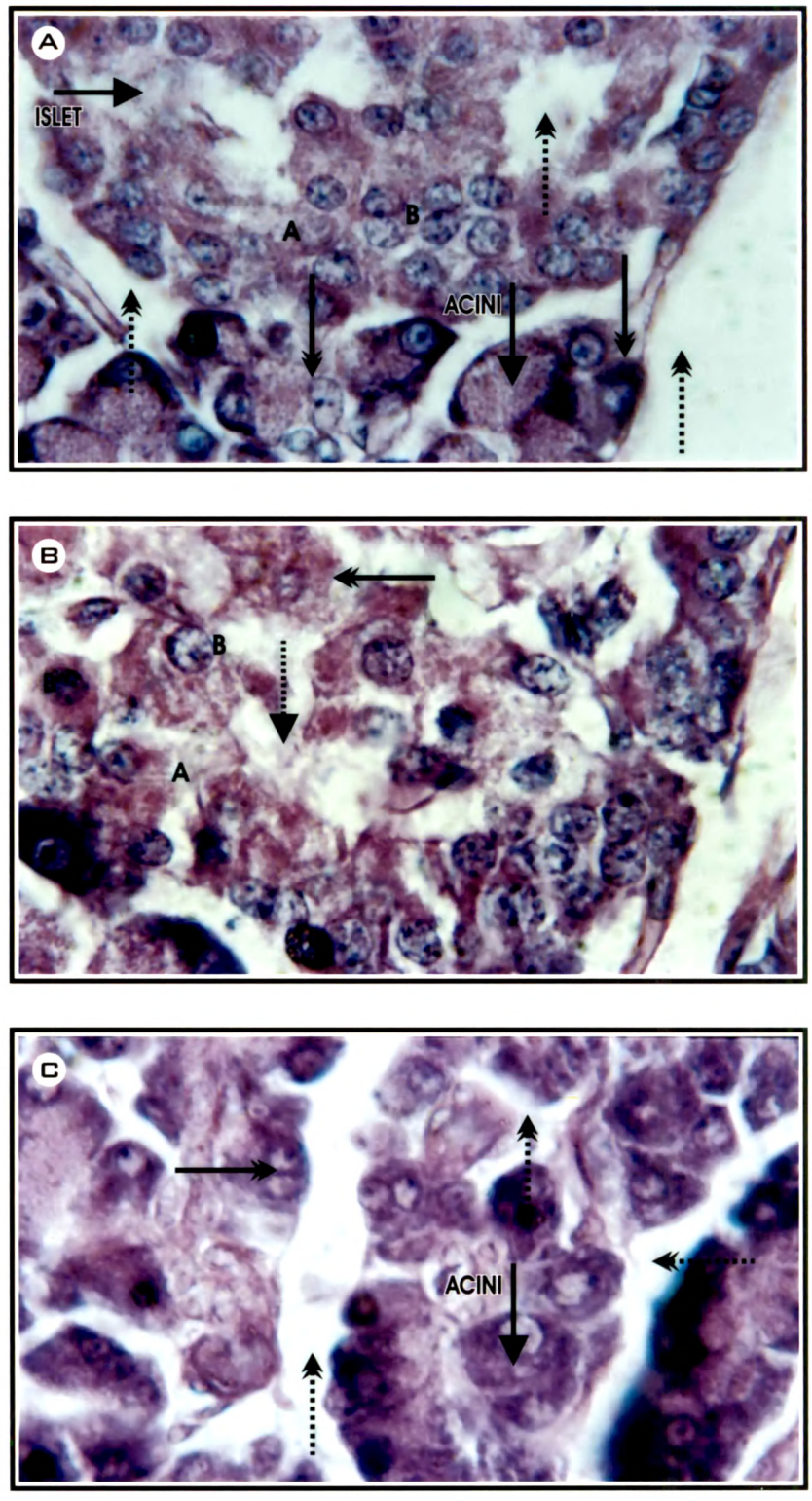


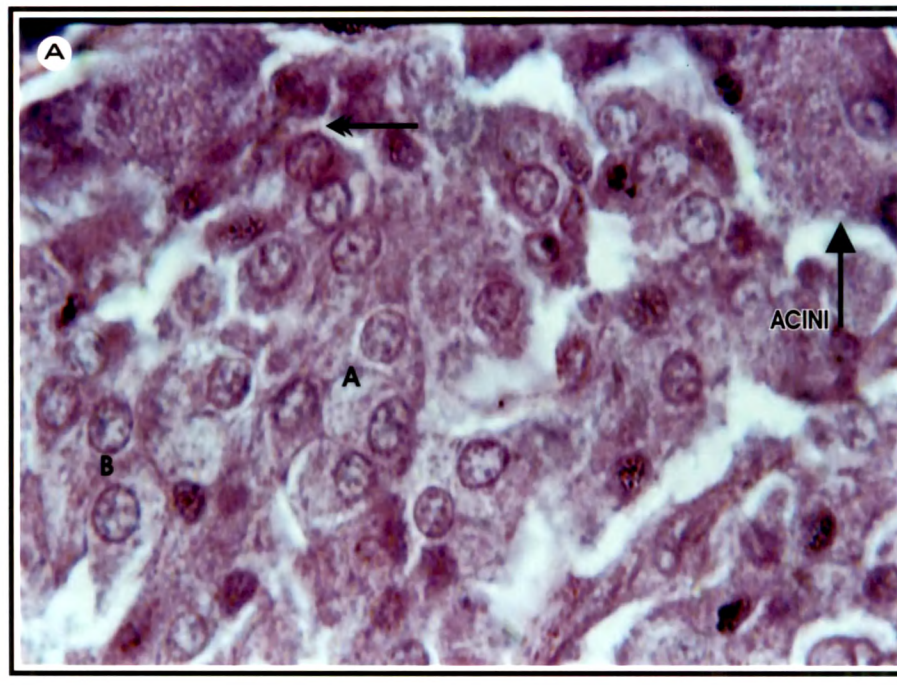
PLATE – 18

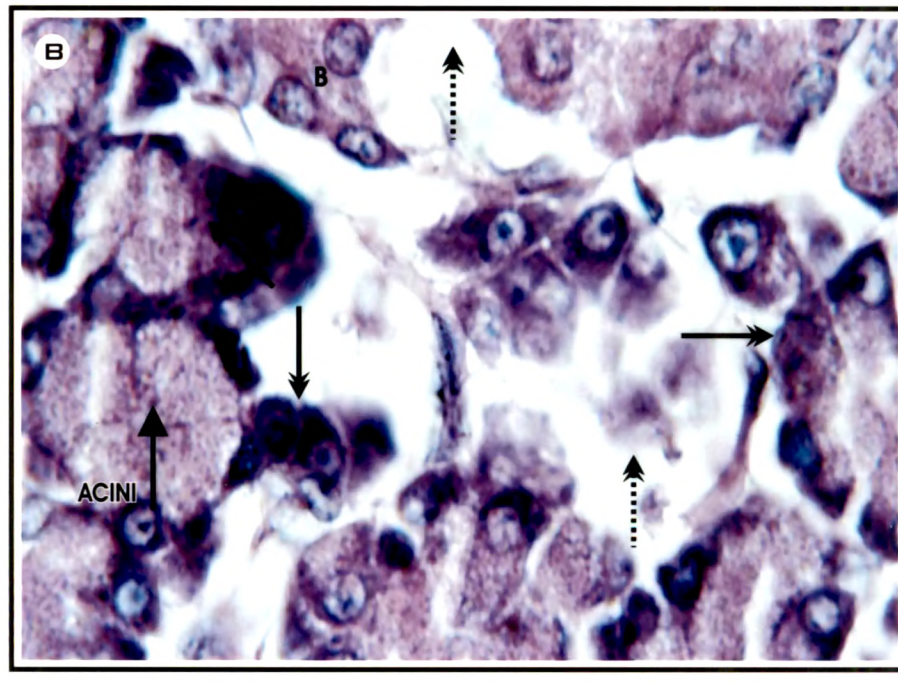
Photomicrographs of sections of pancreas – 1000 X

FIGURE (A): Transverse section of the pancreas of male control alloxanised adult (60^{th} day) rats showing islet and pancreatic acini. Note the increased population of B cells with reduced damage in the form of loss of cells

FIGURE (B): Transverse section of the pancreas of male luzindole alloxanised (75) treated rats on the 60^{th} day showing islet and pancreatic acini. There is an tremendous decrease in the islet size and islet cell number, with an decreased B:A cell ratio. Note the islet periphery showing acinar cells transdifferentiating (double headed arrow) into islet cells to some degree. The clear areas (double headed dotted arrow) show the effect of alloxan in the form of loss of the islet and acinar cells.

PLATE - 18





DISCUSSION:

The results obtained in the present study indicate significant alterations in metabolic status as well as histoarchitecture of the pancreatic islets due to weaning alloxan insult in both control and nL rats with effects in the latter group of animals being more significant. The reduction in body weight in control as well as nL rats, but more significant and dose dependent in latter (Fig. and Tab.; 11.1). Interestingly, the relative weight of pancreas, liver, kidney, spleen and adrenal are all increased in the nL rats while except for spleen the relative weight of all other organs is decreased in the control rats (Fig. and Tab.; 11.8-11.13). It is obvious that there is a favorable influence for organ growth due to neonatal melatonin functional deficiency respectively a retardatory influence on body growth. The underlined cause for the paradoxical effect does not find any valid explanation at this juncture. However, the significantly increased adrenal weights might suggest activation of hypothalamo hypophysial adrenal axis with probably increased corticosterone and catecholamine out put. Three weeks after weaning alloxan treatment (45 days), the nL rats had shown significant hypoglycemia as against hyperglycemia by the control rats paralleled by hyperinsulinemia and hypoinsulinemia respectively (Chapter 10). These changes were related with the increased B cell damage in control rats and remarkable B cell regeneration in nL rats leading to hypoinsulinemia and hyperinsulinemia respectively. However six weeks after alloxan treatment (60 days) the glycemic level increased in nL rats while it decreased in the control rats (Fig. and Tab.; 11.16).

Correspondingly the serum insulin levels have decreased in nL rats and increased in control rats (Fig. and Tab.; 11.35). This reverse in the serum glucose and insulin levels can be understood in the context of histologically observable increased B cell loss in nL rats and increasing recovery in the control rats (Plate, 15-18). Whereas hepatic glycogen content in the control rats is understandable in the context of increased serum glucose level and decreased insulin sensitivity, the more or less unchanged hepatic glycogen content in nL(100) rats with increased serum glucose level and reduced serum insulin levels is suggestive of increased insulin sensitivity Fig. and Tab.; 11.19). A more severe discrepancy is indicated in nL(75) rats by the observed significant decrease in hepatic and muscle glycogen contents with hyperglycemia and hypoinsulinemia (Fig. and Tab.; 11.14, 11.15, 11.35). Absence of protein anabolic influence in nL rats is also inferable by the tendency for reduced tissue protein contents (Fig. and Tab.; 11.17, 11.18). The nL rats also show concomitantly increased hepatic lipid and cholesterol contents as well as increased serum lipid fractions (Triglycerides, Cholesterol and Free Fatty Acids) more pronouncedly in the nL(75) rats (Fig. and Tab.; 11.24, 11.25, 11.30, 11.31, 11.34). Relative to 45 days, the 60 day control animals show remission in serum lipid profiles while the 60 day nL rats showed significant increment. These changes in hepatic and serum lipid levels as well as the observed glycemic and insulinemic status are clearly relatable with the decreased B cell mass with increased degenerative loss reminiscent of a developing of type II diabetes mellitus. It is interesting to record that even rats subjected to

neonatal streptozotocin/alloxan treatment have responded with diabetogenic changes in the adult condition after a period of recovery and normalization in between (Iwase *et al.*, 1987,89; Blondel *et al.*, 1990; Iwase *et al.*, 1991; Iwase, 91; Kodama *et al.*, 1993; Iwase *et al.*, 1994; Bonner Weir, 2000; Arulmozhi *et al.*, 2004). The increased B cell degeneration observable at 60 days could be a long term consequence of reduced neonatal melatonin action resulting in permanent alteration of B cell dynamics and increased generation of oxidative stress. This might suggest the need for an optimal melatonin action during the neonatal period for long term protection of pancreatic B cell and decreased generation of reactive oxygen species (ROS).

A persistence of this low B cell density could be a prognosis for type II diabetes. With reference to the protective effect of melatonin against oxidative stress and scavenging effect of ROS as well on induction of antioxidant activity, enough evidence is available (Bromme *et al.*, 2000; Reiter *et al.*, 2000; Anderson and Sangler, 2001; Stefinova *et al.*, 2002; Anwar and Meki, 2003) and in this light reduced exposure of pancreas to neonatal melatonin might lead to serious long term effects as revealed in the present study. A speculative question that needs to be answered is whether neonatal melatonin exposure is essential for auto induction of its receptors and if so reduce exposure to results in reduced receptor numbers which could contribute to the presently observed B cell loss. It is worth mentioning the report of Mellado *et al*, (1989) of decreased hepatic insulin an glucagon receptor concentrations in pinealectomized rats.

The observed changes in the lipid profiles of nL rats are in consonance with the concept of development of insulin resistance. Recent studies in humans and animals support the idea that liver fat content (independent of body composition or the degree of obesity) may be an important factor influencing insulin resistance (Ryysy *et al.*, 2000; Anderwald *et al.*, 2002). Further Nishida *et al*, (2003) have shown augmented levels of hepatic triglycerides and cholesterol esters in pinealectomized rats and they have also inferred increased lipoprotein production relatable with the condition in type II diabetic rats. Streptozotocin induced diabetic rats have also recorded elevation in plasma glucose, total lipids, triglycerides and cholesterol confirming abnormalities of glucose and lipids in diabetes (Verges, 1991; Manzato *et al.*, 1993; Merzouk *et al.*, 2000). Further treatment of diabetic rats with melatonin decreased blood glucose, triglycerides, total lipids and cholesterol levels (Montilla *et al.*, 1998; Gorgun *et al.*, 2002; Anwar and Meki, 2003). The herein observed changes in the lipid contents and serum glucose level are in confirming with the above reports and suggest a link between neonatal low melatonin status and potentiated diabetogenic inclination. Further searching studies are needed on these lines to take these observations to there logical conclusions.

In conclusion it can be said that neonatal functional melatonin deficiency could render these animals vulnerable to diabetogenic agents, conditions and result in insulin resistance and even type I diabetes by increased B cell loss.

SUMMARY:

Previous studies involving neonatal luzindole treatment and weaning alloxanisation showed increased B cell proliferation and neogenesis with hyperinsulinemia, hypoglycemia and hypolipidemia about 3 weeks after experimental induction of diabetes by alloxan treatment on day 22. It was thought pertinent to study the long term consequences of weaning alloxan treatment on carbohydrate, lipid and protein profiles of tissues and serum, along with serum insulin levels and histoarchitectural patterns of pancreas for possible long term effects about 6 weeks after alloxan treatment. To this end, rat neonates have been treated with Luzindole (An MT_2 receptor blocker) (400 µg/Kg body weight) intra peritoneally from day 1 to day 21 to generate a hypomelatonemic status; and a low (100 µg/kg) dose and a high dose (150 µg/kg) of alloxan were given on the 22^{nd} day and the effects assessed on the 60^{th} day. The body weight of all the alloxanised animals decreased significantly as compared to the control and nLT rats. The relative weight of pancreas of LA(100) and LA(75) rats decreased significantly as compared to the CA(100) and control rats of the same age. The relative weight of liver remained unaltered in all the groups. The relative weight of spleen of LA(75) rats increased significantly as compared to all other groups. The relative weight of kidney of alloxanised animals decreased significantly as compared to controls. The relative weight of adrenals of LA(75) decreased significantly as compared to all other groups other than nLT. The hepatic and muscle glycogen content of LA(100) rats increased

significantly as compared to all other groups while that of LA(75) decreased significantly as compared to nLT and control rats. The serum glucose level of LA(75) rats increased significantly as compared to control rats while that of LA(100) remained unaltered. The serum insulin level of LA(100) and LA(75) decreased significantly as compared to CA(100) and control rats. The muscle protein content of alloxanised animals increased significantly as compared to controls while, the hepatic protein content did not show any significant alterations. The hepatic and muscle glycogen synthetase activity of alloxanised animals decreased significantly as compared to nLT rats. The hepatic glycogen phosphorylase activity of LA(100) rats increased significantly as compared to all other groups. The muscle glycogen phosphorylase activity of LA(75) rats decreased significantly as compared to all other groups. The hepatic glucose-6-phosphatase activity of all the alloxanised rats decreased significantly as compared to nLT and control rats. The hepatic total lipid content of LA(75) rats increased significantly as compared to all other groups while, the hepatic cholesterol content of all the alloxanised groups decreased significantly as compared to the nLT rats. The muscle total lipid content of LA(100) and LA(75) rats decreased significantly as compared to the CA(100) rats. The muscle cholesterol content of LA(100) and LA(75) rats increased significantly as compared to CA(100) and age matched control rats. The total lipid content of adipose tissue of all the alloxanised groups decreased significantly as compared to age matched controls. The adipose tissue cholesterol

content of LA(100) and LA(75) rats decreased significantly as compared to CA(100), nLT and age matched control rats. The serum triglyceride level of all the alloxanised rats decreased significantly as compared to the age matched controls but increased significantly as compared to nLT rats. The serum cholesterol level of LA(100) rats decreased significantly as compared to all other groups. The serum total lipid and free fatty acid levels of the alloxanised rats decreased significantly as compared to the age matched controls. The serum phospholipid level of LA(100) and LA(75) rats decreased significantly as compared to both CA(100) and age matched control rats. It can be concluded from the above observations that neonatal functional melatonin deficiency could render these animals vulnerable to diabetogenic agents or conditions and, result in insulin resistance and even Type 1 diabetes by increased B cell loss.