CHAPTER – 10

LONG TERM PROTECTIVE EFFECT OF NEONATAL MELATONIN ADMINISTRATION ON ALLOXAN INDUCED DIABETES IN WEANING RATS

INTRODUCTION:

Diabetes mellitus is a chronic disease of multiple aetiology characterized by a deficiency of insulin and altered glycemic status. This is a severe metabolic disorder, characterized by hyperglycemia, altered metabolism of lipid, carbohydrates and proteins with an increased risk of vascular disease (Keen and Tang Fui, 1982; Pick up and Williams, 2003). Chronic marker hyperglycemia is the defining feature of diabetes mellitus (Keen and Tang Fui, 1982; Ziv *et al.*, 1999). Despite active research in the field, the molecular mechanisms responsible for the disorder have remained elusive. Dysfunction of B cells is considered to be major cause of development of a diabetes. The limited proliferative potential of adult insulin producing B cells (Hellerstrom *et al.*, 1988) is the major reason for the poor recovery from the diabetic state. It has been shown that the capacity of B cells to divide diminishes with increasing age where glucose intolerance becomes more prevalent (Hellerstrom, 1984; Hellerstrom and Swenne,

1985; Hellerstrom *et al* , 1988). However, expansion of B cell mass by recruitment of B cells to proliferate is a potential mechanism by which organisms can compensate for the loss or dysfunction of B cells occurring in diabetes (Hellerstrom, 1984; Hellerstrom and Swenne, 1985; Weir *et al.*, 1986; Hellerstrom *et al.*, 1988). But B cell regeneration is not a noteworthy feature in either humans or animal models for either of the 2 types of diabetes (Lazarow, 1952; Hellerstrom, 1984; Hellerstrom and Swerne, 1985; Kloppel *et al.*, 1985; Hellerstrom *et al.*, 1988; Portha *et al.*, 1989). Though the adult pancreatic B cell is quiescent, reports have indicated the possibility of their being stimulated to divide or replicate *in vitro* by nutrients and growth factors (Hellerstrom and Swerne, 1985, Kore, 1993; Sjoholm, 1993; Welsh *et al.*, 1993). Since defective insulin secretive response to glucose (Efandic *et al.*, 1981) as well as decreased B cell volume (Westermark and Wilander, 1978; Kloppel *et al* , 1985 Hellerstrom *et al.*, 1988) has been reported in diabetes, identification of factors which can promote insulin secretion and or reduce B cell proliferation would be meaningful.

Experimental induction of diabetes in the rat using chemicals which destroy B cells is used as a convenient tool for studying various aspects of diabetes. Alloxan, 5-6 Di oxy uracil, causes B cell damage by the generation of reactive oxygen species (ROS) and hydroxyl radicals and consequent increase in cytosolic Ca^{++} ion concentration (Szkuldeski, 2001). No experimental animal model is deemed to be identical to any human diabetic syndrome; however n- STZ rat models

(neonatal rats injected with streptozotocin at various ages) have been found to have advantage over other models and is considered to be one of the suitable animal models of type 2 diabetes mellitus (Arulmozhi *et al.*, 2004).

Melatonin, the pineal hormone synthesized and secreted during night (Reiter, 1991) is an effective free radical scavenger (Tan *et al.*, 1993; Reiter *et al.*, 1995; Marshall *et al.*, 1996, Bromme *et al* , 2000) and also a regulator of antioxidant and pro-oxidant enzymes (Barlow-Walden *et al.*, 1995). Being lipophilic, melatonin diffuses readily into cells and hence can also act without receptors (Menendez-Pelaez and Reiter, 1993). Since it has been shown to protect membranes and DNA from oxidative damage, melatonin has been tested in various models of inflammation and oxidative stress (Giushi *et al.*, 1979; Crespo *et al.*, 1999; Cuzzocrea and Kaputi, 1999; Cuzzocrea *et al.*, 1999). Recent studies have shown the potency of melatonin to protect against streptozotocin or alloxan induced damage to pancreatic B cells (Abdel-Wahub and Abet-Allah, 2000; Bromme *et al.*, 2000; Ebelt *et al.*, 2000; Anderson and Sandler, 2001).

In the background reviewed above since we have been studying the immediate and long term effects of neonatal hypermelatonemia on metabolic features, it was decided to test the possible protective action if any of neonatal hypermelatonemic status on long term effects of alloxan induced diabetes in the weaning age. Since experimental induction of diabetes has been carried out in neonates and adults, it was thought interesting to see the effects of induction of diabetes at

weaning age. Body and organ weights as well as metabolic features involving carbohydrates, proteins and lipids have been studied at 45 days of age in control and hypermelatonemic rats injected with alloxan at 22 days of age

MATERIAL AND METHODS: See page Nos. 16 to 37.

RESULTS:

Body and Organs weights: The MA(100) rats showed significantly decreased body weight as compared to CA(100) and control rats. The body weight of MA(150) and CA(100) rats were similar to each other and to the age matched controls. The relative weight of pancreas of the CA(100) rats decreased significantly as compared to MA(150), CA(100) and control rats but was significantly higher than that of nMT rats. However the relative weight of liver of the CA(100) rats decreased significantly as compared to MA(150), MA(100) and control rats. The relative weight of spleen of the MA(100) rats increased significantly as compared to all other groups. The relative weight of kidney of MA(100) rats increased significantly as compared to MA(150) and control rats but was significantly decreased as compared to nMT rats. The relative weight of testes of MA(100) rats decreased significantly as compared to all other groups while, the relative weight of adrenal of all the groups showed no significant alterations (Figure and Table; 10.1-10.13).

Serum glucose and insulin levels: The serum glucose and insulin levels of MA(150), CA(100) and MA(100) rats decreased significantly as compared to control rats (Figure and Table; 10.16, 10.35).

Hepatic glycogen content and the activities of glycogen synthetase, glycogen phosphorylase and glucose-6-phosphatase: The MA(150), CA(100) and MA(100) rats showed an significant decrease in the glycogen content, glycogen synthetase and glycogen phosphorylase activities as compared to nMT and control rats. Whereas the activity of glucose-6-phosphatase increased significantly in the liver of MA(150) and MA(100) rats as compared to CA(100) and control rats (Figure and Table; 10.17, 10.19, 10.21).

Muscle glycogen content and the activities of glycogen synthetase and glycogen phosphorylase: The muscle glycogen content increased significantly in the MA(150), CA(100) and MA(100) rats as compared to nMT and control rats. Whereas, the glycogen synthetase activity was significantly increased in CA(100) rats as compared to MA(150), MA(100) and control rats, the glycogen phosphorylase activity of MA(150), MA(100) and CA(100) rats decreased significantly as compared to the control rats (Figure and Table; 10.15, 10.18).

`Hepatic and muscle protein content: The hepatic and muscle protein content of CA(100) and MA(100) rats increased significantly as compared to control rats (Figure and Table; 10.22, 10.23).

Hepatic total lipid and cholesterol contents: The hepatic total lipid content decreased significantly in the MA(100) rats as compared to all other groups while, the hepatic cholesterol content of MA(100) rats increased significantly as compared to all other groups (Figure and Table; 10.24, 10 25).

Muscle total lipid and cholesterol contents: The muscle total lipid content of MA(100) rats decreased significantly as compared to all other groups while, the cholesterol content in the muscle of MA(100) rats increased significantly as compared to all other groups(Figure and Table; 10.26, 10.27)

Adipose tissue total lipid and cholesterol contents: The adipose tissue total lipid content of MA(150) and MA(100) rats decreased significantly as compared to CA(100), nMT and control rats while, the adipose tissue cholesterol content of CA(100) rats decreased significantly as compared to all other groups(Figure and Table; 10.28, 10.29).

Serum lipid fractions: The serum triglyceride level of CA(100) and MA(100) rats decreased significantly as compared to MA(150), nMT and control rats. However the serum cholesterol level of MA(150) and MA(100) rats increased significantly as compared to CA(100), nMT and control rats. The serum total lipid level of MA(100) rats decreased significantly as compared to all other groups. The serum phospholipid level of MA(100) and CA(100) rats decreased significantly as compared to control rats. The serum free fatty acid level of MA(150), CA(100) and MA(100) rats increased significantly as compared to control rats (Figure and Table; 10.30, 10.31, 10.32, 10.33, 10.34).

Figure 10.1: Body weight of pubertal rats on 45th day subjected to neonatal melatonin treatment and weaning alloxanisation on 22nd day:

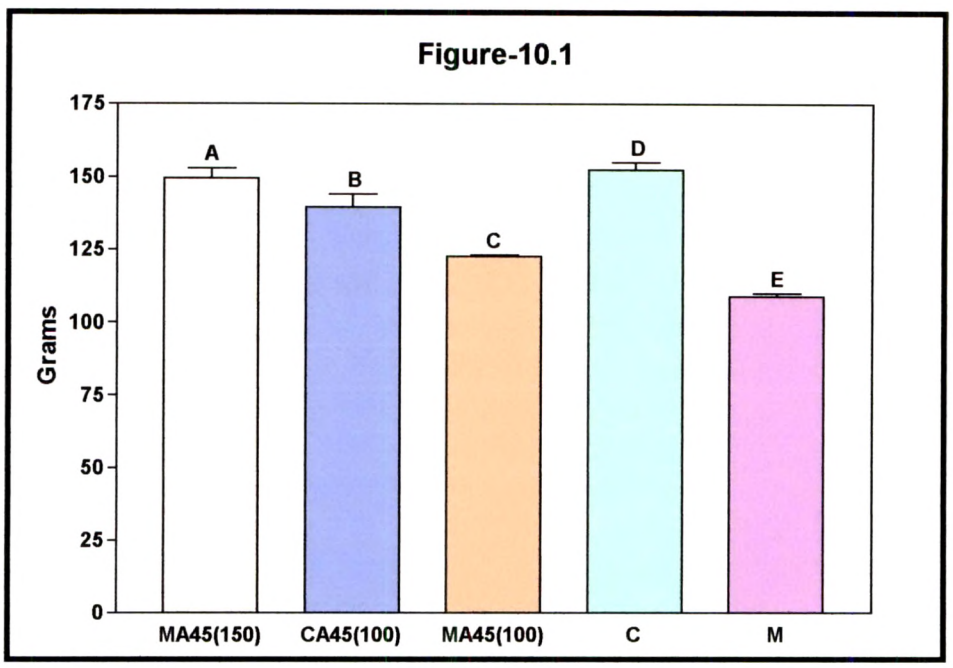


Table 10.1: Body weight of pubertal rats on 45th day subjected to neonatal melatonin treatment and weaning alloxanisation on 22nd day:

	MA(150)(A)	CA(100)(B)	MA(100)(C)	C(D)	M(E)
BODY WEIGHT	149.50 ±3.47	139.50 ±4.51	122.75 ±0.24	152.50 ±2.5	109.00 ±1.00

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

Figure 10.2: Absolute weight of pancreas of pubertal rats on 45th day subjected to neonatal melatonin treatment and weaning alloxanisation on 22nd day:

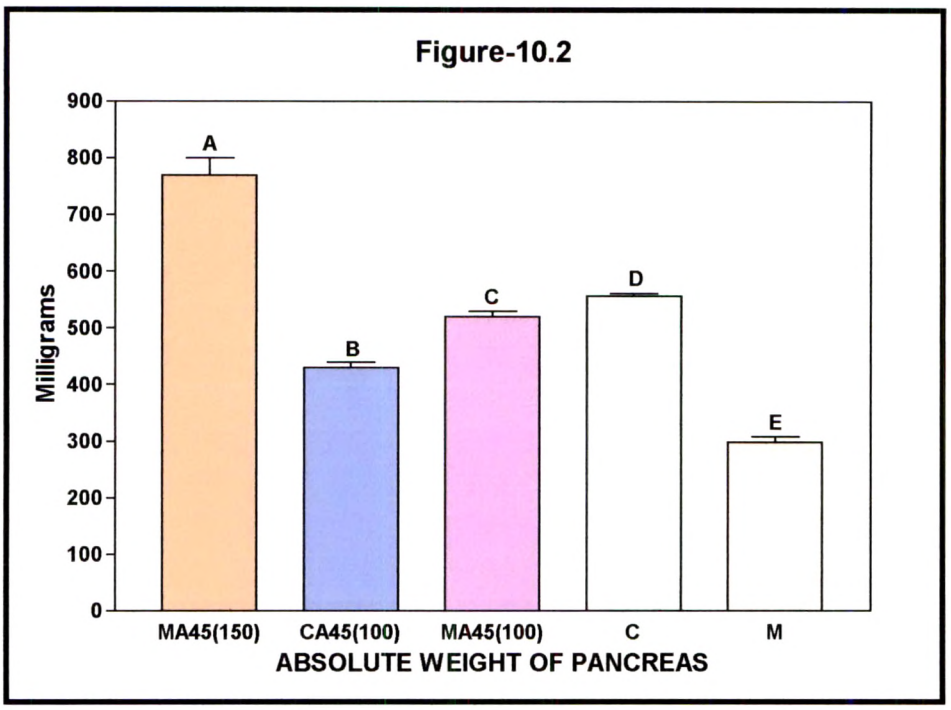


Table 10.2: Absolute weight of pancreas pubertal rats on 45th day subjected to neonatal melatonin treatment and weaning alloxanisation on 22nd day:

	MA(150)(A)	CA(100)(B)	MA(100)(C)	C(D)	M(E)
PANCREAS	770.00 ±30.08	430.00 ±10.02	520.00 ±10.02	557.00 ±5.01	299.00 ±9.02

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 10.3: Absolute weight of liver of pubertal rats on 45th day subjected to neonatal melatonin treatment and weaning alloxanisation on 22nd day:

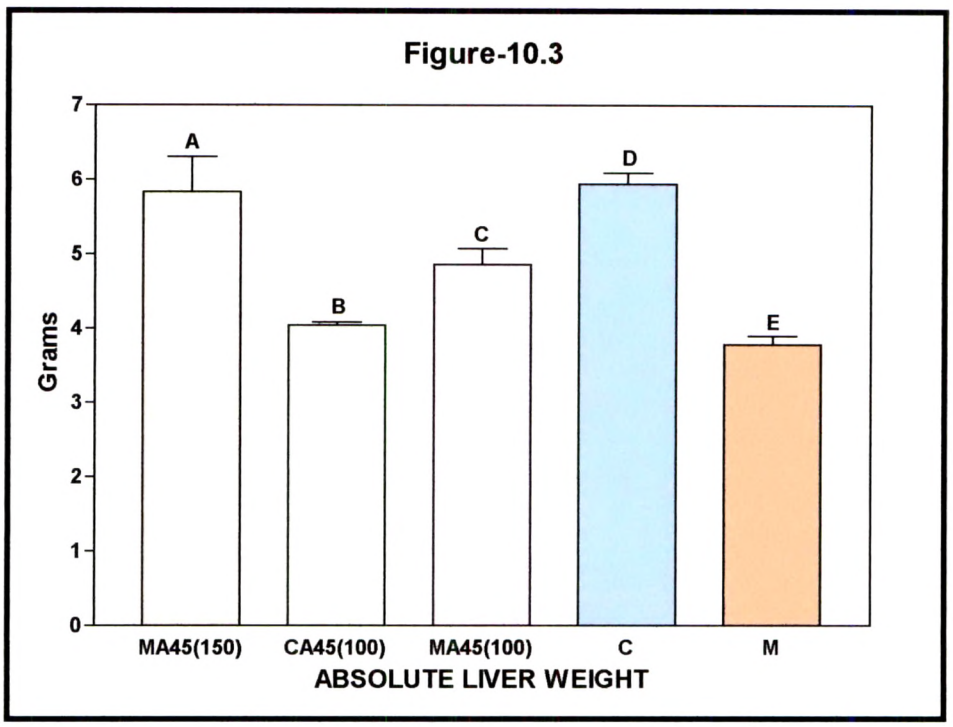


Table 10.3: Absolute weight of liver of pubertal rats on 45th day subjected to neonatal melatonin treatment and weaning alloxanisation on 22nd day:

	MA(150)(A)	CA(100)(B)	MA(100)(C)	C(D)	M(E)
LIVER	5.83 ±0.47	4.04 ±0.039	4.86 ±0.22	5.94 ±0.15	3.79 ±0.11

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 10.4: Absolute weight of spleen of pubertal rats on 45th day subjected to neonatal melatonin treatment and weaning alloxanisation on 22nd day:

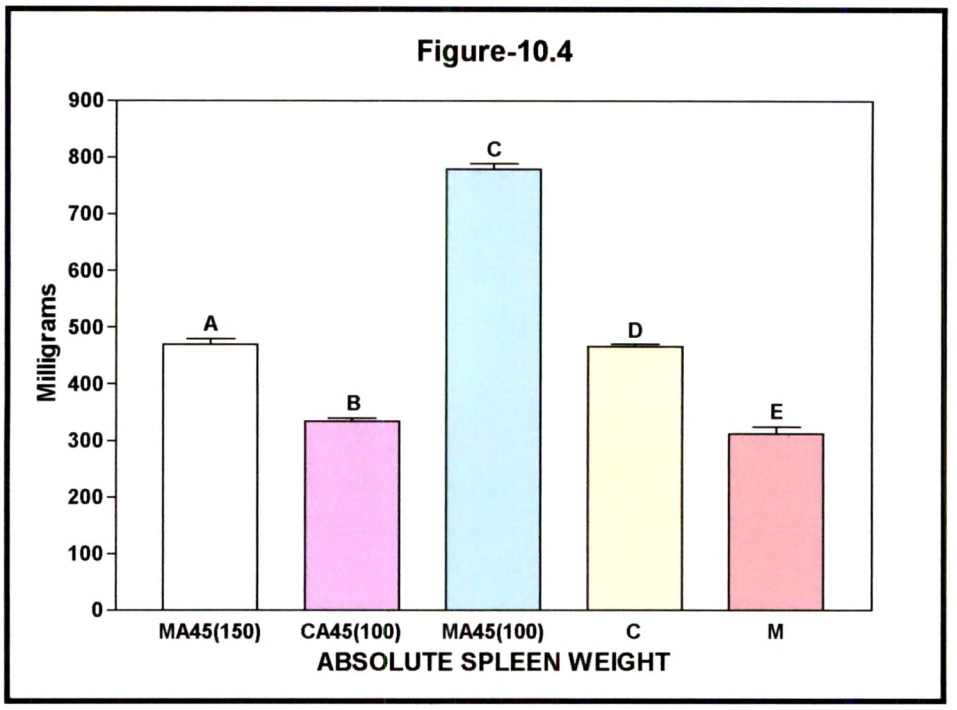


Table 10.4: Absolute weight of spleen of pubertal rats on 45th day subjected to neonatal melatonin treatment and weaning alloxanisation on 22nd day:

	MA(150)$^{(A)}$	CA(100)$^{(B)}$	MA(100)$^{(C)}$	C$^{(D)}$	M$^{(E)}$
SPLEEN	470.00 ±10.02	335.00 ±5.01	780.00 ±10.02	467.5 ±4.51	312.5 ±12.53

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; NSNon Significant

Figure 10.5: Absolute weight of kidney of pubertal rats on 45th day subjected to neonatal melatonin treatment and weaning alloxanisation on 22nd day:

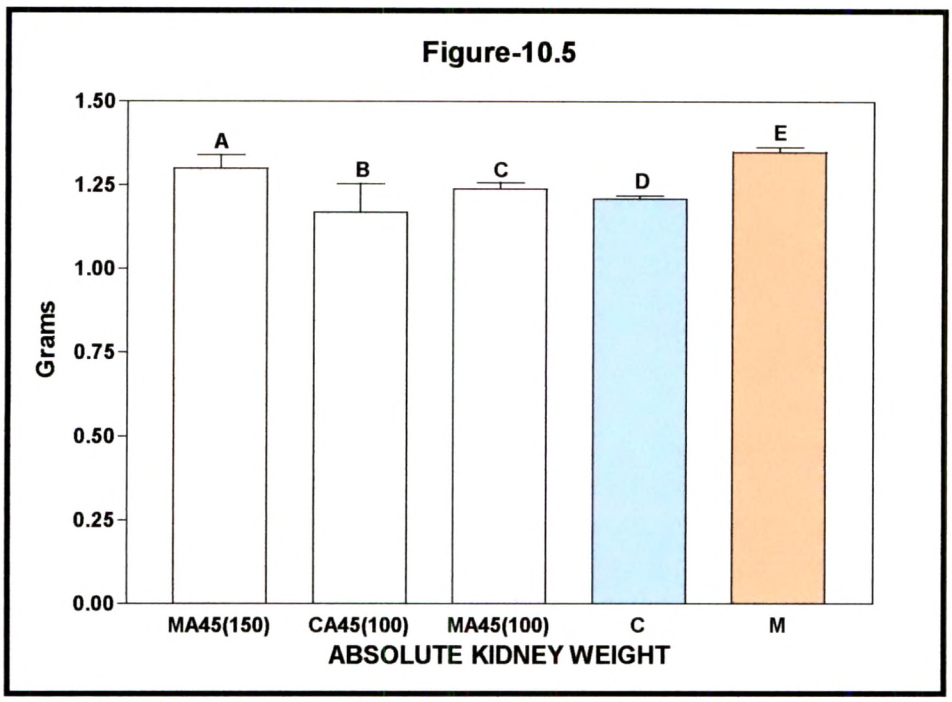


Table 10.5: Absolute weight of kidney of pubertal rats on 45th day subjected to neonatal melatonin treatment and weaning alloxanisation on 22nd weight of day:

	MA(150)[A]	CA(100)[B]	MA(100)[C]	C[D]	M[E]
KIDNEY	1.30 ±0.04	1.17 ±0.085	1.24 ±0.019	1.21 ±0.0099	1.35 ±0.014

Bonferroni's Multiple Comparison Test

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

Values are expressed as mean ± SEM, [NS]Non Significant

Figure 10.6: Absolute weight of testes of pubertal rats on 45th day subjected to neonatal melatonin treatment and weaning alloxanisation on 22nd day:

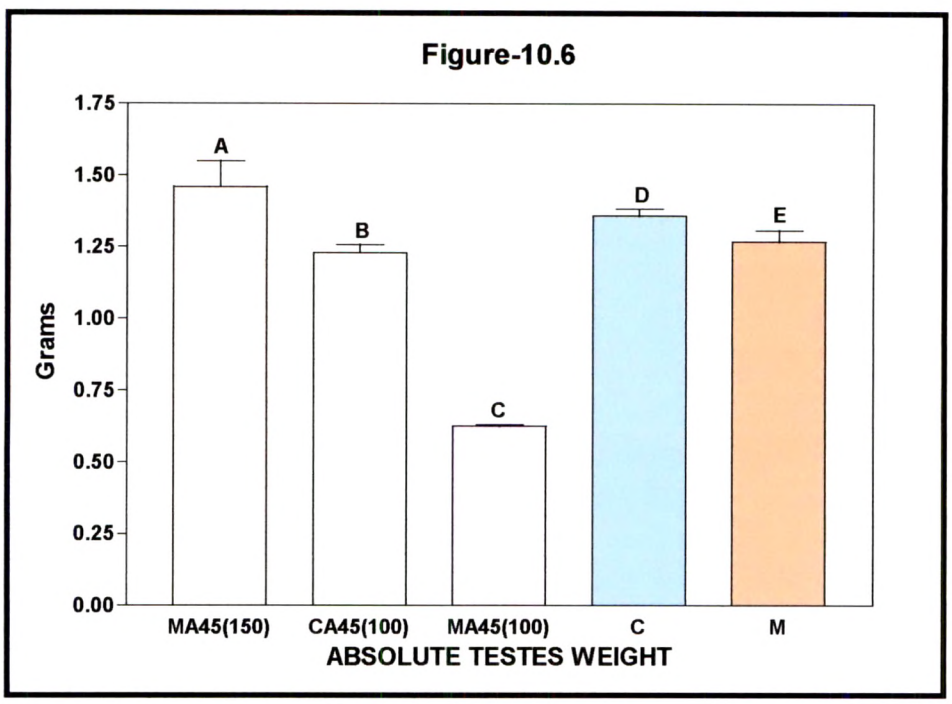


Table 10.6: Absolute weight of testes of pubertal rats on 45th day subjected to neonatal melatonin treatment and weaning alloxanisation on 22nd day:

	MA(150)(A)	CA(100)(B)	MA(100)(C)	C(D)	M(E)
TESTES	1.46 ±0.09	1.23 ±0.028	0.625 ±0.005	1.36 ±0.024	1.27 ±0.04

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 10.7: Absolute weight of adrenal of pubertal rats on 45th day subjected to neonatal melatonin treatment and weaning alloxanisation on 22nd day:

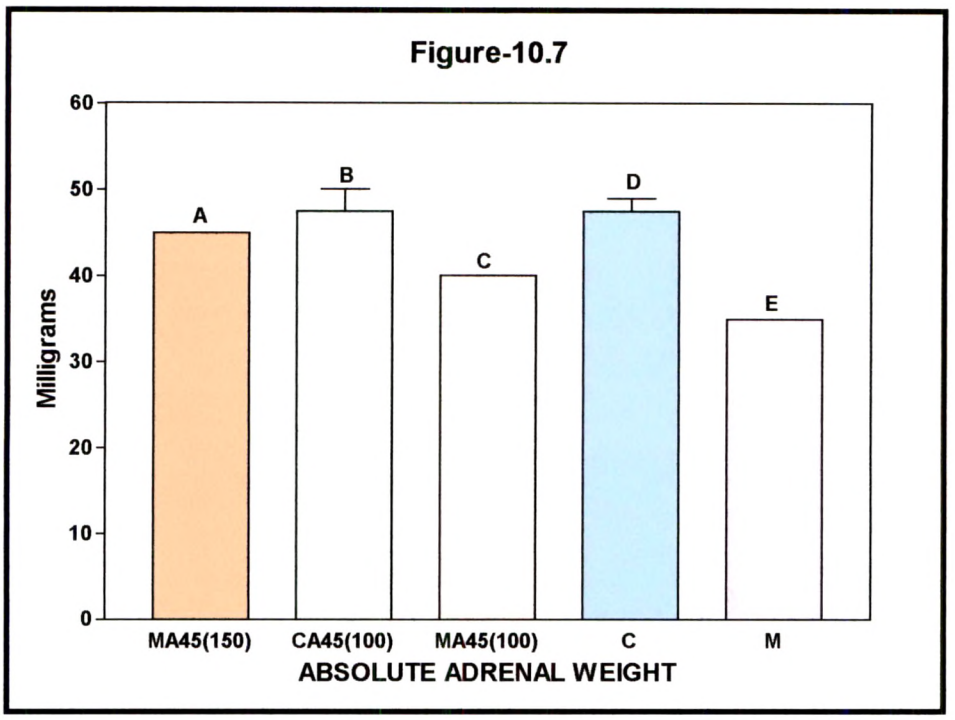


Table 10.7: Absolute weight of adrenal of pubertal rats on 45th day subjected to neonatal melatonin treatment and weaning alloxanisation on 22nd day:

	MA(150)(A)	CA(100)(B)	MA(100)(C)	C(D)	M(E)
ADRENAL	45.00 ±0.00	47.50 ±2.50	40.00 ±0.00	47.50 ±1.50	35.00 ±0.00

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 10.8: Relative weight of pancreas of pubertal rats on 45th day subjected to neonatal melatonin treatment and weaning alloxanisation on 22nd day:

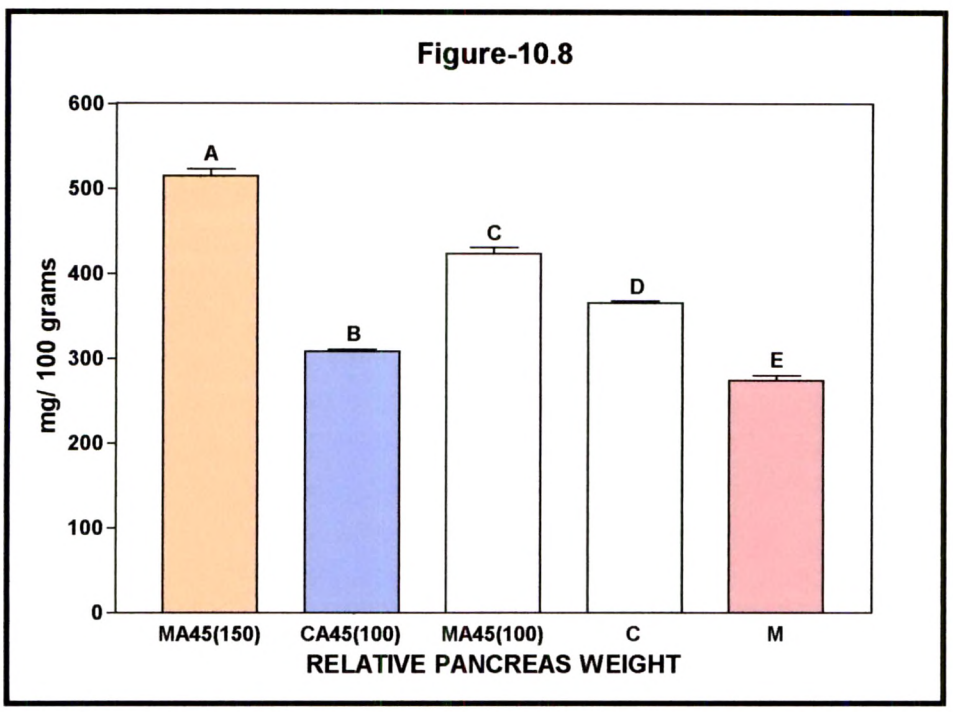


Table 10.8: Relative weight of pancreas of pubertal rats on 45th day subjected to neonatal melatonin treatment and weaning alloxanisation on 22nd day:

	MA(150)(A)	CA(100)(B)	MA(100)(C)	C(D)	M(E)
PANCREAS	514.85 ±8.03	308.33 ±2.78	423.60 ±7.30	365.29 ±2.71	274.25 ±5.75

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;
NSNon Significant

Figure 10.9: Relative weight of liver of pubertal rats on 45th day subjected to neonatal melatonin treatment and weaning alloxanisation on 22nd day:

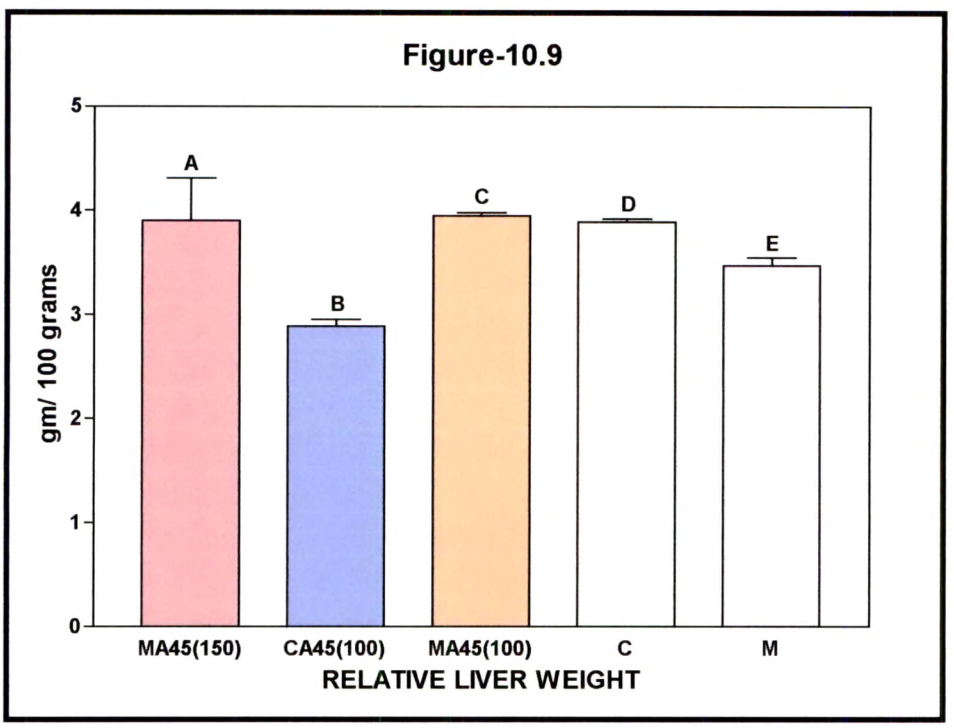


Table 10.9: Relative weight of liver of pubertal rats on 45th day subjected to neonatal melatonin treatment and weaning alloxanisation on 22nd day:

	MA(150)(A)	CA(100)(B)	MA(100)(C)	C(D)	M(E)
LIVER	3.90 ±0.41	2.89 ±0.064	3.95 ±0.19	3.89 ±0.03	3.47 ±0.078

Bonferroni's Multiple Comparison Test

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

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

Figure 10.10: Relative weight of spleen of pubertal rats on 45th day subjected to neonatal melatonin treatment and weaning alloxanisation on 22nd day:

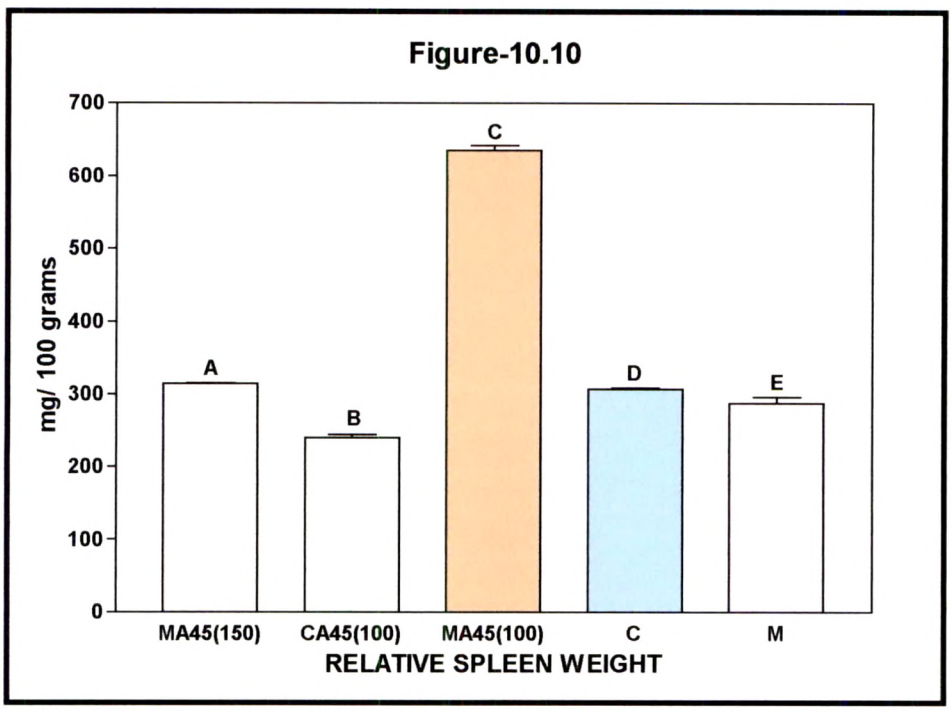


Table 10.10: Relative weight of spleen of pubertal rats on 45th day subjected to neonatal melatonin treatment and weaning alloxanisation on 22nd day:

	MA(150)(A)	CA(100)(B)	MA(100)(C)	C(D)	M(E)
SPLEEN	314.39 ±0.66	240.27 ±4.17	635.42 ±6.86	306.58 ±2.07	286.61 ±8.86

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<0.05;
NSNon Significant

Figure 10.11: Relative weight of kidney of pubertal rats on 45th day subjected to neonatal melatonin treatment and weaning alloxanisation on 22nd day:

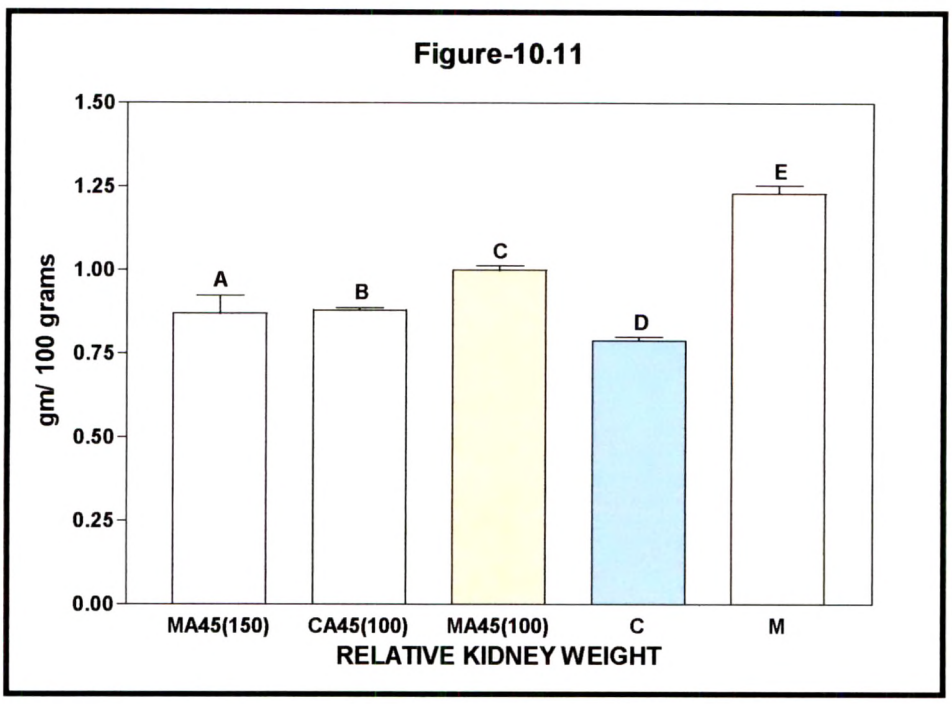


Table 10.11: Relative weight of kidney of pubertal rats on 45th day subjected to neonatal melatonin treatment and weaning alloxanisation on 22nd day:

	MA(150)(A)	CA(100)(B)	MA(100)(C)	C(D)	M(E)
KIDNEY	0.87 ±0.053	0.88 ±0.0064	1.00 ±0.014	0.79 ±0.0099	1.23 ±0.024

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<0.05;
NS Non Significant

Figure 10.12: Relative weight of testes of pubertal rats on 45th day subjected to neonatal melatonin treatment and weaning alloxanisation on 22nd day:

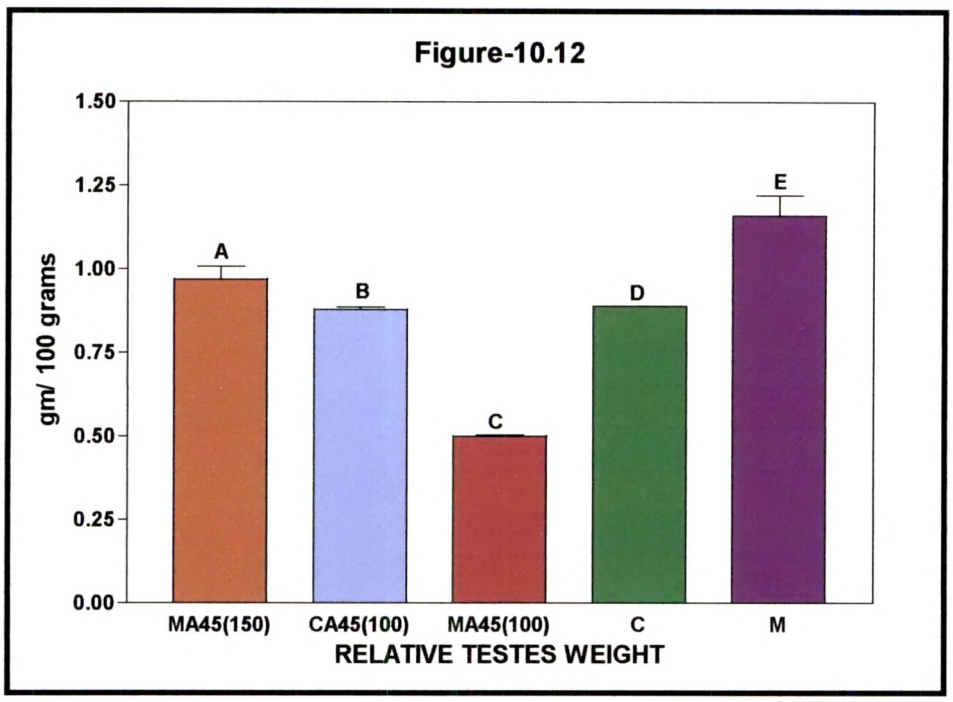


Table 10.12: Relative weight of testes of pubertal rats on 45th day subjected to neonatal melatonin treatment and weaning alloxanisation on 22nd day:

	MA(150)(A)	CA(100)(B)	MA(100)(C)	C(D)	M(E)
TESTES	0.97 ±0.039	0.88 ±0.0064	0.50 ±0.0030	0.89 ±0.00	1.16 ±0.06

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 10.13: Relative weight of adrenal of pubertal rats on 45th day subjected to neonatal melatonin treatment and weaning alloxanisation on 22nd day:

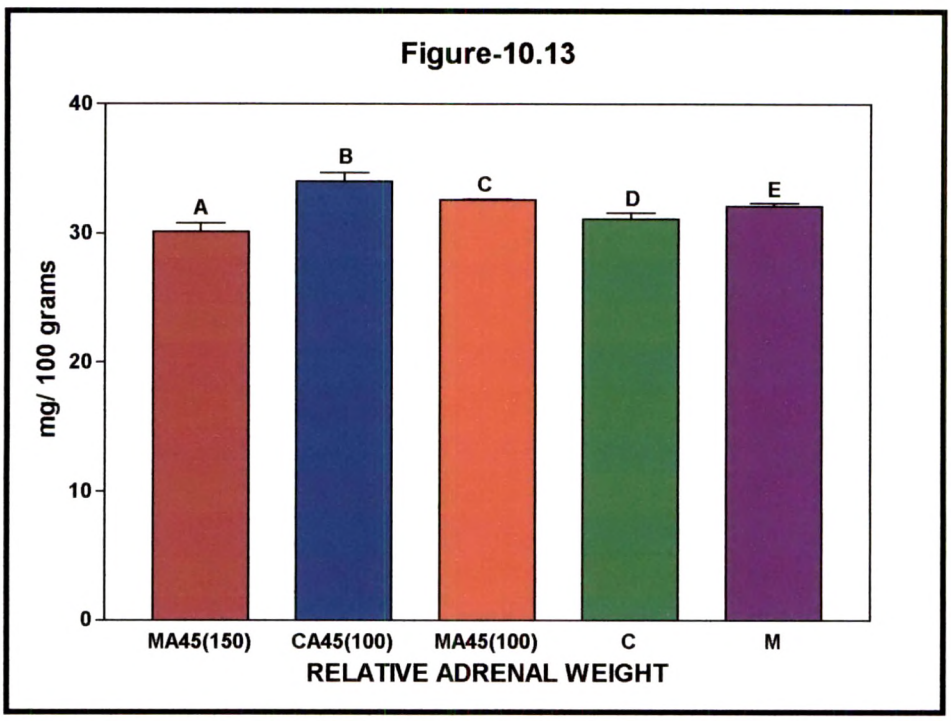


Table 10.13: Relative weight of adrenal of pubertal rats on 45th day subjected to neonatal melatonin treatment and weaning alloxanisation on 22nd day:

	MA(150)(A)	CA(100)(B)	MA(100)(C)	C(D)	M(E)
ADRENAL	30.11 ±0.70	34.02 ±0.69	32.58 ±0.064	31.13 ±0.47	32.10 ±0.29

Bonferroni's Multiple Comparison Test

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

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

Figure 10.14: Hepatic glycogen content of pubertal rats on 45th day subjected to neonatal melatonin treatment and weaning alloxanisation on 22nd day:

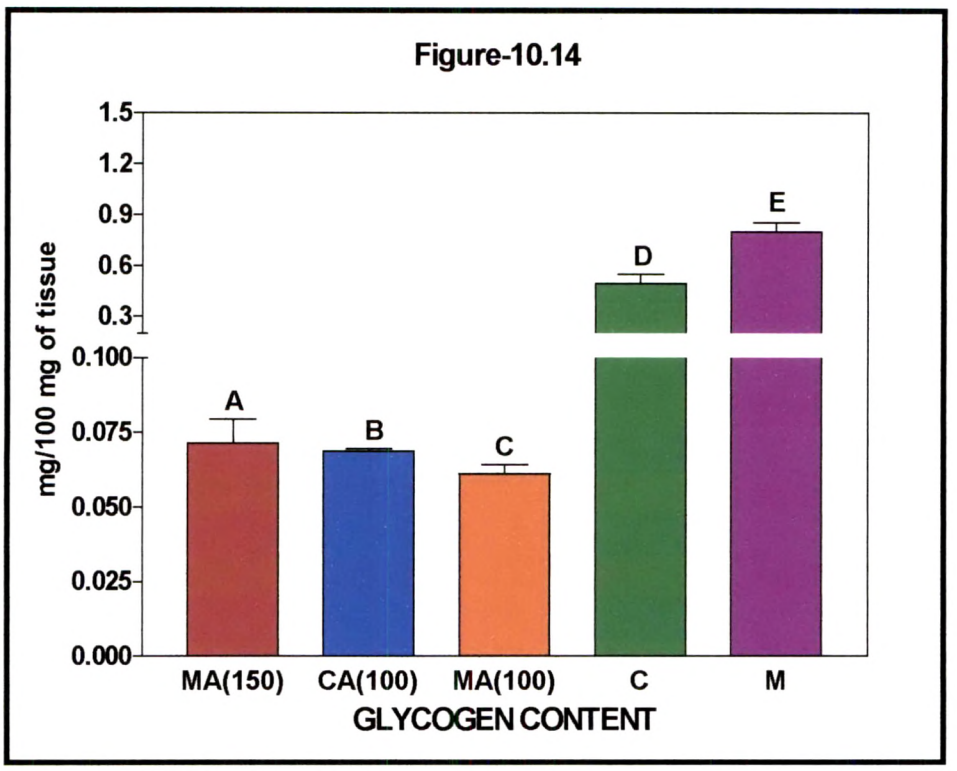


Table 10.14: Hepatic glycogen content of pubertal rats on 45th day subjected to neonatal melatonin treatment and weaning alloxanisation on 22nd day:

	MA(150)(A)	CA(100)(B)	MA(100)(C)	C(D)	M(E)
GLYCOGEN	0.0714 ±0.0028	0.0688 ±0.000705	0.0612 ±0.00106	0.4933 ±0.0275	0.7983 ±0.0274

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 10.15: Muscle glycogen content of pubertal rats on 45th day subjected to neonatal melatonin treatment and weaning alloxanisation on 22nd day:

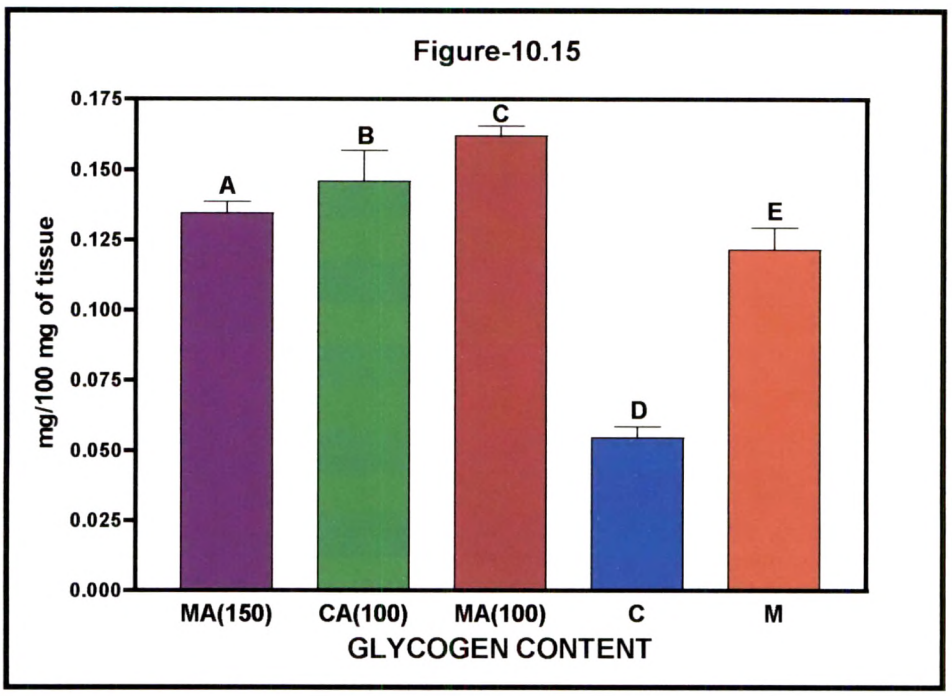


Table 10.15: Muscle glycogen content of pubertal rats on 45th day subjected to neonatal melatonin treatment and weaning alloxanisation on 22nd day:

	MA(150)(A)	CA(100)(B)	MA(100)(C)	C(D)	M(E)
GLYCOGEN	0.1346 ±0.00401	0.146 ±0.01085	0.162 ±0.00353	0.0547 ±0.00395	0.1216 ±0.0079

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;
NSNon Significant

Figure 10.16: Serum glucose level of pubertal rats on 45th day subjected to neonatal melatonin treatment and weaning alloxanisation on 22nd day:

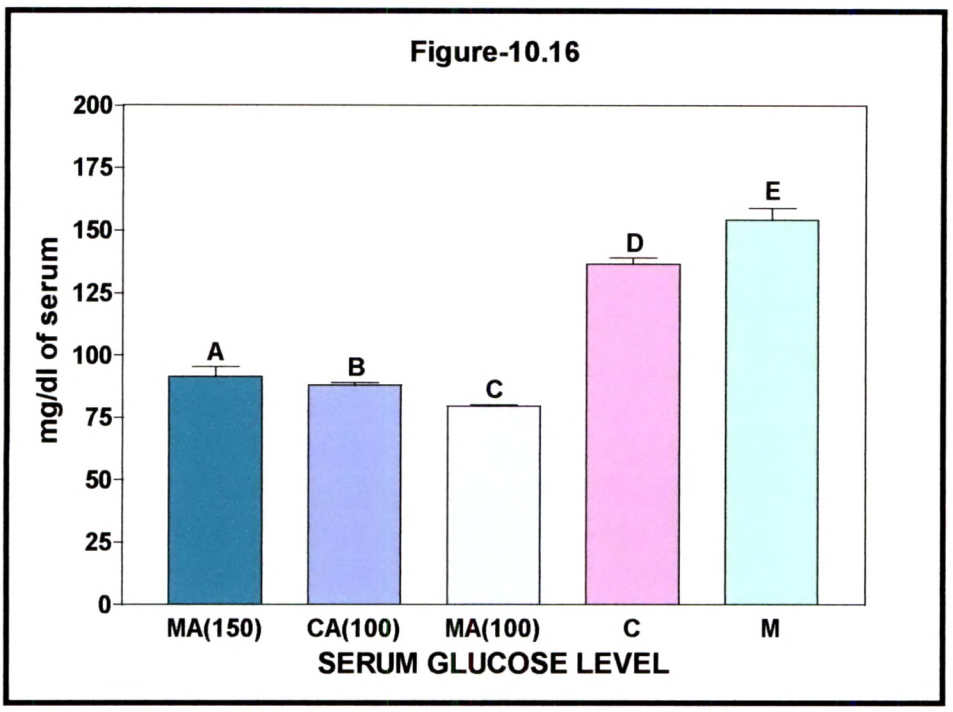


Table 10.16: Serum glucose level of pubertal rats on 45th day subjected to neonatal melatonin treatment and weaning alloxanisation on 22nd day:

	MA(150)(A)	CA(100)(B)	MA(100)(C)	C(D)	M(E)
GLUCOSE	91.40 ±3.9573	87.86 ±1.1224	79.55 ±0.5297	136.72 ±2.3790	154.45 ±4.5840

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 10.17: Hepatic glycogen synthetase activity in pubertal rats on 45th day subjected to neonatal melatonin treatment and weaning alloxanisation on 22nd day:

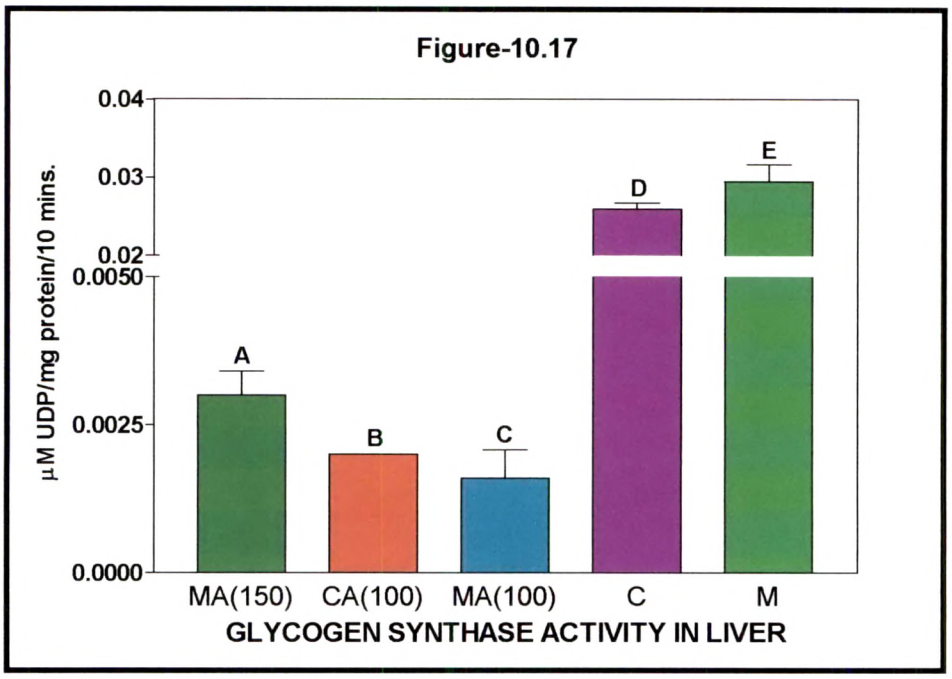


Table 10.17: Hepatic glycogen synthetase activity in pubertal rats on 45th day subjected to neonatal melatonin treatment and weaning alloxanisation on 22nd day:

	MA(150)(A)	CA(100)(B)	MA(100)(C)	C(D)	M(E)
GLYCOGEN SYNTHETASE	0.003 ±0.000405	0.002 ±0.00	0.0016 ±0.000475	0.026 ±0.00075	0.0295 ±0.0022

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; NSNon Significant

Figure 10.18: Muscle glycogen synthetase activity in pubertal rats on 45th day subjected to neonatal melatonin treatment and weaning alloxanisation on 22nd day:

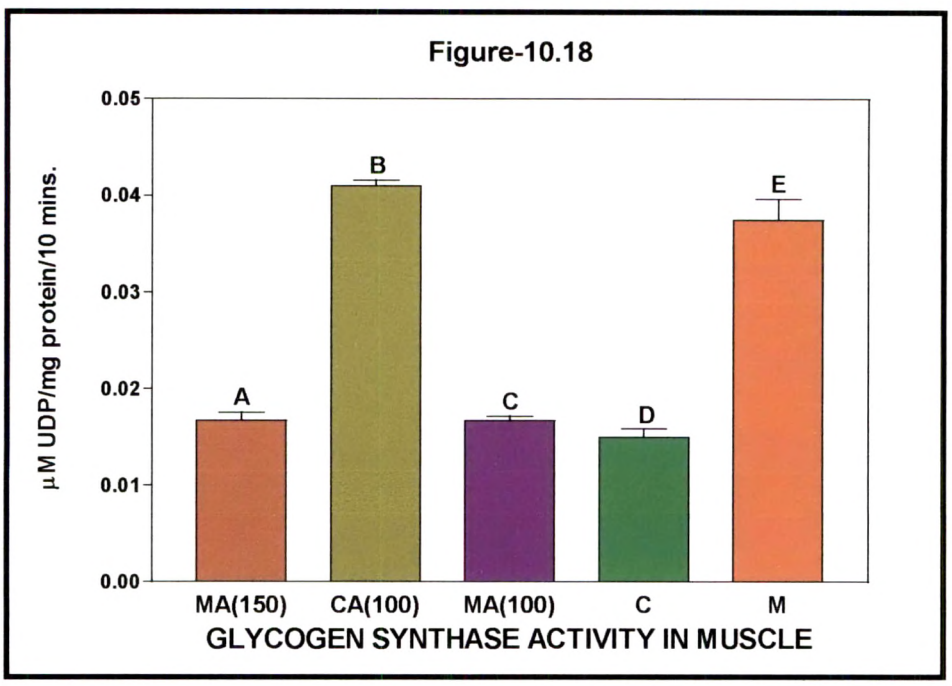


Table 10.18: Muscle glycogen synthetase activity in pubertal rats on 45th day subjected to neonatal melatonin treatment and weaning alloxanisation on 22nd day:

	MA(150)(A)	CA(100)(B)	MA(100)(C)	C(D)	M(E)
GLYCOGEN SYNTHETASE	0.0167 ±0.00085	0.041 ±0.0006	0.0167 ±0.000475	0.015 ±0.00085	0.0375 ±0.00215

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; NSNon Significant

Figure 10.19: Hepatic glycogen phosphorylase activity in pubertal rats on 45th day subjected to neonatal melatonin treatment and weaning alloxanisation on 22nd day:

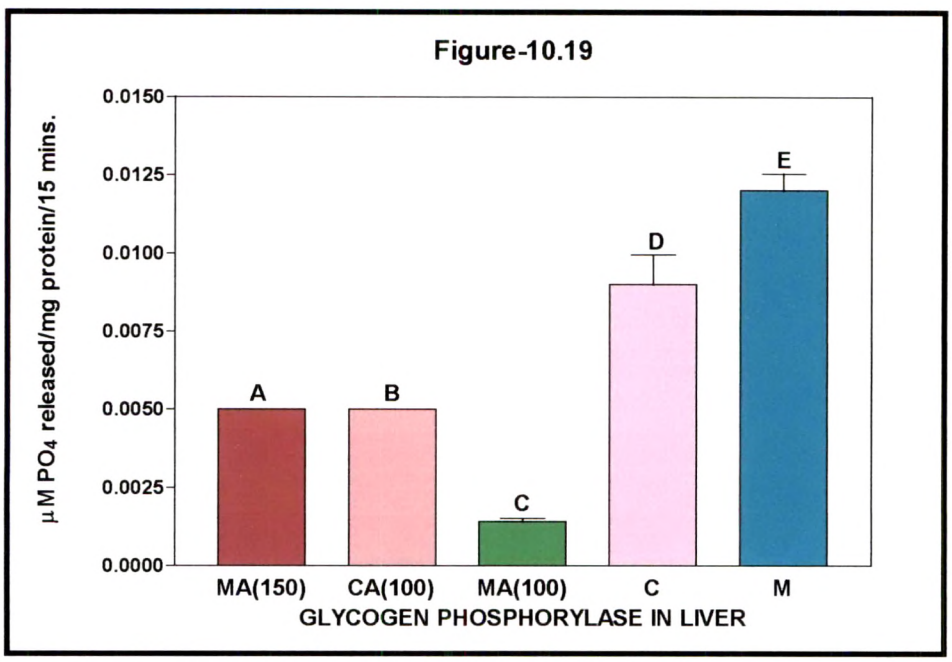


Table 10.19: Hepatic glycogen phosphorylase activity in pubertal rats on 45th day subjected to neonatal melatonin treatment and weaning alloxanisation on 22nd day:

	MA(150)(A)	CA(100)(B)	MA(100)(C)	C(D)	M(E)
GLYCOGEN PHOSPHORYLASE	0.005 ±0.0001	0.005 ±0.0003	0.0014 ±0.0001	0.009 ±0.00095	0.012 ±0.00055

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 10.20: Muscle glycogen phosphorylase activity in pubertal rats on 45th day subjected to neonatal melatonin treatment and weaning alloxanisation on 22nd day:

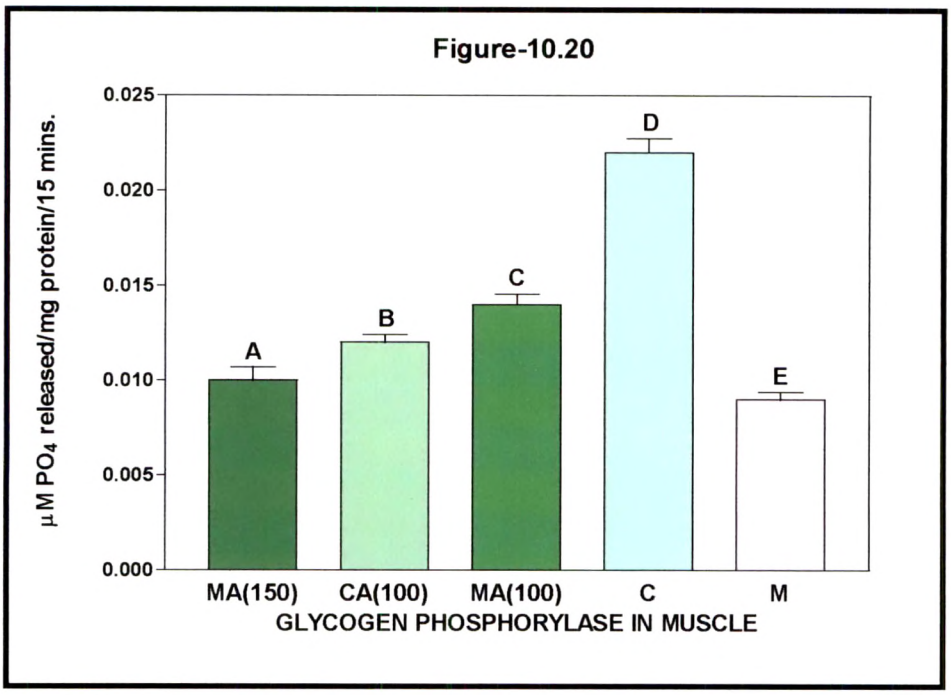


Table 10.20: Muscle glycogen phosphorylase activity in pubertal rats on 45th day subjected to neonatal melatonin treatment and weaning alloxanisation on 22nd day:

	MA(150)(A)	CA(100)(B)	MA(100)(C)	C(D)	M(E)
GLYCOGEN PHOSPHORYLASE	0.010 ±0.0007	0.012 ±0.0004	0.014 ±0.00055	0.015 ±0.00085	0.0375 ±0.00215

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 10.21: Hepatic glucose-6-phosphatase activity in pubertal rats on 45th day subjected to neonatal melatonin treatment and weaning alloxanisation on 22nd day:

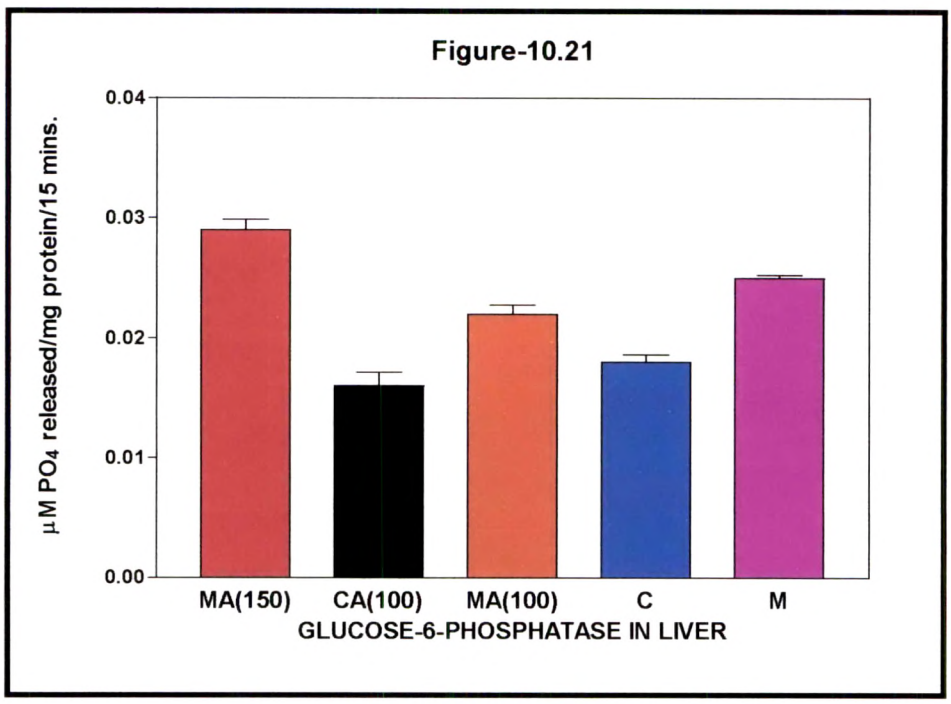


Table 10.21: Hepatic glucose-6-phosphatase activity in pubertal rats on 45th day subjected to neonatal melatonin treatment and weaning alloxanisation on 22nd day:

	MA(150)(A)	CA(100)(B)	MA(100)(C)	C(D)	M(E)
GLUCOSE-6-PHOSPHATASE	0.029 ±0.00085	0.016 ±0.0011	0.022 ±0.00075	0.018 ±0.0006	0.025 ±0.000285

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<0.05; NSNon Significant

Figure 10.22: Hepatic protein content of pubertal rats on 45th day subjected to neonatal melatonin treatment and weaning alloxanisation on 22nd day:

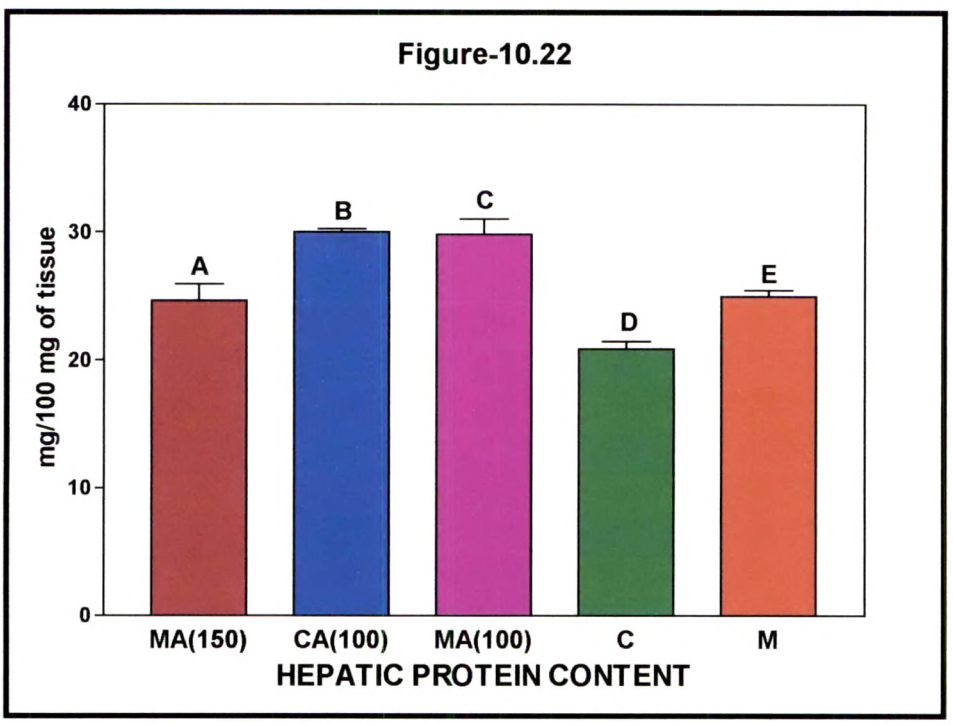


Table 10.22: Hepatic protein content of pubertal rats on 45th day subjected to neonatal melatonin treatment and weaning alloxanisation on 22nd day:

	MA(150)(A)	CA(100)(B)	MA(100)(C)	C(D)	M(E)
PROTEIN	24.66 ±1.255	29.99 ±0.271	29.83 ±1.205	20.92 ±0.5416	24.96 ±0.492

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

Figure 10.23: Muscle protein content of pubertal rats on 45th day subjected to neonatal melatonin treatment and weaning alloxanisation on 22nd day:

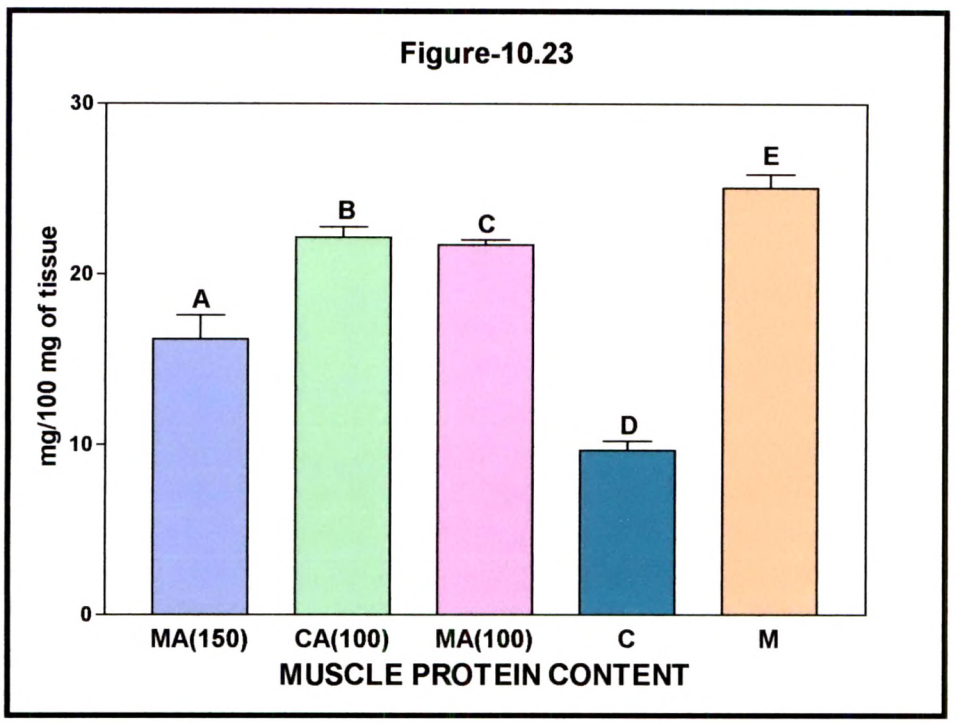


Table 10.23: Muscle protein content of pubertal rats on 45th day subjected to neonatal melatonin treatment and weaning alloxanisation on 22nd day:

	MA(150)(A)	CA(100)(B)	MA(100)(C)	C(D)	M(E)
PROTEIN	16.16 ±1.430	22.16 ±0.617	21.74 ±0.284	9.66 ±0.5385	25.08 ±0.762

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 10.24: Hepatic total lipid content of pubertal rats on 45th day subjected to neonatal melatonin treatment and weaning alloxanisation on 22nd day:

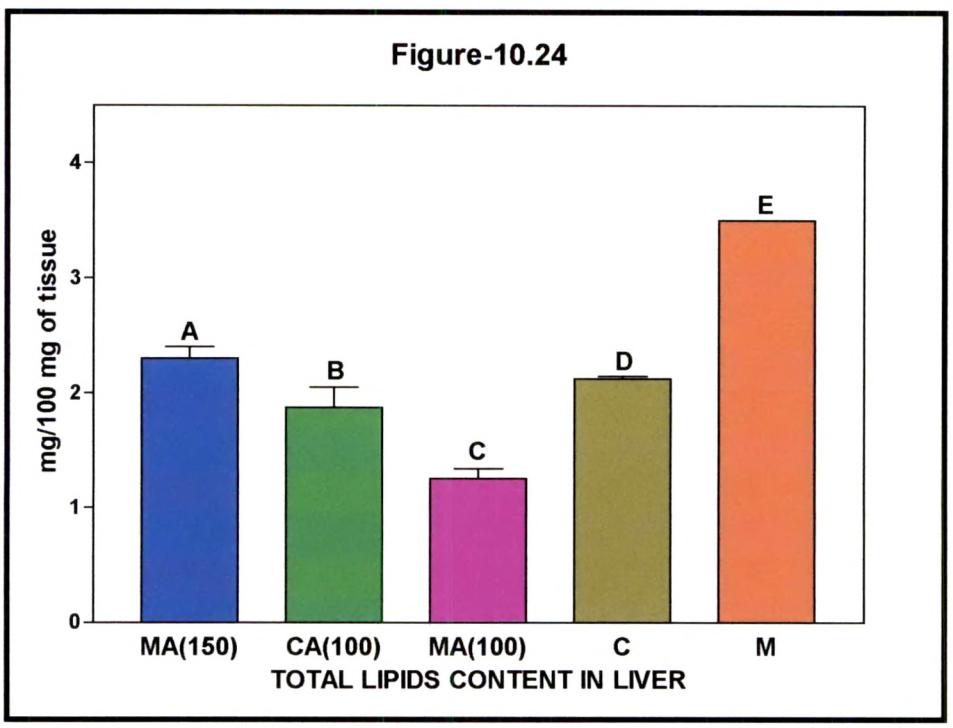


Table 10.24: Hepatic total lipid content of pubertal rats on 45th day subjected to neonatal melatonin treatment and weaning alloxanisation on 22nd day:

	MA(150)(A)	CA(100)(B)	MA(100)(C)	C(D)	M(E)
TOTAL LIPIDS	2.3 ±0.09857	1.875 ±0.175	1.25 ±0.0866	2.125 ±0.017	3.5 ±0.000085

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 10.25: Hepatic cholesterol content of pubertal rats on 45th day subjected to neonatal melatonin treatment and weaning alloxanisation on 22nd day:

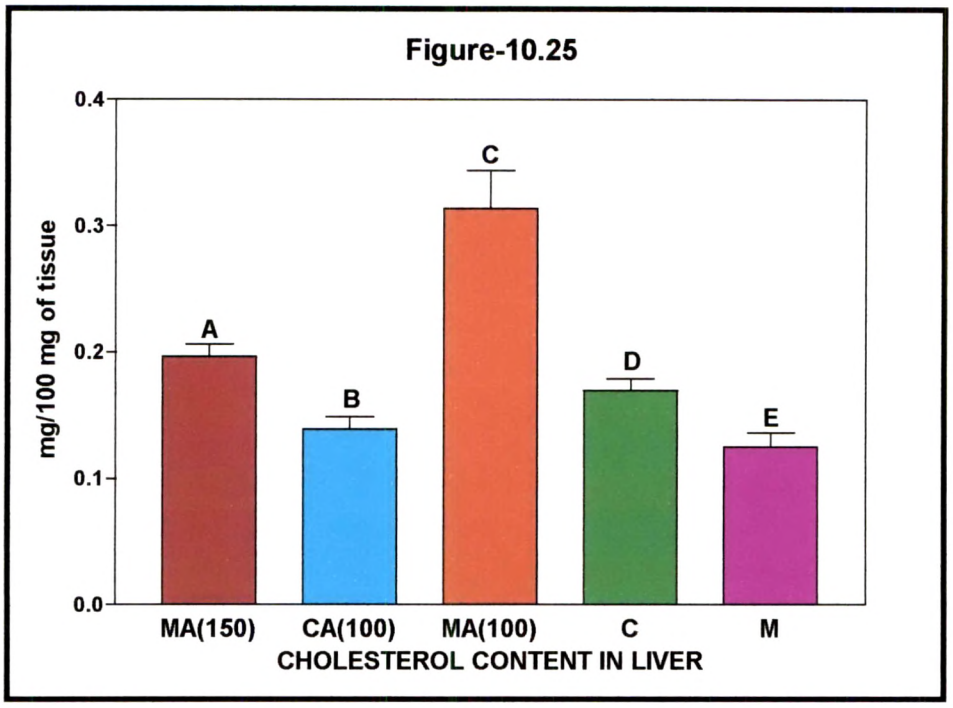


Table 10.25: Hepatic cholesterol content of pubertal rats on 45th day subjected to neonatal melatonin treatment and weaning alloxanisation on 22nd day:

	MA(150)(A)	CA(100)(B)	MA(100)(C)	C(D)	M(E)
CHOLESTEROL	0.1966 ±0.00955	0.1395 ±0.00955	0.3138 ±0.0302	0.17 ±0.0091	0.125 ±0.0115

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;
NSNon Significant

Figure 10.26: Muscle total lipid content of pubertal rats on 45th day subjected to neonatal melatonin treatment and weaning alloxanisation on 22nd day:

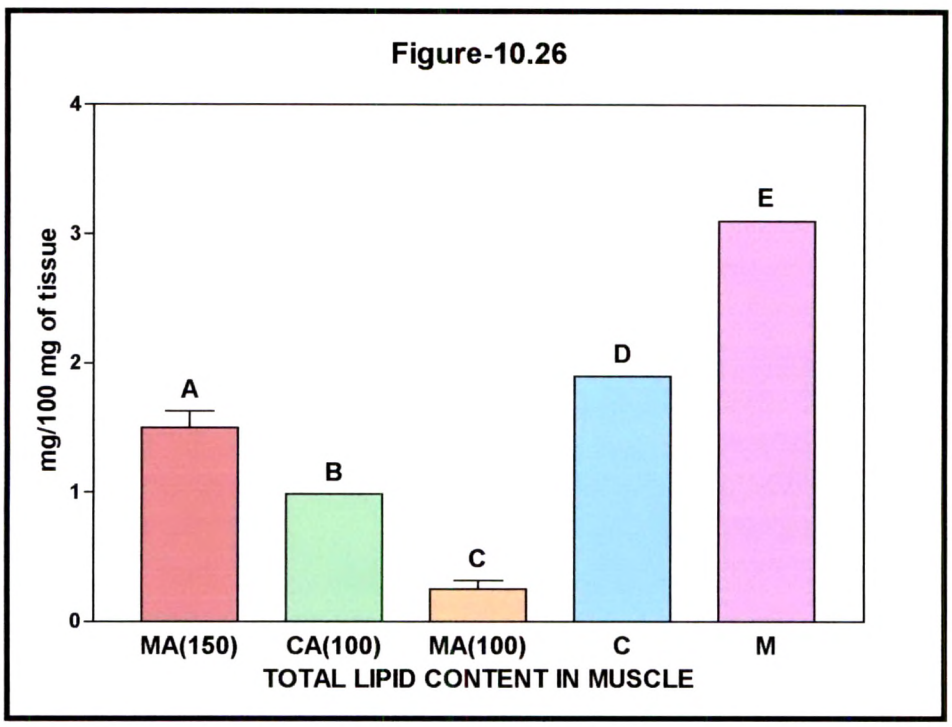


Table 10.26: Muscle total lipid content of pubertal rats on 45th day subjected to neonatal melatonin treatment and weaning alloxanisation on 22nd day:

	MA(150)	CA(100)	MA(100)	C	M
TOTAL LIPIDS	1.5 ±0.129	0.987 ±0.00016	0.25 ±0.0645	1.9 ±0.00004	3.1 ±0.000465

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

Figure 10.27: Muscle cholesterol content of pubertal rats on 45th day subjected to neonatal melatonin treatment and weaning alloxanisation on 22nd day:

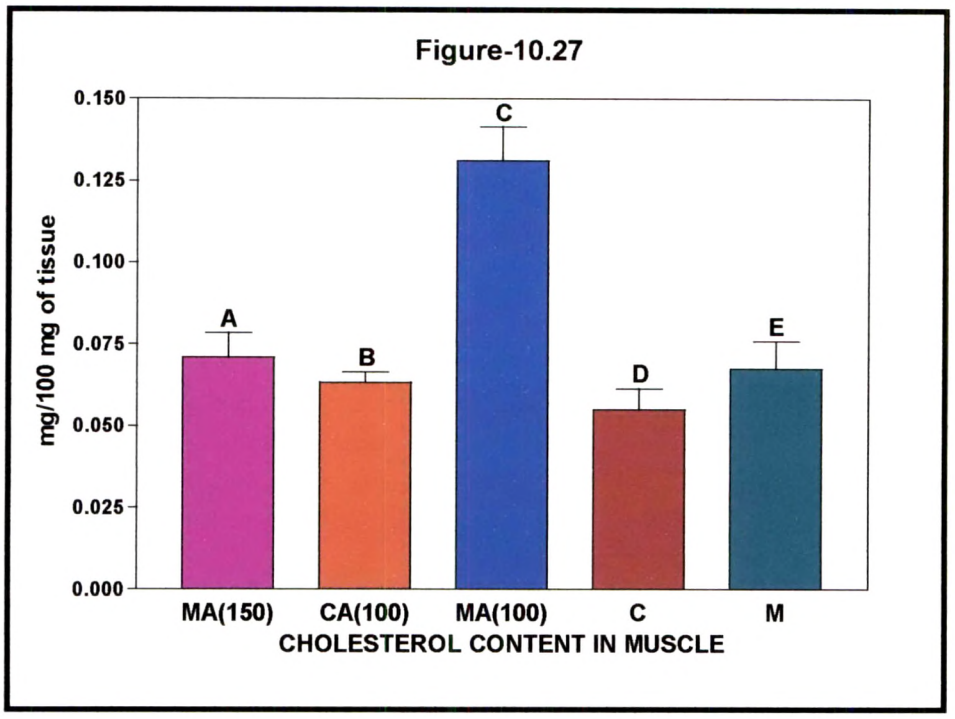


Table 10.27: Muscle cholesterol content of pubertal rats on 45th day subjected to neonatal melatonin treatment and weaning alloxanisation on 22nd day:

	MA(150)	CA(100)	MA(100)	C	M
CHOLESTEROL	0.0708$^{(A)}$ ±0.0077	0.0633$^{(B)}$ ±0.00315	0.1311$^{(C)}$ ±0.0104	0.055$^{(D)}$ ±0.00645	0.0675$^{(E)}$ ±0.0085

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 10.28: Adipose tissue total lipid content of pubertal rats on 45th day subjected to neonatal melatonin treatment and weaning alloxanisation on 22nd day:

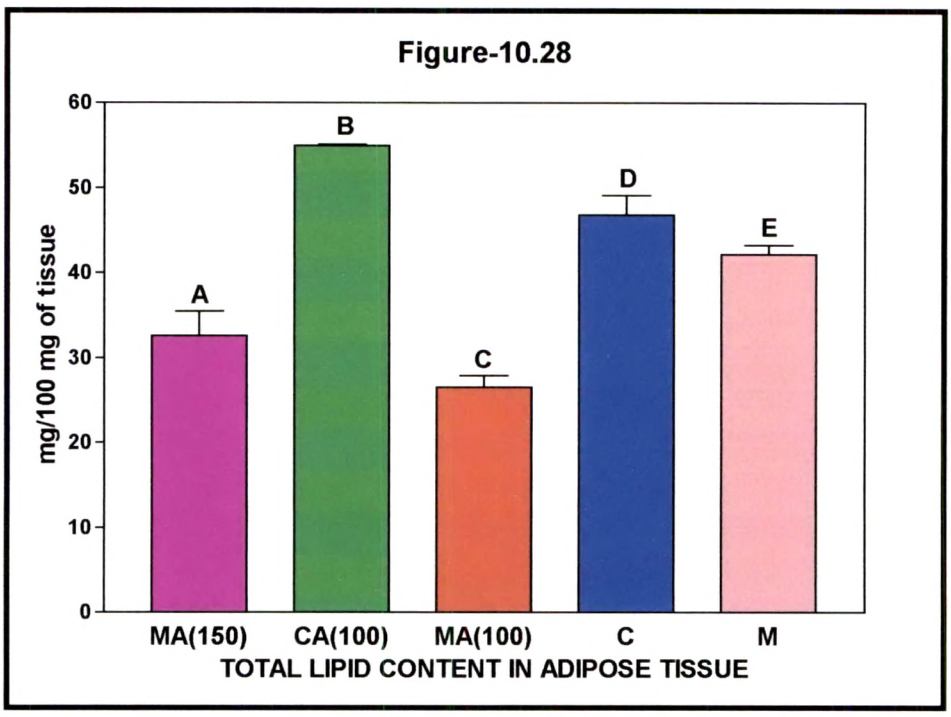


Table 10.28: Adipose tissue total lipid content of pubertal rats on 45th day subjected to neonatal melatonin treatment and weaning alloxanisation on 22nd day:

	MA(150)(A)	CA(100)(B)	MA(100)(C)	C(D)	M(E)
TOTAL LIPIDS	32.525 ±2.9188	54.95 ±0.1499	26.5 ±1.39	46.8 ±2.28	42.15 ±1.085

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<0.05; ■P<0.01; NSNon Significant

Figure 10.29: Adipose tissue cholesterol content of pubertal rats on 45th day subjected to neonatal melatonin treatment and weaning alloxanisation on 22nd day:

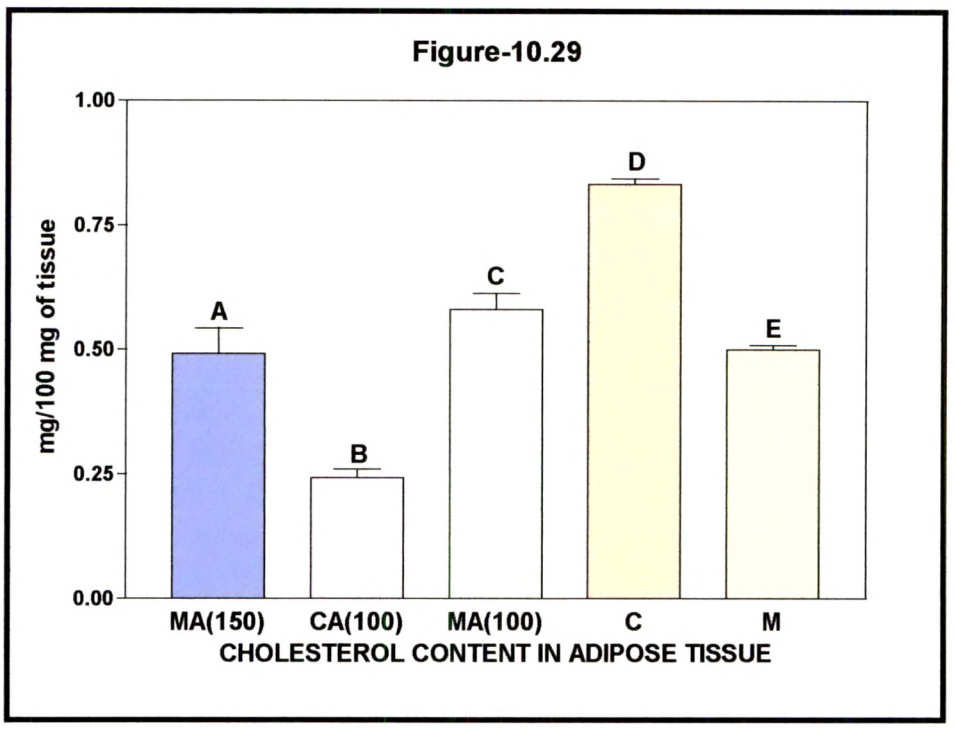


Table 10.29: Adipose tissue cholesterol content of pubertal rats on 45th day subjected to neonatal melatonin treatment and weaning alloxanisation on 22nd day:

	MA(150)(A)	CA(100)(B)	MA(100)(C)	C(D)	M(E)
CHOLESTEROL	0.4916 ±0.051	0.2429 ±0.0173	0.581 ±0.032	0.832 ±0.01105	0.5 ±0.0091

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 10.30: Serum triglyceride level of pubertal rats on 45th day subjected to neonatal melatonin treatment and weaning alloxanisation on 22nd day:

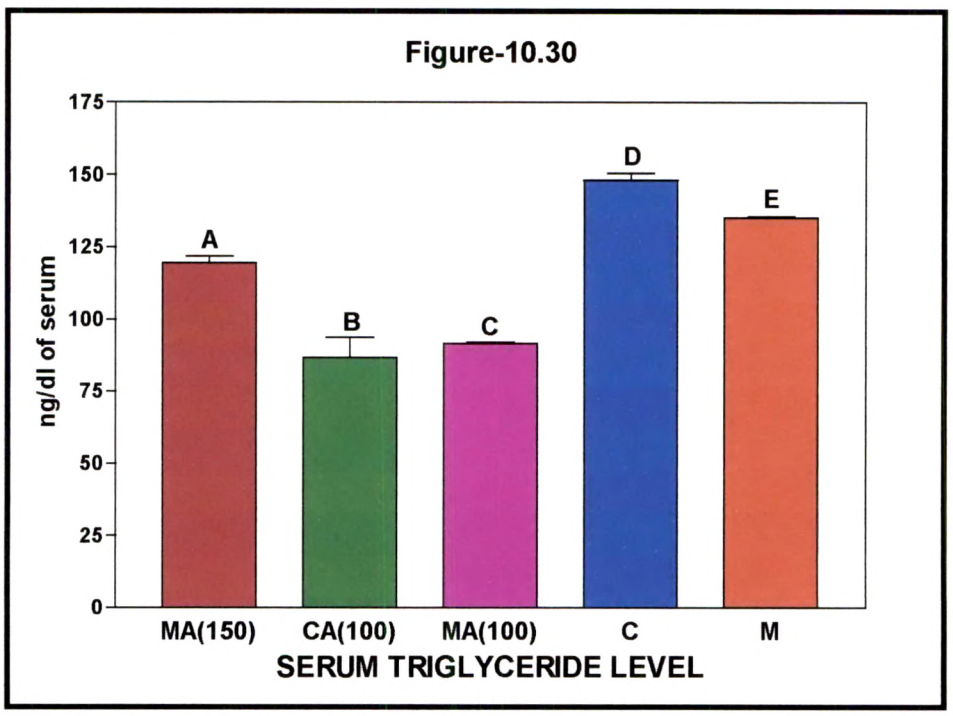


Table 10.30: Serum triglyceride level of pubertal rats on 45th day subjected to neonatal melatonin treatment and weaning alloxanisation on 22nd day:

	MA(150)[A]	CA(100)[B]	MA(100)[C]	C[D]	M[E]
SERUM TRIGLYCERIDE	119.44 ±2.418	86.785 ±6.9035	91.55 ±0.3896	148.187 ±2.3246	135.18 ±0.3795

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 10.31: Serum cholesterol level of pubertal rats on 45th day subjected to neonatal melatonin treatment and weaning alloxanisation on 22nd day:

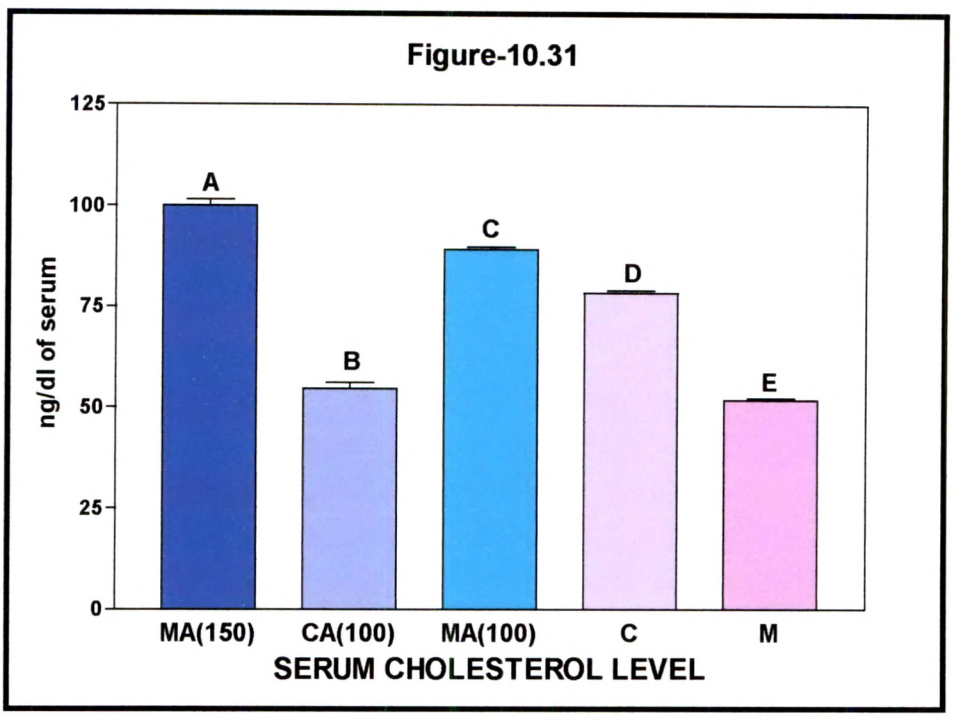


Table 10.31: Serum cholesterol level of pubertal rats on 45th day subjected to neonatal melatonin treatment and weaning alloxanisation on 22nd day:

	MA(150)(A)	CA(100)(B)	MA(100)(C)	C(D)	M(E)
SERUM CHOLESTEROL	100.062 ±1.4993	54.58 ±1.6534	89.29 ±0.5677	78.492 ±0.5883	51.89 ±0.426

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 10.32: Serum total lipids level of pubertal rats on 45th day subjected to neonatal melatonin treatment and weaning alloxanisation on 22nd day:

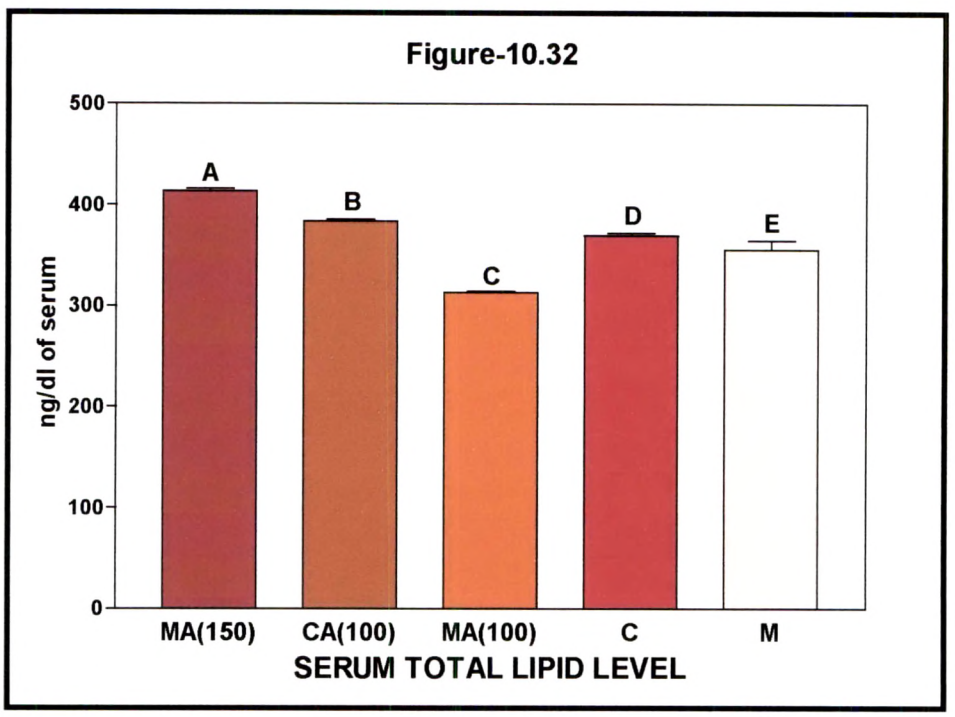


Table 10.32: Serum total lipids level of pubertal rats on 45th day subjected to neonatal melatonin treatment and weaning alloxanisation on 22nd day:

	MA(150)(A)	CA(100)(B)	MA(100)(C)	C(D)	M(E)
SERUM TOTAL LIPIDS	413.50 ±2.63	383.57 ±1.9725	313.16 ±1.378	369.98 ±2.6205	355.74 ±9.388

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;
NS Non Significant

Figure 10.33: Serum phospholipids level of pubertal rats on 45th day subjected to neonatal melatonin treatment and weaning alloxanisation on 22nd day:

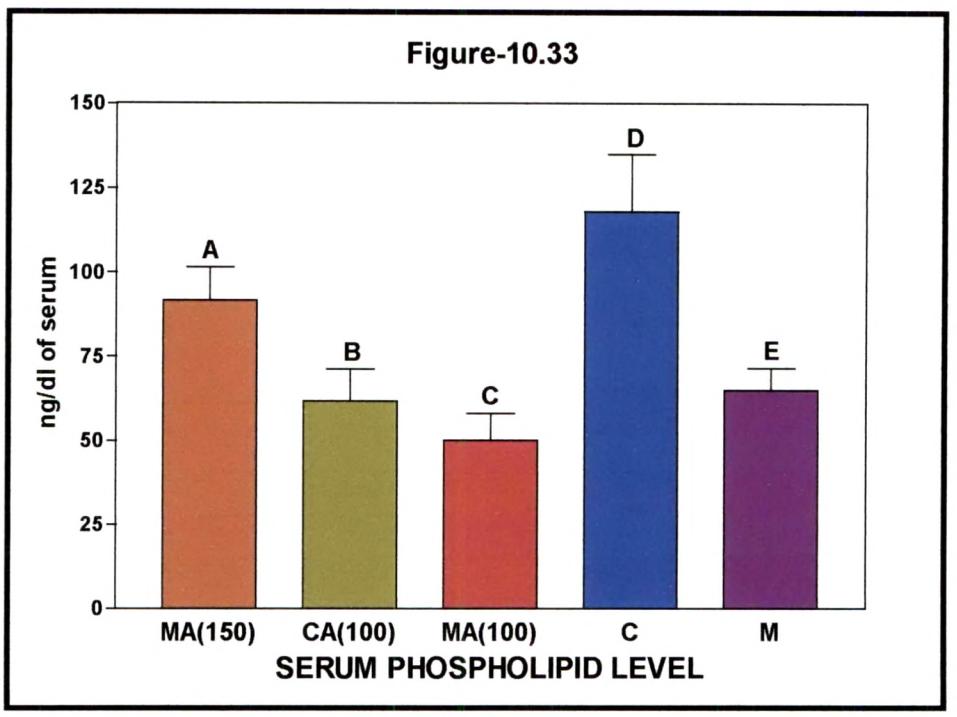


Table 10.33: Serum phospholipids level of pubertal rats on 45th day subjected to neonatal melatonin treatment and weaning alloxanisation on 22nd day:

	MA(150)(A)	CA(100)(B)	MA(100)(C)	C(D)	M(E)
SERUM PHOSPHOLIPIDS	91.67 ±9.746	61.82 ±9.395	50.11 ±8.006	117.97 ±17.0544	65.07 ±6.613

Bonferroni's Multiple Comparison Test

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

Values are expressed as mean ± SEM, NS Non Significant

Figure 10.34: Serum free fatty acid level of pubertal rats on 45th day subjected to neonatal melatonin treatment and weaning alloxanisation on 22nd day:

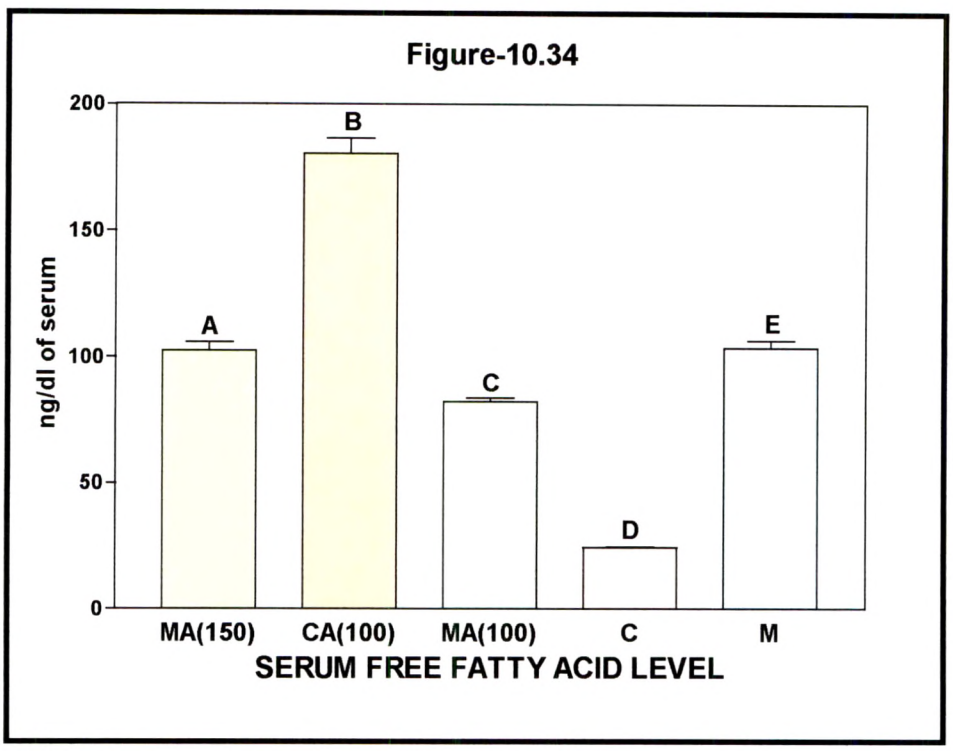


Table 10.34: Serum free fatty acid (FFA) level of pubertal rats on 45th day subjected to neonatal melatonin treatment and weaning alloxanisation on 22nd day:

	MA(150)(A)	CA(100)(B)	MA(100)(C)	C(D)	M(E)
SERUM FFA	102.4 ±3.56	180.39 ±6.28	82.21 ±1.64	24.33 ±0.42	103.6 ±2.94

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

Figure10.35: Serum insulin level of pubertal rats on 45th day subjected to neonatal melatonin treatment and weaning alloxanisation on 22nd day:

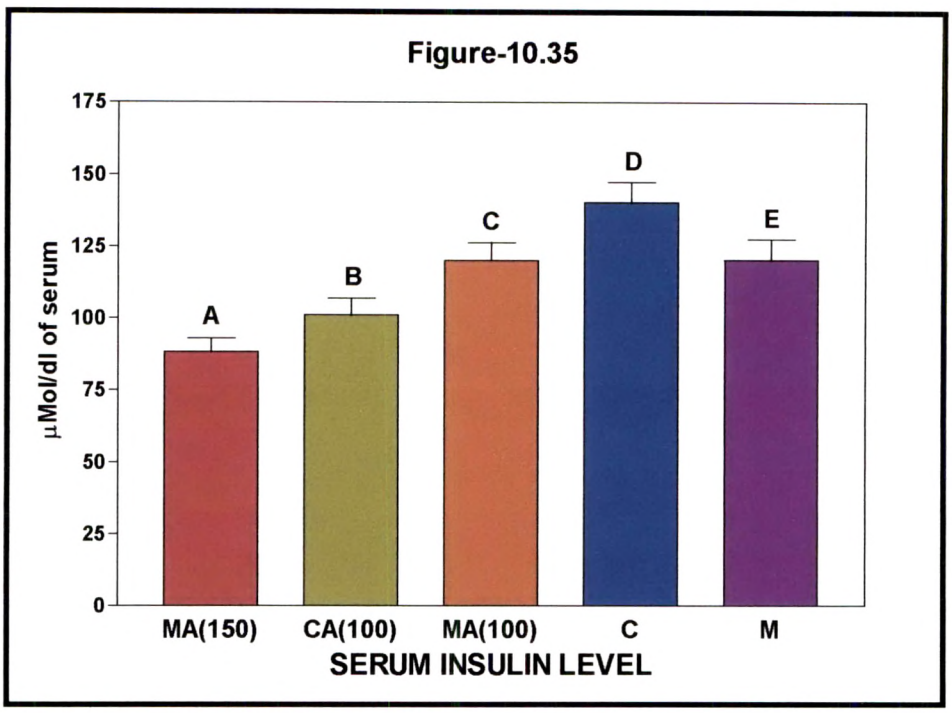


Table10.35: Serum insulin level of pubertal rats on 45th day subjected to neonatal melatonin treatment and weaning alloxanisation on 22nd day:

	MA(150)	CA(100)	MA(100)	C	M
INSULIN	88.26 ±4.73	101.04 ±5.84	120.08 ±6.25	140.27 ±7.0682	120.22 ±7.245

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; NS Non Significant

PLATE – 9

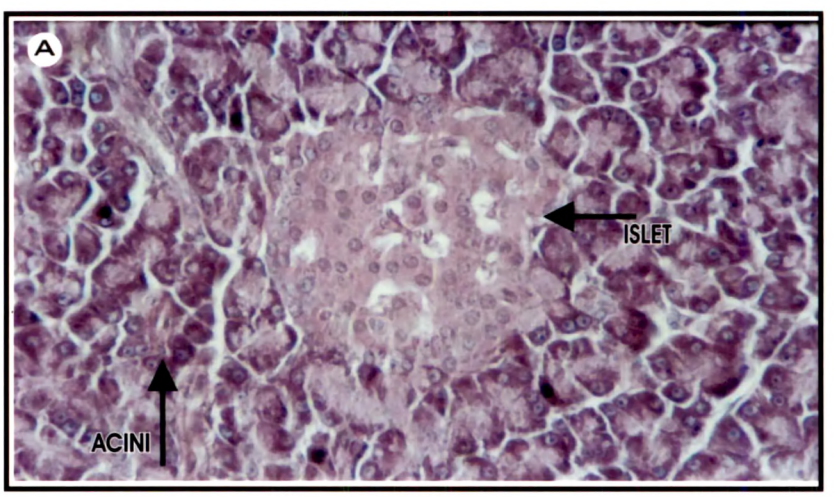
Photomicrographs of sections of pancreas – 450 X

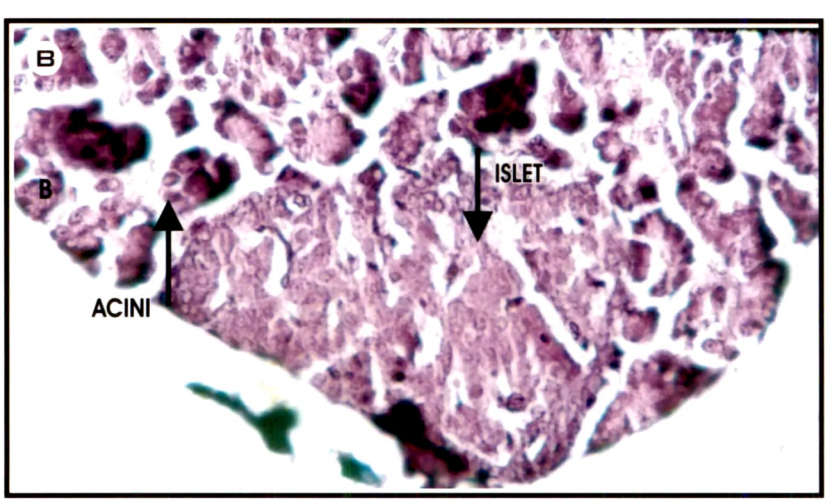
FIGURE (A): Transverse section of the pancreas of male hypermelatonemic alloxanised (100) rats on the 45th day showing islet and pancreatic acini. The islet and acinar cells are loosely packed with empty spaces in between. There is an increase in the B and A cells.

FIGURE (B): Transverse section of the pancreas of male control alloxanised (100) pubertal (45th day) rats showing islet and pancreatic acini. Note the centrally distributed A cells, peripherally distributed B cells with almost an equal number of A and B cells.

FIGURE (C): Transverse section of the pancreas of male hypermelatonemic alloxanised (150) rats on the 45th day showing islet and pancreatic acini. The acinar and islet cells are loosely packed. The A cells can be viewed in the core of the islet area whereas the B cells are prominently seen on the periphery of the islet.

PLATE - 9





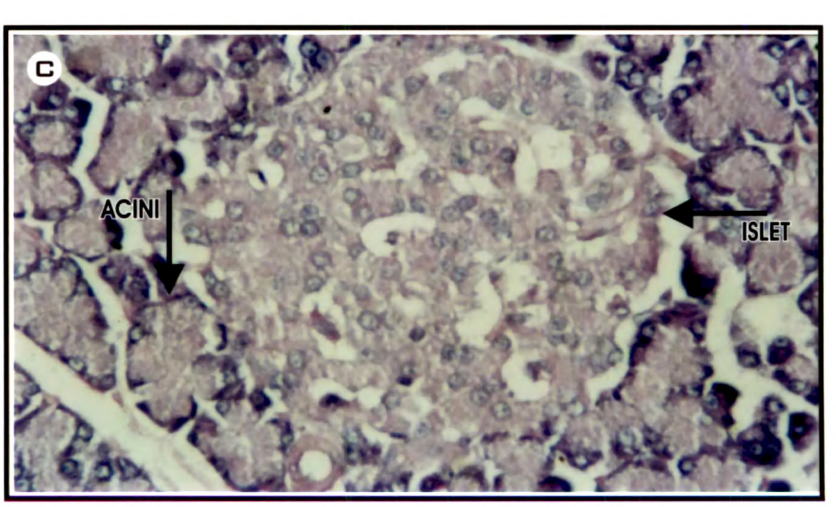


PLATE – 10

Photomicrographs of sections of pancreas – 1000 X

FIGURE (A): Transverse section of the pancreas of male hypermelatonemic alloxanised (100) rats on the 45th day showing islet and pancreatic acini. The islet and acinar cells show less damage. There is an increase in the B:A cell ratio. Note the transdifferentiation (double headed arrow) of the islet cells from the acinar cells.

FIGURE (B): Transverse section of the pancreas of male control alloxanised (100) pubertal (45th day) rats showing islet and pancreatic acini. Note the centrally distributed A cells, peripherally distributed B cells with almost an equal number of A and B cells.

FIGURE (C): Transverse section of the pancreas of male hypermelatonemic alloxanised (150) rats on the 45th day showing islet and pancreatic acini. The acinar and islet cells are loosely packed. The A cells can be viewed in the core of the islet area whereas the B cells are prominently seen on the periphery of the islet.

PLATE - 10

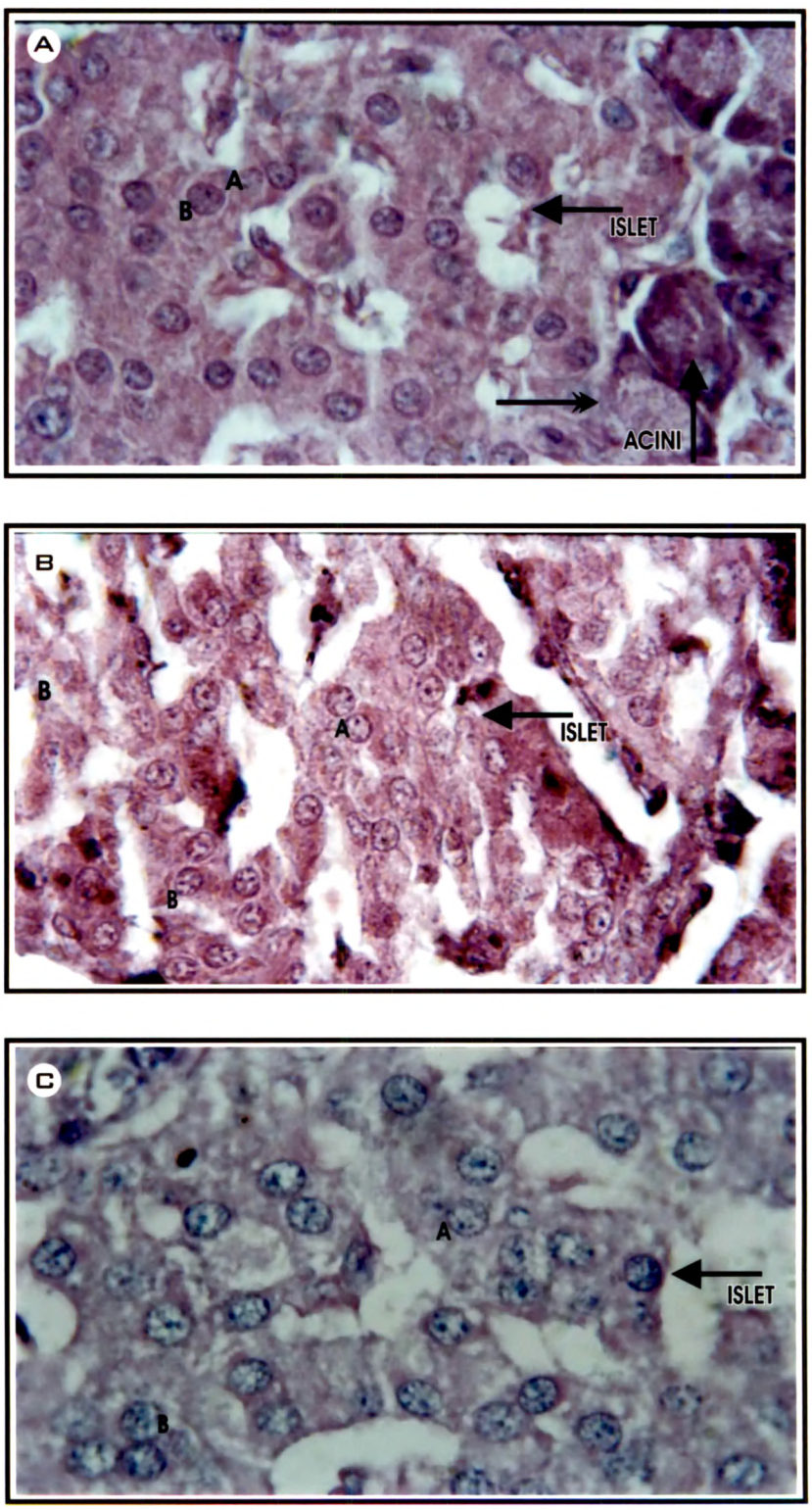


PLATE – 11

Photomicrographs of sections of pancreas – 1000 X

FIGURE (A): Transverse section of the pancreas of male hypermelatonemic alloxanised (100) rats on the 45th day showing islet and pancreatic acini. There is an increase in the B:A cell ratio. Note the transdifferentiation (double headed arrow) of the islet cells from the acinar cells.

FIGURE (B): Transverse section of the pancreas of male control alloxanised (100) pubertal (45th day) rats showing islet and pancreatic acini. Note the centrally distributed A cells, peripherally distributed B cells with almost an equal number of A and B cells The islet cells are loosely packed and show increased damage due to alloxan treatment

FIGURE (C): Transverse section of the pancreas of male hypermelatonemic alloxanised (150) rats on the 45th day showing islet and pancreatic acini. Note the transdifferentiation (double headed arrow) of the islet cells from the acinar cells.

PLATE - 11

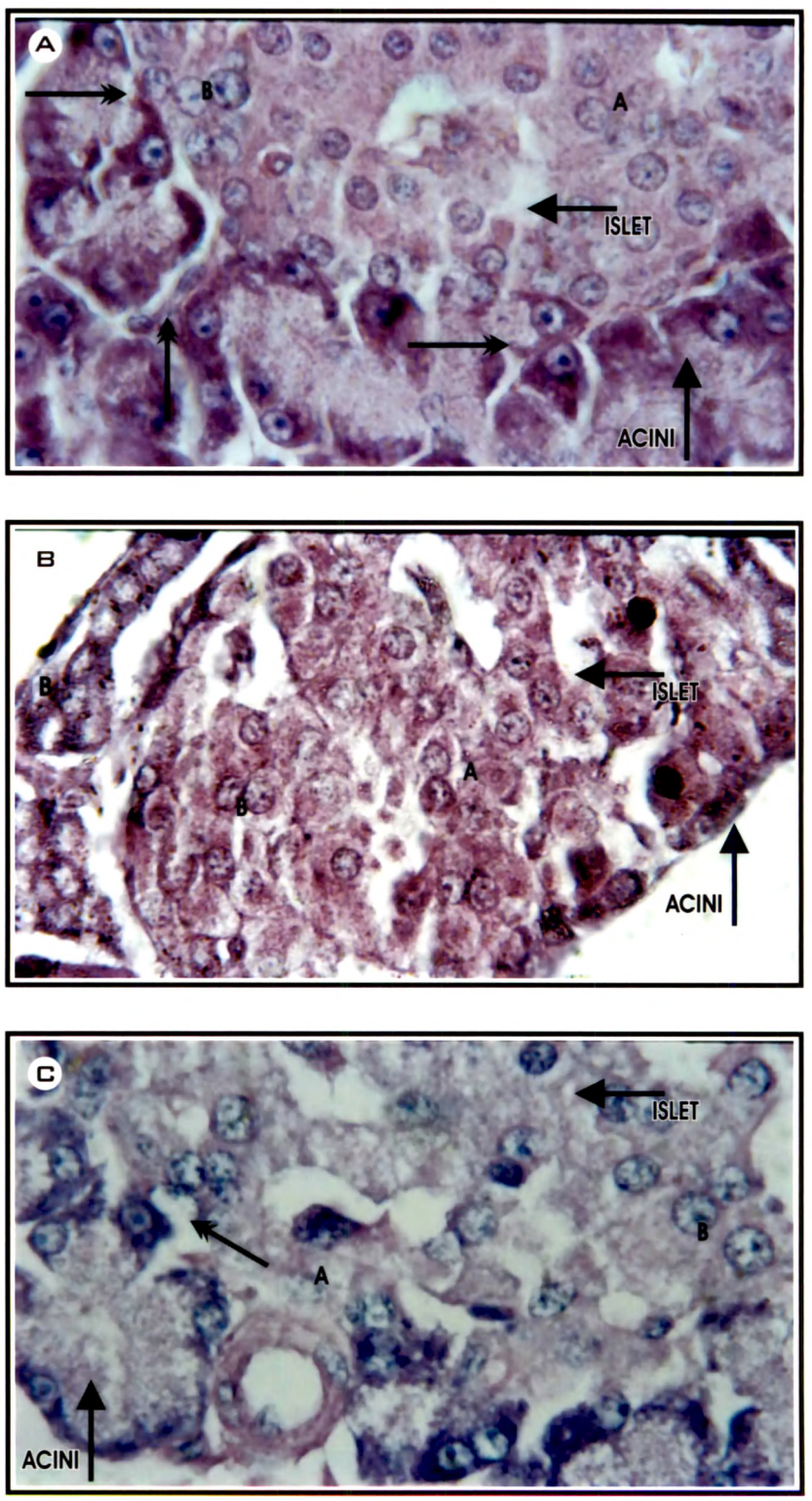


PLATE – 12

Photomicrographs of sections of pancreas – 1000 X

FIGURE (A): Transverse section of the pancreas of male hypermelatonemic alloxanised (100) pubertal (45^{th} day) rats showing islet and pancreatic acini. Note the transdifferentiation (double headed arrow) of the acinar cells into the islet cells.

FIGURE (B): Transverse section of the pancreas of male control alloxanised (100) rats on the 45^{th} day showing islet and pancreatic acini. The acinar and islet cells are loosely packed, the number of B and A cells is decreased. Note the clear areas in the section formed due to the damage caused by alloxan.

PLATE – 13

Photomicrographs of sections of pancreas – 1000 X

FIGURE (A): Transverse section of the pancreas of male hypermelatonemic alloxanised (150) pubertal (45^{th} day) rats showing islet and pancreatic acini. Note the transdifferentiation (double headed arrow) of the acinar cells into the islet cells.

FIGURE (B): Transverse section of the pancreas of male hypermelatonemic alloxanised (150) rats on the 45^{th} day showing islet and pancreatic acini. Note the transdifferentiation (double headed arrow) of the acinar cells into the islet cells. The B:A cell ratio is increased with a prominent hypertrophy of the B cell by neogenesis by transdifferentiation,

Histological observations: The islets of both control and nMT rats show alloxan induced damage marked by loss of cells and formation of large spaces. Both the control and nMT rat islets show B cell neogenesis from acinar cells with the result, the junction between the between the islet and acinar areas have befudged. Melatonin treated rats seem to have a relatively higher number of B cells and a greater B:A cell ratio essentially due to lesser death of B cells (Plate; 9-13).

DISCUSSION:

The present results, apart from showing some significant changes in alloxanised control rats also show dose dependent differential effect of alloxan in rats treated with melatonin in neonatal period. Both neonatal normomelatonemic as well as hypermelatonemic rats show significant differential alteration in body weights and organ weights at the pubertal age due to alloxan treatment on the first weaning day. Neonatal melatonin treated (nMT) rats show a significant decrease in the body weight (285%) while alloxan treatment on first weaning day results in a dose dependent increase in body weight of nMT rats. On the other hand normal rats treated with alloxan on the first neonatal day show a decrease in body weight compared to age matched controls not treated with alloxan (Fig. and Tab.; 10.1). The significant increase in body and pancreas weights of nMT rats treated with alloxan as against decrease in weight in alloxan treated control rats suggest a favorable growth promoting influence due to nMT (Fig. and Tab.; 10.8). The exact basis of these growth promoting influence is not fathomable at this juncture but it might be worth speculating that recovery/regeneration of B cells

Arulmozhi *et al*, 2004). Significant depletion of hepatic glycogen content is an accompanying change but paradoxically, the muscle glycogen contents are increased (Fig. and Tab.; 10 14, 10.15). This would suggest decreased hepatic sensitivity and increased muscle sensitivity to insulin. The glucose moieties seem to be more channalised. In muscle glycogen and apparent protein anabolic influence is also evident, though less evident in high dose alloxan treated nMT rats. The glycogen synthetase: phosphorylase ratio also supports the observed changes in hepatic and muscle glycogen contents. The purported regeneration of B cells is adequately supported by the histologically observable B cell regeneration and B cell density in the islets of alloxan treated control rats and increased islet density with regeneration of B cells in alloxan treated nMT rats (Plate; 9-13). Increased islet neogenesis has been reported earlier in the pancreas of nMT rats (Chapter 7). The present observation on regeneration of B cells is strengthened by the recent understanding of increases in B cell mass through increased B cell replication, increased B cell size and decreased B cell death, and neogenic differentiation of B cells (Finegood *et al.*, 1995). Neogenesis is an important component of B cell mass expansion during development, and also has been shown to contribute to increases in B cell mass in juvenile, neonatal, weaning and adult rats of 3-4 months age (Finegood *et al.*, 1995; Rosenberg, 1995; Bouwens and Kloppel, 1996). Though there are differing reports on the ability of adult pancreas to regenerate or to generate B cells by neogenesis under conditions of pancreatectomy,

hyperglycemia or hyperinsulinemia with no ability (see Sjoholm, 1996) or a definite ability (Fernandez *et al.*, 1997; Bonner Weir, 2000), neonatal or younger rats are shown to have greater capacity for regeneration and neogenesis under various insults which affect the integrity of B cells (Lipsett and Finegood, 2002, Paris *et al.*, 2003; Arulmozhi *et al.*, 2004). In the present study nMT rats seem to resist the diabetogenic effect of alloxan as noted by the relative healthy condition of the islet tissue compared to the non nMT rats. This suggests a protective action of prior melatonin treatment in resisting the effects of B cell damaging agents. This is understandable in the light of known role of melatonin as a free radical scavenger (Bromme *et al.*, 2000; Reiter *et al.*, 2000; Anderson and Sandler, 2001; Rao *et al.*, 2002; Stefinova *et al.*, 2002; Anwar and Meki, 2003) and as such free radicals are now known to be the molecular cause for the alloxan induced B cell death (Bromme *et al.*, 1999, Szkudelski, 2001). However a higher dose of alloxan seems to have more damaging influence, nevertheless healthier islets than those of controls treated with low dose of alloxan as a higher dose resulted in 100% lethality in the non nMT rats Apart from the protective action seen to be provided by melatonin the pancreas of nMT rats subjected to weaning alloxan challenge also show B cell neogenesis by acinar cell transdifferentiation apart from ductal progenitor cells in both adult and neonatal rats, more clearly in the latter (Junod *et al., 1969*; Portha *et al.*, 1979; Okamato, 1981; Weir and Bonner Weir, 1986 Daniel and Portha, 1989; Rosenberg, 1995; Wang *et al.*, 1995. 96; Fernendes *et*

al., 1997; Bouwens and Pipeleers, 1998; Lipsett and Finegood, 2002; Paris *et al.*, 2003; Arulmozhi *et al.*, 2004).

The body lipid profile shows a generalized ability of the weaning animals to resist diabetes related increase in lipid content. The reduced tissue lipid and serum lipid fractions in both nMT and non nMT rats relative to non alloxanised controls is probably indicative of reduced insulin level/action in the post alloxan treatment period (Fig. and Tab.; 10 31-10 34) This is substantiated by the observed insulin status in these rats (Fig. and Tab.; 10.35) However, the relatively higher tissue lipid contents and higher serum profiles in the nMT rats treated with higher dose of alloxan is indicative of the developing insulin resistance favored by neonatal melatonin treatment. It is likely that nMT rats with a higher dose of alloxan would develop insulin resistance and NIDDM in the later periods.

It is concluded from the present observations that neonatal melatonin treatment has a protective influence on alloxan induced B cell damage and also favors islet B cell neogenesis by acinar transdifferentiation, but a higher dose of alloxan can lead to hyperlipidemia and insulin resistance.

SUMMARY:

Previous studies involved evaluation of immediate and long term effects of neonatal hypermelatonemia on metabolic features, which showed subtle alterations. In the present study, the protective effect if any of neonatal hypermelatonemic status on long term effects of alloxan induced diabetes in the weaning age has been assessed.

Since experimental induction of diabetes has been carried out in neonates and adults, it was thought interesting to see the effects of induction of diabetes at weaning age. To this end, rat neonates have been treated with melatonin in graded doses of 200 µg/animal from day 1 to day 7; 400 µg/animal from day 8 to day 14 and 600 µg/animal from day 15 to day 21 to generate neonatal hypermelatonemic status; a low (100 µg/kg) dose and a high dose (150 µg/kg) of alloxan were given on the 22nd day and the effects assessed on the 45th day. The hypermelatonemic alloxanised and control alloxanised rats showed decrease in body weight as compared to age matched controls. The relative weight of pancreas, liver and spleen increased significantly in the melatonin treated alloxanised (both low and high dose) rats, while the relative weight of kidneys remained unaltered. Whereas the hepatic glycogen content decreased significantly in all the alloxanised animals as compared to age matched controls, the muscle glycogen content increased significantly in all the alloxanised groups. The serum glucose and insulin levels decreased significantly in all the alloxanised groups as compared to age matched controls. Whereas the hepatic glycogen synthetase and glycogen phosphorylase activities decreased significantly in all the alloxanised animals, the muscle glycogen synthetase activity increased significantly in the control alloxanised rats and remained unaltered in hypermelatonemic alloxanised (both low and high dose) rats. The hepatic protein content increased significantly in control alloxanised and melatonin alloxanised (low dose) rats but, remained unaltered in melatonin alloxanised (high dose) animals as

compared to age matched controls. The muscle protein content increased significantly in all the alloxanised animals as compared to age matched controls. The hepatic total lipid content decreased significantly in the hypermelatonemic alloxanised (low dose) rats as compared to control alloxanised rats while, the hepatic cholesterol contents increased significantly in the hypermelatonemic alloxanised (low dose) rats as compared to control alloxanised animals. The muscle total lipid content decreased significantly in all the alloxanised rats as compared to the age matched controls. The muscle cholesterol content increased significantly in the hypermelatonemic alloxanised (low dose) as compared to control alloxanised and age matched control rats. Whereas the adipose tissue total lipid content decreased significantly in hypermelatonemic alloxanised rats as compared to control alloxanised rats, the adipose tissue cholesterol content increased significantly in hypermelatonemic alloxanised rats. The serum triglyceride level decreased significantly in all the alloxanised rats as compared to age matched controls. The serum cholesterol level increased significantly in the hypermelatonemic alloxanised rats as compared to control alloxanised rats. The serum total lipid level increased significantly in the hypermelatonemic alloxanised (high dose) rats as compared to control alloxanised and controls of the same age while hypermelatonemic alloxanised (low dose) rats showed a significant decrease as compared to the other two alloxanised groups and age matched controls. The serum phospholipid level decreased significantly in all the alloxanised rats as compared to control rats of the

same age. The serum free fatty acid level increased significantly in all the alloxanised rats as compared to age matched controls. The islets of both control and nMT rats show alloxan induced damage marked by loss of cells and formation of large spaces. Both the control and nMT rat islets show B cell neogenesis from acinar cells with the result, the junction between the islet and acinar area has got befudged. It is concluded from the present observations that neonatal melatonin treatment has a protective influence on alloxan induced B cell damage and also favors islet B cell neogenesis by acinar transdifferentiation, but a higher dose of alloxan can lead to hyperlipidemia and insulin resistance.