Sci. J. Fac. Sci. Minufia Univ., 2009, Vol. XXIII, 69-84

HISTOLOGICAL AND ULTRASTRUCTURAL INVESTIGATIONS ON LOW PROTEIN DIET INDUCED-HEPATOTOXICITY IN ALBINO RATS

BAYOMY, M.F.F., ABDEL-SAMIE, H.A., ZARAD, A.M.E.

Department of Zoology, Faculty of Science, Menoufiya University, Egypt

ABSTRACT

Protein is an essential material for maintaining normal organs' functions and body development. Protein malnutrition is responsible for arrested growth and disturbed metabolism. This work was designed to test effects of feeding on low protein diet on the liver histology of male albino rats studied by light and electron microscopes as well as the biochemical changes in the levels of AST and ALT in the serum. In this work, rats fed on a diet containing 10% protein for 4, 6, and 8 weeks exhibited morphological alterations in their livers and elevation in some serum parameters. Animals were divided into two groups, one group was fed on a standard rodent diet and served as control. The second group was fed on low protein diet. Specimens were investigated after 4, 6, and 8 weeks then two weeks upon return to feed on normal diet and served as recovery group. The low protein diet caused reduction in body weight of animals throughout the experimental and recovery periods. Feeding on the low protein diet induced time-dependent histopathological alterations in the liver in the form of cytoplasmic vacuolization of hepatocytes, nuclear changes leucocytic infiltration, blood vessels congestion, Kupffer cells activation, enlargement of bile ductules. The mitochondria possessed closely packed cristae while others showed degenerated cristae and the rough endoplasmic reticulum was reduced. The nucleus exhibited side projections, wider nuclear pores and dissipation of heterochromatin into smaller clumps. Most of these changes disappeared after two weeks of returning to normal diet. Serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) exhibited statistical significant increase ($P<0.05$) in all periods. The only exception was in AST in the recovery period where it showed highly significant decrease in comparison to control.

Keywords: Low protein diet, body weight, liver, histology, ultrastructure, liver function enzymes.

INTRODUCTION

A balanced diet is necessary for maintaining the normal health and for the normal functioning of animals' organs. Contradictory to this, improper composition of the diet through variation of its constituents either elevating or decreasing such constituents resulted in defects in the normal functions of vital systems such as the immune, nervous and other body systems. The functional integrity of the immune system and the metabolism of xenobiotics are interrelated (Germolec *et al.*, 1997) and both are dependent upon the nutritional status (Banerjee, 1999; Handy *et al.*, 2002).

Protein malnutrition is the most apparent form of nutritional disorder encountered in developing and underdeveloped countries that in many cases caused many serious health problems (Pitkanen *et al.*, 2000). In this concern, Onis *et al.* (1993) indicated that about 43 % of children in developing countries suffered from malnutrition during some periods in their life. The significance of protein in the diet emerges from the fact that dietary protein is the sole source of essential amino acids that the body can not synthesize them. Moreover, it was recorded by Gershwin *et al.* (1985) that protein deficiency generally depresses the immune system, and with DDT exposure at least, exacerbates immunotoxicity.

Meanwhile, the effect of supporting pregnants upon protein-deficient diet on the offspring couldn't be ignored. In this regard, Shrader *et al.* (1977b) indicated delay in thyroid follicle formation and reduction in gland area, follicle number, colloid space and cell size in thyroid tissue of fetal and neonatal pups from protein-deprived dams. In addition, Rocha-de-Melo and Guedes (1997) indicated abnormality in neural development in the offspring of mother rats maintained on protein-poor diet during lactation period. Barsotti *et al.* (1983) and Zeller *et al.* (1991) found that feeding on low protein diet caused reduction in glomerular capillary pressure and filtration.

Inadequate nutrition either in pre-or postnatal periods alters brain development resulting in biochemical, physiological and anatomical changes that in turn caused behavioral abnormalities and impairment of the central nervous system function. Krause *et al.* (2000) declared that protein malnutrition induced reduction in the number of synapses in some rat's cortical areas and changed animal's behavior. In addition, Andrade *et al.* (2002) recorded that malnutrition affected the hipocampal formation. Results of the work of Cheema *et al.* (2005) clarified the action of low protein diet on the heart during pregnancy. The results showed that low protein diet caused depression of the contractile function of the neonatal heart in rats. McMullen and Langley-Evans (2005) demonstrated that the offsprings of Wistar rats fed a low protein diet during pregnancy had reduced

number of nephrons and suffered hypertension in postnatal life. 10 % maternal protein elevated maternal serum corticosterone, oestradiol and testosterone concentrations at 19 days gestation, while luteinizing hormone in male offspring, body weight and testis weight were reduced (Zambrano *et al.*, 2005).

According to the hazardous effects associated with feeding on low protein diet, this work was essentially planed to examine histological, ultrastructural and biochemical effects emerged in male albino rats from feeding on low protein diet.

MATERIALS AND METHODS

A- Experimental animals:

Healthy adult male albino rats (*Rattus norvegicus*) approximately two months old and weight ranges between 120 ± 5 g were used in the present investigation. Rats were maintained in the laboratory for at least one week before starting the experiment. Animals were housed in plastic rodent cages in animal's house in Faculty of Science, Menoufia University, under constant temperature (28± 2 °C) and were exposed to 12: 12 hour dark- light cycle and relative humidity 45% ±5 and fed on standard commercial rodent diet before starting experimentation with free access to water.

Rats were divided into two groups:

1- Control group: Rats in this group were fed on standard diet composed of 20% casein, 15% corn oil, 55% corn starch, 5% salt mixture and 5% vitamins starch throughout the experimental period.

2- Low protein diet fed group: Rats in this group were fed on diet contains 10% protein for a total of 8 weeks then returned to feed on normal diet for two weeks.

Specimens were taken from control and low protein fed rats after 4, 6, and 8 weeks and after two weeks of stopping the treatment. Halothane-anesthetized rats were quickly dissected and small pieces of livers were taken. Sera from control and low protein-fed rats were collected in centrifuge tubes for biochemical determination of liver function enzymes (ALT and AST). Results were statistically analyzed to determine the difference from control at $P<0.05$ level of significance.

Histological and electron microscopic preparations:

Small pieces of livers were fixed in 10 % neutral formalin for 24 hours, washed, dehydrated, cleared and mounted in Parablast. Sections of 5μm thickness were cut using rotary microtome (Leica, model Rm 2125, Germany) and mounted on clean slides. Sections were stained with Ehrlich's hematoxylin and counterstained with eosin following the method of Lillie and Fulmer (1976).

For electron microscope examination, liver specimens were fixed in glutaraldehyde, washed, post-fixed in osmium tetroxide, washed, dehydrated and embedded in resin according to the method adopted by Karnovsky (1965). Ultrathin sections were stained with uranyl acetate and lead citrate and finally examined and photographed under JEOL JEM-100S Electron Microscope, Unit of Electron Microscope, National Cancer Institute, Egypt.

Biochemical analysis:

1- Liver function enzymes:

Alanine-aminotransferase (ALT) and aspartate aminotransferase (AST) were determined following the method of Gella *et al.* (1985) using Spinreact Kit (S. A. Ctra. Santa Coloma, Spain).

RESULTS

Changes in body weight

Feeding on the low protein diet induced statistical significant reduction ($P<0.01$) in body weight of rats in all time periods (Table 1). Moreover, such depress in body weight was still found during the recovery period.

Table 1: Effect of low protein diet on body weight (g).

Treatment period	Control group	Low protein diet group
	Mean ± SE	Mean ± SE
4 weeks	141± 1.31	131.6 ± 1.07*
6 weeks	142 ± 1.51	122.6 ± 2.33*
8 weeks	141.8 ± 1.57	122.8 ± 1.32*
2 weeks post-treatment	143.2 ± 2.08	130.2 ± 1.51*

n=5 animals for each group.

* Significant at ($P<0.01$) in comparison with control.

Histological examinations

Inspection of liver sections obtained from control rats at any experimental period showed normal histological structure (Fig. 1). In low protein diet-fed rats (10 % protein), liver sections showed many histopathological alterations. These changes were time-dependent. Regarding to this, after 4 weeks had passed, sections manifested leucocytic infiltration around portal veins that appeared widened. Bile ductules appeared enlarged and the cytoplasmic vacuolization of hepatocytes was evident (Figs. 2 and 3). When 6 weeks had lapsed, liver sections exhibited more pronounced damage where congestion of central veins, cytoplasmic vacuolization of hepatocytes and lecucocytic infiltration were observed (Fig. 4). Still later, animals' liver sections kept in these conditions for 8

weeks indicated an increased degree of the cytoplasmic vacuolization, activation of Kupffer cells, and enlarged central veins (Fig. 5). After two weeks of stop feeding such diet and returning to feed on the normal diet, an observable improvement in the liver histology has attained. Thus, liver regained its normal histological picture to high extent, hepatic cells arranged into its characteristic pattern, the cytoplasmic vacuolization of hepatocytes became less manifested and no leucocytic infiltration was demonstrated (Fig. 6).

Ultrastructurally, livers of normal rats have normal-structured organelles (Fig. 7). After 4 and 6 weeks, little change in nuclear chromatin and in the shape of mitochondria was observed (Figs. 8 and 9). At the end of the 8th week, the amount of rough endoplasmic reticulum was reduced and the mitochondrial cristae became closely packed. The nucleoli in most cells were well-organized and the euchromatin was nearly normal but the heterochromatin was dissipated into smaller clumps (Fig. 10). However, some nuclei exhibited side projections and widened nuclear pores. During the recovery period an advanced degree of improvement was reached, where nearly normal mitochondria and rough endoplasmic reticulum were observed. In addition, slight cytoplasmic vacuolization of hepatocytes was still encountered (Fig. 11).

Liver function enzymes:

1- Serum aspartate aminotransferase (AST):

Feeding animals on the low protein diet resulted in a highly significant increase in serum aspartate aminotransferase activity. However, an advanced degree of decreased activity was achieved in the recovery period (Table 2).

Table (2): Effects of low protein diet on serum aspartate aminotransferase (AST)(U/L).

Period of treatment	Control group	Low protein diet group
	Mean ± SE	Mean ± SE
4 weeks	83.4 ± 1.4	131 ± 19.1*
6 weeks	87.51 ± 1.1	121.21 ± 8*
8 weeks	88.1 ± .97	113.1 ± 7.5*
2 weeks post-treatment	89.5 ± 1.74	72.3 ± 5.13*

n=5 animals for each time period.

* Significant difference ($P<0.01$) in comparison with control group.

2- Serum alanine aminotraransferase (ALT)

Keeping animals in the conditions previously described for AST enzyme, induced general increase in ALT activity while decrease in such activity was

encountered in the two-weeks- post treatment in comparison to the previous periods (table 3).

Table (3): Effects of low protein diet on serum alanine aminotransferase (ALT)(U/L).

Periods of treatment	Control group	Low protein diet group
	Mean ± SE	Mean ± SE
4 weeks	38.32 ± 2.2	72.2 ± 2.4*
6 weeks	39.8 ± 0.32	59.6 ± 1.9*
8 weeks	39.2 ± 1.2	61.6 ± 0.77*
2 weeks post-treatment	37.4 ± 0.11	40.8 ± 1.1*

n=5 animals for each time period.

* Significant difference ($P<0.01$) in comparison with control group.

DISCUSSION

Malnutrition is a serious health problem especially in poor countries. Many problems were encountered from such situation. Among these defects that were announced by Torrens *et al.* (2003) that protein restriction during development impairs vascular endothelial function in adult offspring. However, it is worthy mentioning that, food restriction sometimes offers undeniable functions, as it prolongs the mean and maximal life spans, activates immunity (Gartner *et al.*, 1992), reduces the incidence of and delays onset of tumors and other age-associated diseases (El Hadri *et al.*, 2004), lowers some of the signs of radiation nephropathy in pigs (Robbins *et al.*, 1993), aids renal function in insulin-dependent diabetics (Brouhard and LaGrone, 1990) as well as, delays the occurrence of many degenerative diseases (Masoro, 2005).

In the present work, feeding on the low protein-containing diet (10%) caused decrease in body weight of rats. Therefore, maintenance of the correct ratio of protein in the diet is of great importance as dietary proteins are the sole source of essential amino acids. Protein deficiency may affect metabolism that was reflected as loss of weight and arrested development (Bayomy and Taie, 1991). A possible explanation for this weight reduction might be the reduced appetite of the tested animals. Running parallel to the present results that were declared by Richard and Helen (1963) who found loss of body weight in rats fed on 3.5 % protein diet. Verma (1980) recorded reductions in weight gain, total plasma proteins and albumin, hepatic total and microsomal proteins, cytochrome

P450 and protein: DNA ratio upon feeding male albino guinea pigs on 5 % protein for 4 weeks. Moreover, Darmon *et al.* (1993) found decrease in body weight of male weanling Sprague-Dawley rats after feeding on 6 % protein diet for 4 weeks. In addition, Iglésias-Barreira *et al.* (1996) registered decrease in body weight in newborn rats when their mothers fed on 8 % protein for 27 days.

The protein concentration used in this work was similar or close to that used by other authors. Regarding to this, Okawa *et al.* (2006) documented similar results as they found lowered body weight gain in Wistar rats fed on 10 % protein. In a more recent study, a decrease in body weight of New Zealand rabbits fed on diet containing 8 % of fish proteins for a total period of 8 weeks was indicated (Oboh *et al.*, 2007).

In the present work, low protein diet caused many histopathological changes in the liver of rats such as the presence of abnormal-shaped hepatocytes, leucocytic infiltration, congestion in blood vessels, cytoplasmic vacuolization of hepatocytes, abnormal nuclei with condensed chromatin, activated Kupffer cells and abnormality in cell membrane. Similar results were encountered by many investigators; among them were Cho *et al.* (2001). They demonstrated that protein-calorie malnutrition altered liver morphology and caused nuclear shrinkage in rat hepatocytes. When rats fed on low protein diet for 21 days, Cervinková *et al.* (1987) recorded cell hypertrophy and occurrence of number of binucleate hepatocytes. Moreover, Mandell *et al.* (1992) reported that when Weanling rats fed on 5% protein diet, they exhibited necrosis and bile duct proliferation. In addition, Dudrick and Kavic (2002) recorded that significant malnutrition caused liver failure, cholestatic liver disease, steatosis and cirrhosis. Alfawwaz and Alhamdan (2006) found that Wister rats given 6% protein for one month exhibited increased deposition of fat globules and proliferation of bile canaliculi.

Ultrastructural inspection of ultrathin sections of livers obtained from protein-deficient-fed rats revealed alterations both in the cytoplasm and nucleus. These changes intensified as time passed i.e. time-dependent. Few mitochondria were degenerated and little amount of rough endoplasmic reticulum and small vacuoles were observed in the cytoplasm. The nucleus possessed reduced amount of heterochromatin. Two weeks following stopping treatment, advanced degree of restoration in the ultrastructure was observed. Bearing similarity to the present results that was declared by Enwonwu *et al.* (1977) who found marked fragmentation and vesiculation of the rough endoplasmic reticulum, distension of nuclear membrane, mitochondrial polymorphism, increased accumulation of glycogen particles in male pigtail monkeys fed on 2% protein for 3 and 5 months. When rats fed on 4% protein for 6 weeks, dilatation of sarcoplasmic reticulum,

swelling of mitochondria and widening of interstitial spaces were recorded (Rossi and Zucoloto, 1982). Moreover, when rats fed on 8% protein for 4 weeks, expanded Golgi bodies and autophagic vacuoles were recorded (Isozaki *et al.*, 1993). Finally, it was indicated that diet deficient in protein decreased mitochondrial reactive oxygen species production (Ayala *et al.*, 2007).

Regarding changes in AST and ALT activities, results obtained herein demonstrated increased activity in these two enzymes as a result of feeding on the low protein diet. This result reinforced the work of Simek *et al.* (1986). The authors showed that rats fed for 3 weeks on low protein diet exhibited significant elevation in serum ALT and AST concentration and alkaline phosphatase activity. Meanwhile, a recent study conducted by Sidhu *et al.* (2005) on Sprgue Dawley rats fed on 8% protein, showed an increase in serum ALT and AST levels. In addition, Oboh *et al.* (2007) recorded an increase in serum ALT and AST level in rabbits fed on 8% fish protein for a period of 8 weeks. This increase in liver function enzymes pointing out to defective hepatic function output. Again, such elevation in these enzymes is acceptable since it is associated with the induced histological alterations both on the light and on the ultrastructural levels.

In conclusion, protein malnutrition exerts harmful effects upon the histology and function of the liver as appeared from light and electron microscopic examination and from some serum liver function enzymes in albino rats. Accordingly, scientific researches should be continued and focused so as to explore such hazards in more finer details especially that malnutrition is a case commonly encountered in Egypt.

Legends of figures

Fig. 1: Section in the liver of a control rat showing the basic histological structure where the hepatic cords radiating from the central vein (CV) and are separated from each other by blood sinusoids (S). Kupffer cells: K. X400

Fig. 2: Section in the liver of a rat fed on protein-deficient diet for 4 weeks exhibiting corruption of the normal hepatic architecture. The liver lost the characteristic pattern of the hepatic strands arrangement that caused disappearance of sinusoids. Some hepatocytes have macronuclei (Mn) while others possess micronuclei (Mu). Most cells suffered from the cytoplasmic vacuolization (V) and slight leucocytic infiltration (Li). In addition, slightly enlarged portal vein was also observed (PV). Bile ductule: Bd; Kupffer cells: arrowhead. X400

Fig. 3: Liver section of another rat maintained in the same conditions showing also loss of normal architecture with cytoplasmic vacuolization of hepatic cells, leucocytic infiltration (Li) around widened portal vein (PV). Slightly enlarged bile ductule (Bd) and activated Kupffer cells (arrowhead) were observed. Most cells had condensed chromatin and round vacuoles (V). X400

Fig. 4: Liver section of a rat fed on the low protein diet for 6 weeks showing congested central vein (CV), abnormal-sized sinusoids (S) and leucocytic infiltration (Li). X400

Fig. 5: Section in the liver of a rat after 8 weeks of feeding on low protein diet showing disruption of normal hepatic structure with the presence of ill-defined cell membranes between some cells, cytoplasmic vacuolization (V), few kupffer cell (arrowhead) and leucocytic infiltration (Li). X400

Fig. 6: Section in a rat's liver 2 weeks post-cessation of feeding on the protein-deficient diet showing improvement in histological structure where normal central vein (CV), nearly normal hepatocytes, but still some widened sinisoids (S) were observed. X400

Fig. 7: Ultrathin section of a hepatocyte of a control rat showing the presence of rough endoplasmic reticulum (RER) and mitochondria (M). The hepatocyte cytoplasm contains glycogen particles. In addition, the nucleus (N) contains two nucleoli (Nu) and is surrounded by nuclear envelope and provided with nuclear pores (NP). The two types of chromatin mainly heterochromatin (Hc) and euchromatin (Ec) are also represented. X1000

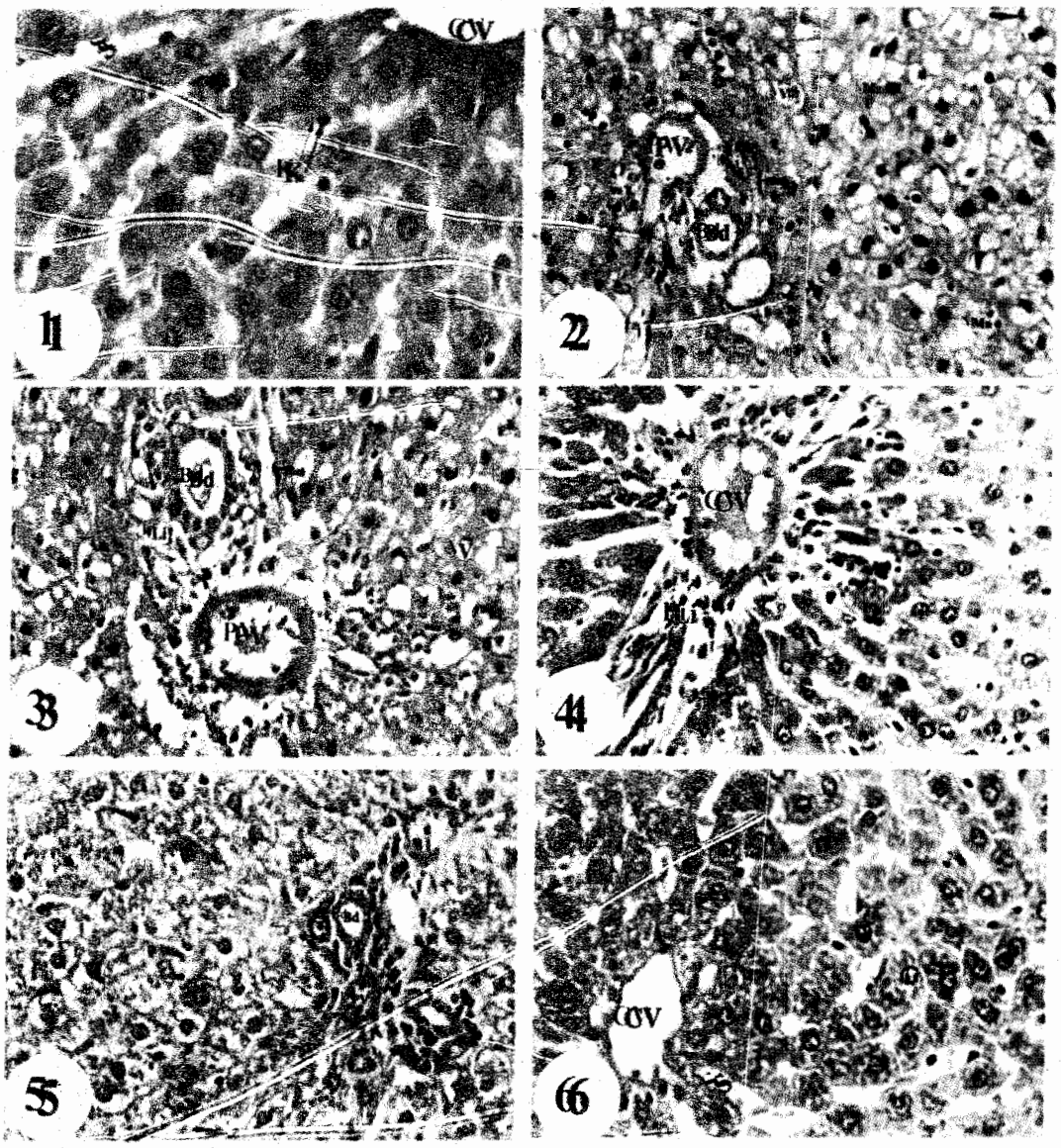
Fig. 8: Electron micrograph of a rat's hepatocyte fed on low protein diet for 6 weeks showing the presence of some mitochondria (M) with degenerated cristae. The rough endoplasmic reticulum (RER) is fairly developed. The

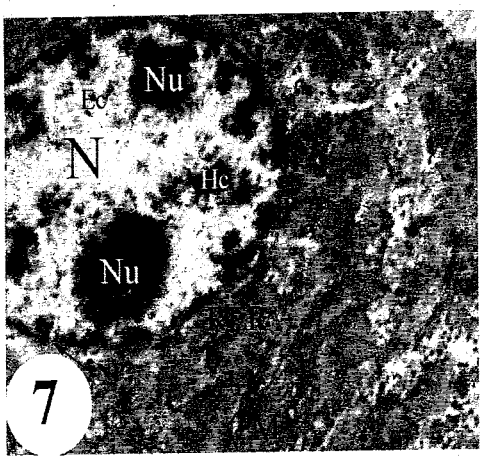
nucleus (N) suffered from loss of most its euchromatin. Nucleolus: Nu; nuclear pores: NP. X2000

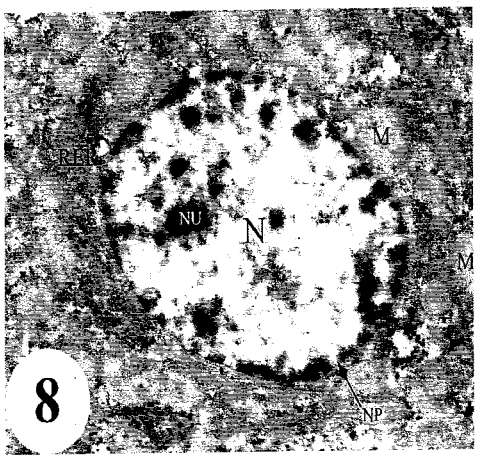
Fig. 9: Electron micrograph of a hepatocyte of another rat subjected to the same treatment showing the presence of various-sized mitochondria (M) having dark matrices and highly affected nucleus (N) that possesses abnormal morphological feature where projections or outgrowths (arrowhead) with disturbance in the quantity and distribution of the nuclear chromatin. Nuclear pores: NP; vacuoles: V. X3000

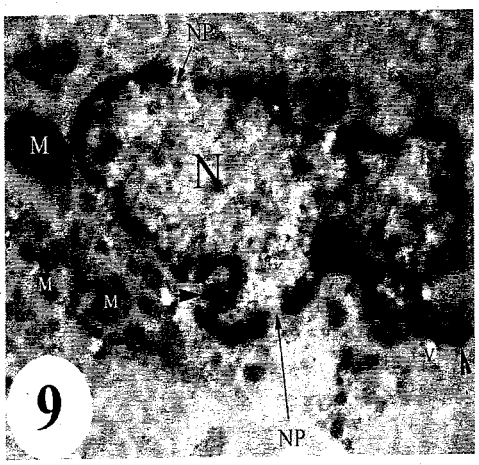
Fig. 10: Electron micrograph of a hepatocyte obtained from a rat's liver after 8 weeks of feeding on the low protein diet showing the presence of large mitochondria (M) some with degenerated cristae. The nucleus (N) lost most of its heterochromatin (Hc) in addition to the existence of large nucleolus (Nu). X2000

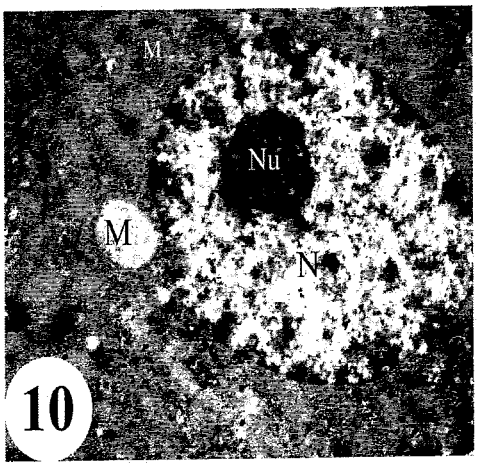
Fig. 11: Electron micrograph of a hepatocyte of a rat two-weeks upon cessation of feeding low protein diet showing nearly normal ultrastructural features. The rough endoplasmic reticulum (RER) and Golgi apparatus (G) are observed. The nucleus (N) contains a nucleolus (Nu), heterochromatin (Hc) and euchromatin (Ec). X2000.

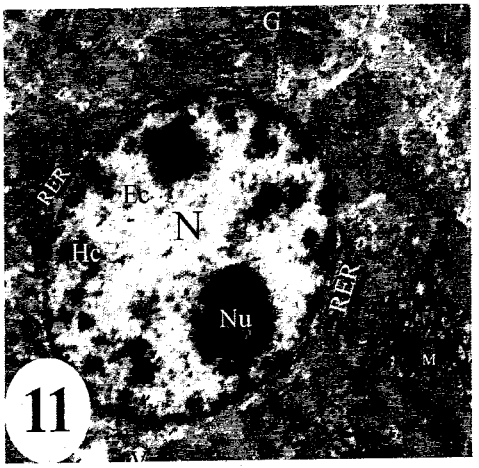

COV
PV
COV
PV
COV
COV


Nu
Ec
N
Hc
Nu
7


M
RER
NU
N
M
8
NP


NP
M
N
M
M
9
NP


M
Nu
M
N
10


G
RER
Ec
N
Hc
RER
Nu
M
11

REFERENCES

AlFawwaz, R.A. and Alhamdan, A. A. (2006): The modulatory effect of N-acetyl cysteine supplementation on hepatic glutathione concentration and lipid peroxidation status in old rats fed a low- protein diet. Pakistan. J. Nutr., 5(2): 156-165.

Andrade, J. P.; Lukoyanov, N.V. and Paula-Barbosa, M. M. (2002): Chronic food restriction is associated with subtle dendretic alterations in granule cells of the rat hippocampal formation. Hippocampus, 12:149-164.

Ayala, V.; Naudí, A.; Sanz, A.; Caro, P.; Portero-Otin, M.; Barja, G. and Pamplone, R. (2007): Dietary protein restriction decrease oxidative protein damage, peroxidizability index, and mitochondrial complex I content in rat liver. J. Gerontol. A Biol. Sci. Med. Sci., 62(4): 352-360.

Banerjee, B.D. (1999): The influence of various factors on immune toxicity assessment of pesticide chemicals. Toxicol. Lett., 107: 21-31.

Barsotti, G.; Moreli, E.; Giannoni, A.; Guinucci, A.; Lupetti, S. and Giovanetti, S. (1983): Restricted phosphorus and nitrogen intake to slow the progression of chronic renal failure: a control trial. Kidney. Int. 24: Supple. 16:S278-S284.

Bayomy, M.F.F. and Taie, H.T. (1991): Effects of fasting, refeeding and restricted feeding on the young rabbit. I. Influence on body organ weights, nucleic acids and protein contents. Egypt. J. Rabbit Sci., 1(2): 159-171.

Brouhard, B.H. and LaGrone, L. (1990): Effect of dietary protein restriction on functional renal reserve in diabetic nephropathy. Am. J. Med., 89 (4):427-431.

Cervinková, Z.; Bgatova, N.P.; Shorina, T.G.; Holecek, M.; Subrtová, D.; Vosvrdová, H.; Shkurupy, V.A. and Simek, J. (1987): Structural and functional changes after the administration of tetrachlormethane in the liver of rats fed on diets with different protein contents. Physiol. Bohemoslov., 36(4): 349-59.

Cheema, K.K.; Dent, M.R.; Aroutionova, N. and Tappia, P.S. (2005): Prenatal exposure to maternal under nutrition induced adult cardiac dysfunction. Br. J. Nutr., 93: 471-477.

Cho, M.K.; Kim, Y.G.; Lee, M.G. and Kim, S.G. (2001): Prevention of c-Jun/activator protein-1 activation and microsomal epoxide hydrolase induction in the rat liver by cysteine during protein-calorie malnutrition. Biochem. Pharmacol., 61(1): 15-24.

Darmon, N.; Pèlissier, M.A.; Heyman, M.; Albrecht, R. and Desjeux, J.F. (1993): Oxidative stress may contribute to the intestinal dysfunction of weanling rats fed on low protein diet. J. Nutr., 123: 1068-1075.

Dudrick, S.J. and Kavic, S.M. (2002): Hepatobiliary nutrition: history and future. J. Hepatobiliry. Pancreat. Surg., 9(4): 459-468.

El Hadri, L.; Garlich, J.D.; Qureshi, M.A.; Ferket, P.R. and Odetallah, N.H. (2004): Glucose and electrolyte supplementation of drinking water improve the immune response of poults with inanition. Poultry Sci., 83(5): 803-809.

Enwonwu, C.O.; Worthington, B.S. and Jacobson, K.L. (1977): Protein-energy malnutrition in infant non-human primates (*Macaca nemestrina*). I. Correlation of biochemical changes with fine structural alterations in the liver. Br. J. Exp. Pathol., 58(1): 78-94.

Gartner, A.; Castellon, W.T.; Gallon, G. and Simondon, F. (1992): Total dietary restriction and thymus, spleen, and phenotype and function of splenocytes in growing mice. Nutrition, 8(4): 258-265.

Gella, F.J.; Olivella, T.; Cruz-Pastor, M.; Arenas, J.; Moreno, R.; Durban, R. and Gomez, J.A. (1985): A simple procedure for routine determination of aspartate aminotransferase and alanine aminotransferase with pyridoxal phosphate . Clin. Chem. Acta, 153: 341-247.

Germolec, D.R.; Adams, N.H. and Luster, M.I. (1997): The importance of enzymatic biotransformation in immunotoxicology. Rev. Toxicol., 1:33-51.

Gershwin, M.E.; Beach, R.S. and Hurley, L.S. (1985): Nutrition and immunity. Academic Press, Orlano, pp. 156-284.

Handy, R.D.; Abdel Samei, H.A.; Bayomy, M.F.F.; Mahran, H.A.; Abdeen, A.M. and El-Elaimy, E.A. (2002): Chronic diazinon exposure: pathologies of spleen, thymus, blood cells and lymph nodes are modulated by dietary protein or lipid in the mouse. Toxicology, 172:13-34.

Iglésias-Barreira, V.; Ahn, M.T.; Reusens, B.; Dahri, S.; Hoet, J.J. and Remacle, C. (1996): Pre-and postal low protein diet affected pancreatic islet blood flow and insulin release in adult rats. Endocrinology, 137(9): 3797-3801 .

Isozaki, T.; Verlander, J.W. and Sands, J.M. (1993): Low protein diet alters urea transport and cell structure in rat initial inner medullary collecting duct. J . Clin. Invest., 92(5): 2448-2457.

Karnovsky, M.J. (1965): A formaldehyde-glutraldehyde fixative of high osmolarity for use in electron microscopy. J. Cell. Biol., 27: 137A.

Krause, M.V.; Mahan, L.K. and Escott-Stump, S. (2000): Krause's food, nutrition and diet therapy. Philadelphia, Saunders.10th edi., Pp, 242.

Lillie, R.D.; and Fulmer, H.M. (1976): Histopathological Technique and Practical Histochemistry. 4th edit. New York, McGraw Hill.

Mandell, H.G.; Judah, D.J. and Neal, G.E. (1992): Effects of dietary protein level on aflatoxin B1 actions in the liver of weanling rats. Carcinogenesis, 13 (10): 1853-1857.

Masoro, E.J. (2005): Overview of caloric restriction and ageing. Mech. Ageing Dev., 126: 913-922.

McMullen, S. and Langley-Evans, S.C. (2005): Sex-specific effects of prenatal low-protein and carbenoxolone exposure on renal angiotensin receptor expression in rats. Hypertension, 46(6): 1374-1380.

Oboh, H.A.; Omofoma, C.O.; Olumese, F.E. and Eiya, B. (2007): Effects of high carbohydrates low fats Nigerian-like diet on biochemical indices in rabbits. Pakistan. J. Nutr., 6(4): 399-403.

Oboh,H.A.; Omofoma, C.O.; Olumese, F.E. and Eiya, B.(2007): Effects of high carbohydrates low fats Nigerian –like diet on biochemical indices in rabbits. Pakistan. J. Nutr., 6(4): 399-403.

Okawa, H.; Morita, T. and Sugiyama, K. (2006): Increased plasma homocysteine concentration in rats from a low casein diet. Biosci. Biotechnol. Biochem., 70(12): 3050-3053.

Onis, M.; Monteiro,C. and Clugeston,G. (1993): The world magnitude of protein-energy malnutrition: An overview from the WHO Global Data-base on Children Growth. B. World Health Organ., 71: 703-712.

Pitkanen, A.; Maria Pikkarainen,M.; Nurminen,N.; Yinen,A. (2000): Reciprocal connections between the amygdala and hippocampal formation, perirhinal cortex and postrhinal cortex in rats. Ann. NY. Acad. Sci., 911: 369-391.

Richard, R.R. and Helen, L.M. (1963): Effects of several low protein diets on the serum iron, total iron-binding capacity, and serum cholesterol of rats. J. Nutr., 81: 63.

Robbins, M.E.; Bywaters, T.; Jaenke, R.S.; Rezvani, M.; Golding, S.J; Whitehouse, E. and Hopewell, J.W. (1993): Influence of a low protein diet on radiation nephropathy in pig. Int. J. Rad. Biol., 64(4): 407-416.

Rocha-de-Melo, A.P. and Guedes, R.C.A. (1997): Spreading depression is facilitated in adult rats previously submitted to short episodes of malnutrition during the lactation period. Braz. J. Med. Bio. Res., 30: 663-669.

Rossi, M.A. and Zucoloto, S. (1982): Ultrastructural changes in nutritional cardiomyopathy of protein-calorie malnourished rats. Br. J. Exp. Pathol., 63(3):242-253.

Shrader, R.E.; Hasting-Roberts, M.M.;Ferlatte, M.I. and Zeman, F.J. (1977b): Effects of prenatal protein deprivation on fetal and neonatal thyroid morphology in the rats. J. Nutri., 107(2): 213-220.

Sidhu, P.; Garg, M.L. and Dhawan, D.K. (2005): Protective effects of zick on oxidative stress enzymes in liver of protein-deficient rats. Drug Chem. Toxicol., 28(2): 211-230.

Simek, J.; Shkurupy, V.A.; Shorina, G.N.; Bgatova, N.P.; Cervinkova`, Z. and Holecek, M. (1986): Effect of long-term administration of a high protein or low protein diet on rat liver. Morphological and biochemical findings. Physiol. Bohemoslov., 35(2): 127-133.

Torrens, C.; Brawley, L.; Barker, A.C.; Itoh, S.; Poston, L. and Hanson, M.A. (2003): Maternal protein restriction in the rat impairs resistance artery but not conduit artery function in pregnant offspring. J. Physiol., 547: 77-84.

Verma, D.R. (1980): Myocardial effects and pharmacokinetics of digoxin and quabain in protein-deficient guinea pigs. Can. J. Physiol. Pharmacol., 58(5): 564-567.

Zambrano, E. R.; Guzmán, G.L.; García-Becerra, C.; Boeck, L.; Díaz, L. Menjivar, M.; Larrea, F. and Nathanielsz, P.W. (2005): A maternal low protein diet during pregnancy and lactation in the rat impairs male reproductive development. J. Physiol., 563(Pt 1): 275-284.

Zeller, K.; Whittaker, E.; Sullivan, L.; Raskin, P. and Jacobson, M.R. (1991): Effects of restricting dietary protein on the progression of renal failure in patients with insulin dependent diabetes mellitus. New Engl. J. Med., 34: 78-84.