Journal of Food and Drug Analysis

Volume 20 | Issue 2 Article 18

2012

Effects of diethylcarbamazine (DEC) on hepatocytes of C57BL/6J mice submitted to protein malnutrition

Journal of Food and Drug Analysis, Vol. 20, No. 2, 2012, Pages 524-531 doi:10.6227/jfda.2012200214

Effects of Diethylcarbamazine (DEC) on Hepatocytes of C57BL/6J Mice Submitted to Protein Malnutrition

SURA WANESSA SANTOS ROCHA[1], ANA CELIA OLIVEIRA DOS SANTOS[2]*, BRUNA DOS SANTOS SILVA[1], DILENIA DE OLIVEIRA CIPRIANO TORRES[1], EDLENE LIMA RIBEIRO[1], KARLA PATRICIA SOUSA BARBOSA[1], FABIANA DE OLIVEIRA GOMES[1] AND CHRISTINA ALVES PEIXOTO[1,3]

[1.] *Ultrastructure Laboratory, Institute Aggeu Magalhães - FIOCRUZ, Pernambuco, Brazil*
[2.] *University of Pernambuco, Pernambuco, Brazil*
[3.] *Microscopy and Microanalysis Laboratory, Center for Strategic Technology Northeastern - MCT, Pernambuco, Brazil*

(Received: May 24, 2011; Accepted: April 3, 2011)

ABSTRACT

Even though diethylcarbamazine (DEC) is widely used in the treatment for filaricide, its pharmacological potential is little explored. Protein malnutrition is highly incident in many populations in developing and under-developed countries and some of these populations are affected by lymphatic filariasis. The objective of the present study was to analyze the DEC effect in the hepatocytes of malnourished mice. Forty-eight male C57BL/6 mice were separated into groups: a control group (C, D25 and D50) and a malnourished group (M, MD25 and MD50). After being induced to malnutrition, the mice were submitted to 12 days of treatment with DEC solutions in concentrations of 25 and 50 mg/kg which were orally administered. Biochemical analyses were performed and liver fragments were processed for light microscopy and transmission electron microscopy. For the enzymatic dosages, it was possible to observe a significant reduction in serum albumin in group M (2.52 ± 0.415 g/dL) when compared with the control group (C) (3.27 ± 0.427 g/dL) which received no treatment. The histological analysis of the M group showed evident hepatocellular damage. However, groups MD25 and MD50 returned to basal levels. Groups M, MD25 and MD50 presented a significant increase in alkaline phosphate when compared with the control group (C). Histological analysis of group M showed evident hepatic steatosis, which characterizes hepatocellular damage. Groups MD25 and MD50 showed a decrease in steatosis. An ultrastructural analysis of the hepatocytes in groups MD25 and MD50 confirmed a reduction of lipid droplets. It is possible that the DEC effects have contributed to the reduction of hepatic changes caused by malnutrition.

Key words: diethylcarbamazine, hepatocytes, malnutrition

INTRODUCTION

Diethylcarbamazine is the most widely used filaricide in the treatment of lymphatic filariasis and induces a rapid disappearance of microfilariae from the blood circulation[1,2].

Maizels and Denhan[3] have presented a hypothesis to explain the mechanism of *in vivo* DEC microfilaricide. The most consistent interpretation for the DEC mechanism is that it alters the metabolism of arachidonic acid in the host's microfilaria and endothelial cells. Such changes would lead to vasoconstriction, which would increase endothelial adhesion, consequently causing the immobilization of the parasite stock. This would increase adherence and the host's cytotoxic activity of platelets and granulocytes. These events could bring about the activation of nonspecific innate immune system, regardless of the antigen-specific adaptive immune response.

DEC also has anti-inflammatory properties for asthma conditions[4,5,6]. According to Florêncio[7], after 12 days of treatment with DEC, type II pneumocytes showed a substantially greater number of mature secretion vesicles, and lung macrophages presented in several characteristics of cellular activation. These results could explain the relief of asthma symptoms after treatment with DEC as previously observed by Liu *et al*.[8]

According to Queto *et al*.[9], DEC has important roles in blocking the pulmonary eosinophilic inflammation in mice that are sensitized with ovalbumin. In addition, DEC effectively prevented the effects of subsequent airway resistance, Th1/Th2 cytokine production, pulmonary eosinophil

* Author for correspondence. Tel: 55-81-34531291;
Fax: 55-81-31833301; E-mail: anaceliaoliveira@bol.com.br

accumulation and *in vivo* and *ex vivo* eosinophilopoiesis. DEC directly suppressed IL-5–dependent eosinophilopoiesis in naive bone marrow[9].

Malnutrition represents a significant worldwide health problem affecting both developing and developed countries. According to the Food and Agriculture Organization (FAO), there are 1.02 billion undernourished people worldwide[10]. Starvation, which affects one out of five people in underdeveloped countries, is an impediment to individual and social advancement[11].

Reactions to protein malnutrition include the removal of protein amino acids from the tissues, so that the liver can maintain homeostasis, and the amount of albumin synthesized by malnourished mice livers is significantly lower than that synthesized by mice that are fed with a normal diet[12,13].

Metabolic and morphologic changes in various organs, resulting from these situations, are well known by the scientific community and general population. As these situations exist and are significant, the study of their effects on liver functions and the DEC effect is of great importance, given how widely used filaricide is.

Therefore, it is necessary to clarify whether there are important metabolic and histopathological changes in several cell types of the individual under treatment, who may be malnourished. This study investigated DEC's effects, through morphological (histological and ultrastructural) and biochemical analysis, on undernourished hepatocytes mice for possible analogies with human cases.

MATERIALS AND METHODS

I. *Animals and Diets*

Forty-eight 30 days old male C57BL/6J mice weighing 15 - 16 g were used in all experiments. The health status of the mice were examined and they were acclimated to the laboratory environment at 23°C and exposed to 12 h of light and 12 h of dark photoperiod. The mice were submitted to two nutritional treatments with a protein sufficient diet (Pragsoluções®, São Paulo) containing 20% protein, or a protein deficient diet containing 4% protein[14] and water *ad libitum*. The composition diets are shown in Table 1. After 30 days under a low-protein diet, a group of mice had a loss of 20% of their body mass as malnourishment was observed. The animals were weighed daily. All experiments were carried out according to ethical guidelines (LW32/2010 CEUA-FIOCRUZ).

Table 1. Experimental diet composition[1]

Ingredients	Control Diet (g/kg diet)	Restriction Diet (g/kg diet)
Casein (>85% ptn)	200	40
Sucrose	100	100
Fiber	10	10
Corn oil	80	80
Minerals mixture[2]	40	40
Vitamins mixture[2]	10	10
DL- methionine	1.5	1.5
Choline bitartrate	2.5	2.5
Cornstarch	556.5	716.5

[1] Isocaloric diets providing 1.716.3 kJ/100g (410.6 kcal/100g).
[2] Minerals and vitamin mixtures were prepared according to the AIN-93 recommendations from the American Institute of Nutrition for mice[30].

II. *Diethylcarbamazine Solutions*

The solutions were composed of distilled water and DEC (Farmanguinhos, BR) adjusted according to the body weight of each animal. The groups that were treated received 25 mg/kg (**D25**) and 50 mg/kg (**D50**) of DEC for twelve days through gavage (200 µL). The control group (**C**), which consisted of 8 animals, received only distilled water using the same administration method[15]. Classical metabolic studies describe the total metabolic rate of a 30-g mouse as 961 kJ per kg body weight, which is approximately seven times the total metabolic rate of a 70-kg human, 138 kJ per kg[16]. In the present study, the choice of using 50 mg/kg as the maximum dose of DEC was based on lymphatic filariasis therapeutic dose regimens recommended by WHO (6 mg/kg for 12 days).

III. *Experimental Design*

After a week of acclimation, 30 days-old C57BL/6 mice were separated into six groups (n = 8): 1) Control group (**C**), 2) DEC-treated 25 mg/kg group (**D25**), 3) DEC-treated 50 mg/kg group (**D50**), all of the above groups fed with a protein sufficient diet; 4) Malnourished group (**M**), 5) Malnourished DEC-treated 25 mg/kg group (**MD25**), and 6) Malnourished DEC-treated 50 mg/kg group (**MD50**), all of the above groups fed with a low-protein diet. After 30 days of being fed these diets, the mice were submitted to 12 days of treatment with oral DEC solutions in concentrations of 25 and 50 mg/kg. The control (**C**) and malnourished (**M**) groups received only water for 12 days. Subsequently, the mice were anesthetized, the blood was collected and the serum was separated for biochemical analyses. Anesthetized mice were killed and the liver was quickly dissected and processed by an optical and electronic microscopy.

IV. *Optical Microscopy*

Liver fragments were fixed in 10% formalin for 24 h, processed and embedded in paraffin. Sections of 4 - 5 µm were cut and mounted on glass slides. Slices were stained with hematoxylin-eosin and analyzed with the help of an inverted microscope (Observer Z1, Zeiss MicroImaging GmbH) equipped with a camera (AxionCam MRm Zeiss) at a magnification of 400x, and coupled to the area of the 4.7.4 image analysis program (Zeiss).

V. *Ultrastructural Assays*

The fragments of liver were fixed in a solution containing 2.5% glutaraldehyde and 4% formaldehyde in 0.1 M cacodylated buffer. After fixation, the samples were washed twice in the same buffer, and post-fixed in a solution containing 1% osmium tetroxide, 2 mM of $CaCl_2$ and 0.8% potassium ferricyanide in 0.1 M cacodylate buffer, pH 7.2, dehydrated in acetone, and embedded in Epon 812 resin (Sigma Company, St Louis, USA). Polymerization was performed at 60°C for 2 days. Ultrathin sections were collected on 300-mesh copper grids counterstained with uranyl acetate and lead citrate, and examined with a Morgani FEI transmission electron microscope.

VI. *Biochemical Assays*

Serum was assessed for lipid profile (total cholesterol (TC), high-density lipoprotein (HDL), low-density lipoprotein (LDL) and triglycerides (TG) and hepatic damage test alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), bilirrubin and albumin. Serum concentrations were measured photometrically in an automatic analyzer (Cobas Integra 400, Roche, Mannheim, Germany), using Roche kits.

VII. *Statistical Analysis*

Data was expressed as mean ± standard deviation. The differences between the control group and the treated groups were analyzed using analysis of variance (ANOVA) and Dunnet and Tukey's tests were used as *post hoc* tests. GraphPad Prism software, version 5 was used. The differences between means were considered significant at $p < 0.05$.

RESULTS

I. *Body Weight*

The animals which were fed a low-protein diet showed significant reduction in body weight (M) (18.46 g ± 0.209 g) when compared to the control group (**C**) (24.03 g ± 0.758 g). Malnourished animals DEC-treated, **MD25** (18.47 g ± 0.142 g) and MD50 (19.03 g ± 0.126 g), also showed a significant reduction in body weight (Table 2).

II. *Metabolic Changes*

No significant differences in lipid profile (total cholesterol, LDL, HDL and triglycerides) could be observed between DEC-treated groups (**D25** and **D50**), neither in the malnourished group DEC-treated (**M, MD25** and **MD50**) (Table 3).

The serum alkaline phosphatase (ALP) levels of the malnourished group M (198.5 ± 39.69 IU/L), **MD25** (174.8 ± 38.29 IU/L) and **MD50** (165.5 ± 40.43 IU/L) were statistically higher when compared with control group C (104.2 ± 5.79 IU/L) (Table 4). Serum ALT, AST and bilirubin showed no significant differences between the above mentioned groups.

In relation to the levels of serum albumin, only group **M** (2.520 ± 0.414 g/dL) showed significantly lower values

Table 2. Body weight variations in control mice (C, D25 and D50) and malnourished mice (M, MD25 and MD50)

Parameter *n = 8/group*	C	D25	D50	M	MD25	MD50
Body weight (g)	24.03 ± 0.758	23.65 ± 0.772	24.49 ± 0.751	18.46 ± 0.209*	18.47 ± 0.142*	19.03 ± 0.126*

Mean ± SD values for change in body weight of control (C, D25 and D50) and malnourished (M, MD25 and MD50) mice.
* Indicates a significant difference between the control group and the malnourished group [ANOVA and Dunnet and Tukey's tests ($p < 0.05$)].

Table 3. Lipid profiles of control mice (C, D25 and D50) and malnourished mice (M, MD25 and MD50)

Parameter *n = 8/group*	C	D25	D50	M	MD25	MD50
Total Cholesterol (mg/dL)	123.3 ± 23.53	126.4± 31.22	109.1± 36.58	136.0 ± 31.24	126.7 ± 85.52	101.2 ± 37.80
HDL Cholesterol (mg/dL)	104.4 ± 13.5	107.6 ± 24.86	92.74 ± 27.43	119.0 ± 22.48	104.7 ± 72.33	82.50 ± 25.25
LDL Cholesterol (mg/dL)	8.854 ± 8.132	4.905 ± 5.025	11.60 ± 13.25	7.947 ± 2.998	9.448 ± 5.506	5.775 ± 2.297
Triglycerides (mg/dL)	78.83 ± 17.62	90.14 ± 23.25	60.00 ± 15.85	73.00 ± 14.14	77.50 ± 22.96	51.00 ± 17.55

Mean ± SD values of control group (C, D25 and D50) and malnourished groups (M, MD25 and MD50). No significant difference between the lipid profiles of the groups (ANOVA and Dunnet and Tukey's tests).

Table 4. Liver enzymes and serum albumin concentrations of control mice (C, D25 and D50) and malnourished mice (M, MD25 and MD50).

Parameter *n = 8/group*	C	D25	D50	M	MD25	MD50
AST (IU/L)	80.11 ± 11.85	110.1 ± 11.12	144.0 ± 19.40	117.8 ± 21.12	85.28 ± 12.28	132.9 ± 2.57
ALT (IU/L)	26.10 ± 9.78	27.18 ± 7.13	47.40 ± 21.23	31.44 ± 9.57	22.74 ± 5.28	46.54 ± 17.62
ALP (IU/L)	104.2 ± 5.79	128.5 ± 6.16	126.5 ± 7.46	198.5 ± 39.69***	174.8 ± 38.29**	165.5 ± 40.43*
Bilirubin (mg/dL)	0.128 ± 0.028	0.126 ± 0.069	0.138 ± 0.053	0.102 ± 0.118	0.248 ± 0.172	0.170 ± 0.161
Albumin (g/dL)	3.27 ± 0.427	3.10 ± 0.379	3.22 ± 0.407	2.52 ± 0.414*	2.86 ± 0.207	2.70 ± 0.975

Mean ± SD values for change of control (C, D25 and D50) and malnourished mice (M, MD25 and MD50).
* Indicates significant values of malnourished groups when compared to the control group (ANOVA and Dunnet and Tukey's tests
* $p < 0.05$; ** $p < 0.02$; *** $p < 0.01$).

when compared with group **C** (3.267 ± 0.427 g/dL). **MD25** (2.860 ± 0.207 g/dL) and **MD50** groups (2.700 ± 0.975 g/dL) showed lower levels of serum albumin compared to the control group (**C**), without, however, being statistically significant (Table 4).

III. *Histological Changes*

Histological analysis of liver fragments in group **C** showed well-preserved tissue with a standard aspect. No histological changes were observed in the hepatocytes of the animals from this group (Figure 1A). After treatment with DEC for 12 days (**D25** and **D50**), we could observe the presence of some ballooning degeneration in the liver parenchyma. The blood vessels showed a typical pattern (Figures 1B and C).

In the histological sections of the malnourished groups of mice (**M, MD25, MD50**), the most striking finding was the presence of multiple lipid droplets dispersed in the cytoplasm of hepatocytes, characterizing the process of steatosis (Figures 1D, E and F). Besides steatosis, hydropic degeneration could be observed among groups **M, MD25** and **MD50** (Figures 1D, E and F).

IV. *Ultrastructural Changes*

The ultrastructural analysis of hepatocytes of the control group showed a characteristic morphology with rough endoplasmic reticulum and evident mitochondria (Figure 2A). The hepatocytes in the group treated for 12 days with 25 mg/kg and 50 mg/kg DEC (**D25** and **D50**) showed a proliferation of rough endoplasmic reticulum membranes. However, the other organelles showed similar characteristics to the control group (Figures 2B and C).

In relation to malnourished groups, the control group (**M**) showed large lipid droplets, and the steatosis a compatible process with malnutrition. Few endoplasmic reticulum and peroxisomes were visualized and there was evident cytoplasmic degradation (Figure 2D). Malnourished animals treated with 25 mg/kg and 50 mg/kg of DEC (**MD25** and **MD50**) showed similarities with the malnourished control group (**M**), with few lipid droplets dispersed in the cytoplasm and a reduction in the damage caused by malnutrition (Figures 2E and F).

DISCUSSION

Changes in diet and nutritional status may influence neurological and metabolic changes and induce inflammatory reaction due to increased susceptibility to infectious agents[17,18,19]. We have analyzed the biochemical, histopathological and ultrastructural changes in the liver cells of undernourished mice undergoing treatment with DEC.

Animals fed a low-protein diet consumed less protein than controls, which resulted in a significant reduction in body weight. Protein restriction used in this study caused a reduction of more than 20% weight loss when compared with the control groups and that is in line with literature data and indicates the adequacy of the proposed model[16,17].

In relation to the serum levels of alkaline phosphatase (ALP), Metwalli *et al.*[20] noticed some aspects of this enzyme in the liver of mice subjected to protein-energy malnutrition and found increased protein synthesis of this enzyme in these malnourished animals. Another study showed that elevations of ALP are common abnormalities in biochemical tests of malnourished dogs[21]. Similarly, Osborne *et al.*[22], when administering protein-restricted diets in mice, could observe an increased ALP. Based on these data, protein deficiency should be considered a cause for mild or moderate hepatobiliary damage. Corroborating these studies, our results have demonstrated a significant increase in alkaline phosphatase in the animals of malnourished groups **M, MD25** and **MD50** in relation to the control group (**C**). However, it should be noted that exposure to DEC did not significantly alter serum levels of ALP among the malnourished groups.

Hypoalbuminemia is related to the lack of amino acids for albumin synthesis or a lower capacity of the liver tissue to synthesize this protein. It was observed that only the malnourished control group (**M**) showed a significant decrease of albumin in relation to the control group (**C**). This finding indicates that this type of malnutrition may be related to a decrease in albumin gene transcription expressed in the liver[23,24]. However, malnourished animals treated with

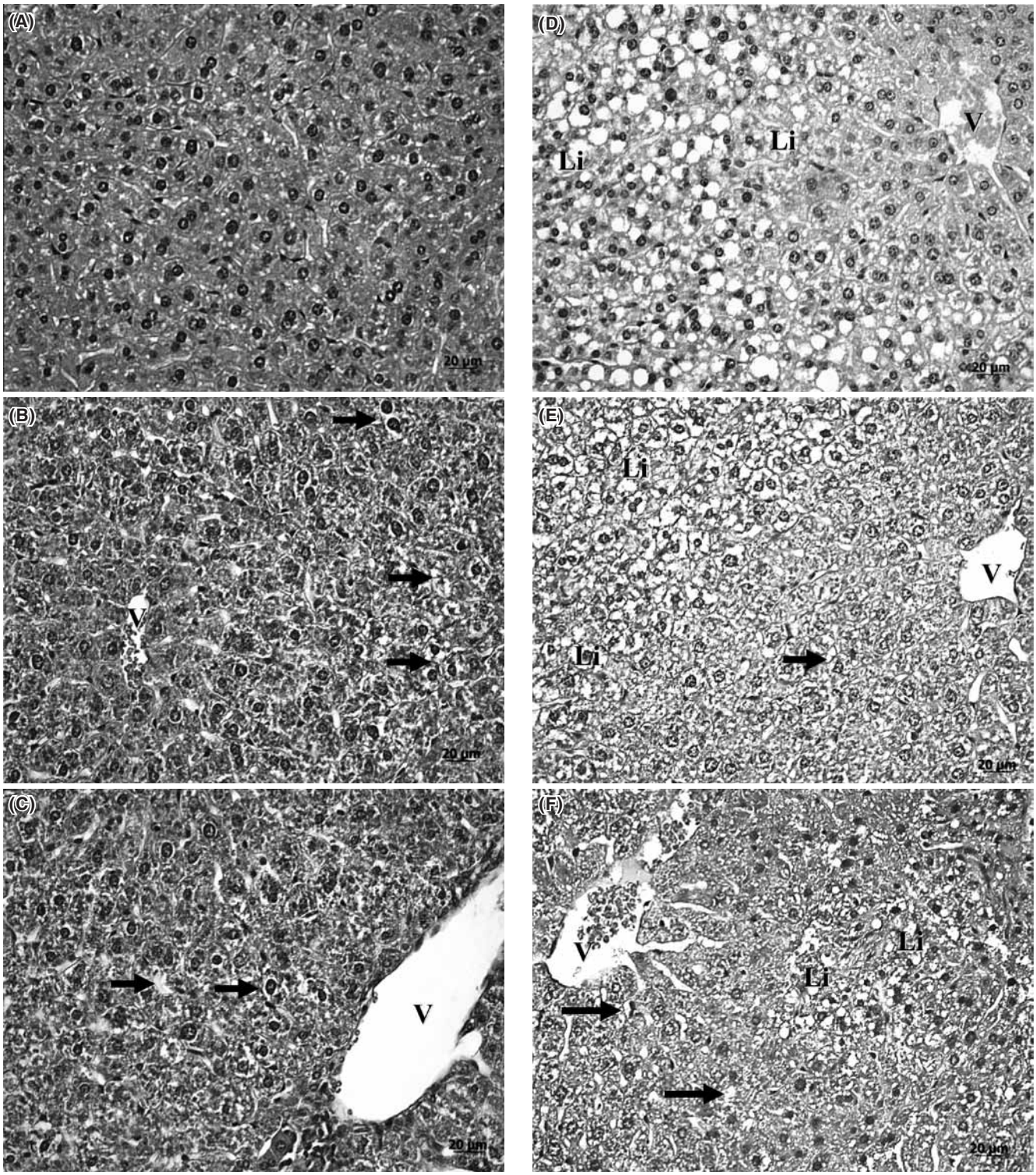

Figure 1. Micrograph of hepatocytes. A: Control group (C) showing typical morphology, B: Group treated with 25 mg/kg DEC (D25), C: Group treated with 50 mg/kg DEC (D50), D: Malnourished control group (M), E: Malnourished 25 mg/kg DEC-treated group (MD25), F: Malnourished 50 mg/kg DEC-treated group (MD50). Blood vessel (V), hydropic degeneration (arrow), lipid droplets (Li). HE staining. Bar = 20 µm.

Journal of Food and Drug Analysis, Vol. 20, No. 2, 2012

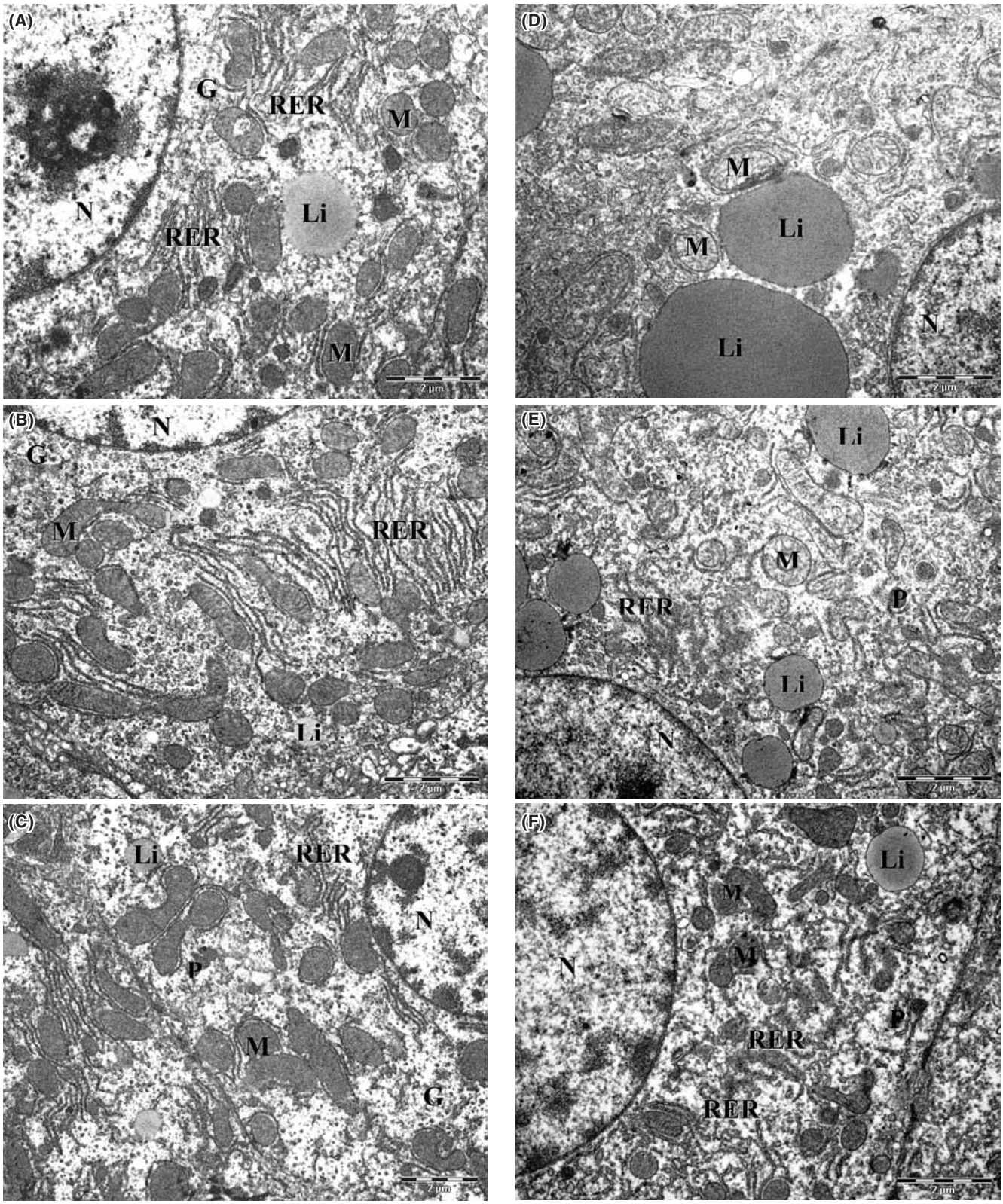

Figure 2. Ultrathin sections of hepatocytes. A: Control group (C), B: 25 mg/kg DEC-treated group (D25), C: 50 mg/kg DEC-treated group (D50), D: Hepatocytes of malnourished control group (M), E: Malnourished plus 25 mg/kg DEC-treated group (MD25), F: Malnourished plus 50 mg/kg DEC-treated group (MD50). Mitochondria (M), glycogen (G), nucleus (N), rough endoplasmic reticulum (RER), lipid droplets (Li), peroxisomes (P). Bar = 2 μm.

DEC (**MD25** and **MD50**) showed no significant decrease in serum albumin in relation to the control group.

The histological analysis of the malnourished control group (**M**) resulted in an accumulation of lipids in the cytoplasm of hepatocytes, characterizing a steatosis process. Joshi and colleagues[25] have observed that the deposition of lipids in the liver of animals fed a protein-restricted diet was higher than that observed in control animals. This is due to a reduced synthesis of lipoproteins that transport cholesterol and triglycerides and removal of endogenous liver tissue[26]. Thus, the main causative evidence of hepatic steatosis in the animals studied was possibly due to reduced protein carrier and subsequent accumulation of triglycerides and cholesterol in hepatocytes. However, the analysis of **MD25** and **MD50** groups showed reduced accumulation of lipid droplets in hepatocytes when compared with the control group (**M**). These results are in agreement with Gonzàlez *et al.*[27], who showed that rats with acute inflammation induced by CCl_4 had a reduction of liver damage after DEC treatment at 25 and 50 mg/kg concentrations, such as, well-preserved organelles and membrane system of hepatocytes, showing that DEC had a protective effect. These studies suggested that DEC inhibited the products of arachidonic acid metabolism, which include lipoxygenases (LOX) and cyclooxygenases (COX) during the development of liver injury[3,27].

Several investigators have studied the ultrastructural alterations in the liver in experimental protein deficiency. The ultrastructural analysis of the liver of the malnourished group (**M)** confirmed the presence of large lipid droplets dispersed in the cytoplasm. Moreover, we observed reduced endoplasmic reticulum and few peroxisomes, besides cytoplasmic degradation. According to Brooks *et al.*[28], after ultrastructurally analyzing the liver tissue of children suffering from protein-energy malnutrition, they could not only observe several lipid droplets, but also swollen mitochondria, glycogen sparing, reduced peroxisomes and endoplasmic reticulum and cytoplasmic changes. The decrease of peroxisomes can stop the oxidation of long-chain fatty acids and increase the accumulation of lipids. Moreover, a reduction in the concentration of catalase reduces the detoxification of free radicals. As the concentrations of other antioxidants, especially glutathione, are also reduced, free radical damage can occur. That may lead to lipid peroxidation of membranes, mitochondrial damage and influx of water and electrolytes into the cell[29]. In this study, after treatment with DEC, malnourished animals of groups **MD25** and **MD50** had a clear reduction of liver damage generated by protein restriction. Recent studies have emphasized the importance of treatment with DEC in pulmonary eosinophilia, reducing infiltration and cytokine levels, and migration of eosinophils[9]. These studies have facilitated the understanding of the DEC immunomodulatory, cytoprotective and anti-inflammatory effects. DEC possibly acted as a hepatoprotective agent, reducing the levels of free radicals and consequently decreasing lipid peroxidation due to suppression of inflammatory mediators.

Further investigations are underway in our laboratory in order to verify the mechanisms of action of DEC on the liver cells of mice subjected to protein restriction. Thus, we will be able to confirm the beneficial effect of DEC against liver injuries.

ACKNOWLEDGMENTS

This study was supported by the Fundação de Amparo à Ciência e Tecnologia do Estado de Pernambuco (FACEPE) and Aggeu Magalhães Research Center of the Oswaldo Cruz Foundation in Recife, Brazil (CPqAM/FIOCRUZ).

REFERENCES

1. Freedman, O. D., Plier, A. D., Almeida, B. A., Oliveira, A., Miranda, J. and Braga, C. 2001. Effect of aggressive prolonged diethylcarbamazine therapy on circulating antigen levels in bancroftian filariasis. Trop. Med. Int. Health. 6: 37-41.
2. Dreyer, G. and Norões, J. 1997. Dietilcarbamazina no tratamento da filariose bancroftiana. Rev. Soc. Bra. Med. Trop. 30: 229-240.
3. Maizels, R. M. and Denham, D. A. 1992. Diethilcarbamazine (DEC): immunopharmacological interactions of an anti-filarial drug. Parasitology. 105: 849-860.
4. Salazar-Mallén, M. 1971. Treatment of intractable asthma with diethylcarbamazine citrate. Annals of Allergy. 23: 534-537.
5. Srinivas, H. V. and Antani, J. 1971. Diethylcarbamazine in bronchial asthma. Annals of Allergy. 29: 418-421.
6. Thiruvengadam, K. V. *et al.* 1974. Diethylcarbamazine citrate in bronchial asthma. J. Indian Med. Assoc. 63: 278-281.
7. Florêncio, M. S., Saraiva, K. L. A. and Peixoto, C. 2005. The effects of diethylcarbamazine on ultrastructure of lung cells in vivo. Tissue Cell. 37: 241-246.
8. Liu, M., Wang, L., Li, E. and Enhorning, G. 1996. Pulmonary surfactant given prophylactically alleviates an asthma attack in guinea pigs. Clin. Exp. Allergy. 26: 270-275.
9. Queto, T., Xavier, P., Gardel, M. A., Luca, B., Barradas, M., Masid, D., Silva, P. M. and Peixoto, C. A. *et al.* 2010. Inducible Nitric Oxide Synthase/CD95L-dependent Suppression of Pulmonary and Bone Marrow Eosinophilia by Diethylcarbamazine. Am. J. Respir. Crit. Care Med. 181: 429-437.
10. Food and Agriculture Organization of the United Nations (FAO). 2009. The State of Food Insecurity in the World. http://www.fao.org/docrep/012/i0680e/i0680e.pdf/
11. Evans, G.W. 2004. The environment of chilghood poverty. Am. Psychol. 59: 77-92.
12. Kirdch, R., Frith, I., Black, E. and Hoffenberg, R. 1968. Regulation of albumin synthesis and catabolism by alteration of dietary protein. Nature. 217: 578-179.
13. Tovar, A. R., Santos, A., Halhali, A., Bourges, H.

and Torres, N. 1998. Hepatic histidase gene expression responds to protein rehabilitation in undernourished growing rats. J. Nutr. 128: 1631-1635.

14. Fock, R. A., Vinolo, M. A. R., Crisma, A. R., Nakajima, K., Rogero, M. M. and Boreli, P. 2008. Protein-Energy malnutrition modifiles the production of interleukin-10 in response to lipopolysaccharide (LPS) in murine model. J. Nutr. Sci. Vitaminol. 54: 371-377.
15. Saraiva, K. L. A., Junior-Silva, V. A., Dias, E. S. F. and Peixoto, C. A. 2006. Morphological changes in the testis induced by diethilcarbamazine. Reprod. Toxicol. 22: 754-759.
16. Terpstra, A. H. M. 2001. Differences between humans and mice in efficacy of the body fat lowering effect of conjugated linoleic acid: role of metabolic rate. J. Nutr. 131: 2067-2068.
17. Borelli, P., Mariano, M. and Borojevic, R. 1995. Protein malnutrition: effect on myeloid cell production and mobilization into inflamatory reactions in mice. Nutr. Res. 15: 1477-1485.
18. Vituri, C. L., Borelli, P., Alvarez-Silva, M. and Tretin, A. Z. 2001. Alteration of the bone marrow in extracellular matrix in mice undernourished. Baz. J. Med. Biol. Res. 33: 889-895.
19. Xavier, J. G., Favero, M. E., Vinolo, M. A., Rogero, M. M., Dagli, M. L., Aranachavez, V. E., Borojevic, R. and Borelli, P. 2007. Protein-energy malnutrition alters histological and ultrastructural characteristics of the bone marrow and decreases haematopoiesis in adult mice. Histol. Histopathol. 22:651-660.
20. Metwalli, O. M., Galal, O. M. and Salem, S. I. 1978. Some aspects on liver enzymes in protein-energy malnutrition. Z. Ernähungswiss. 17: 206-209.
21. Davehport, D. J., Mostarda, R. A., Richardsoh, D. C., Gross, K. L., Greenej, K. L. and Blair, K. 1994. Protein-deficient diet alters serum alkaline phosphatase, bile acids, proteins and urea nitrogen in dogs. J. Nutr. 124: 2677S-2679S.
22. Osborne, C. A., Polzin, D. J., Abdullahi, S. U., Leininger, J. R., Clin ton, C. W. and Griffith, D. P. 1985. Struvite urolithiasis in animals and man: Formation, detection, and dissolution. Adv. Vet. Sci. 29: 1-101.
23. Ogawa, A., Yano, M., Tsujinaka, T., Ebisui, C., Morimoto, T., Kishibuchi, M. and Morita, J. F. S. *et al.* 1997. Gene expression of albumina and liver-specific nuclear transcription factores in liver of protein-deprived rats. J. Nutr. 127: 1328-32.
24. Marten, N. W., Sladek, F. M. and Straus, D. S. 1996. Effect of dietary protein restriction on liver transcription factores. Biochem. J. 317: 361-70.
25. Joshi, S., Garole, V., Daware, M., Girigosavi, S. and Rao, S. 2003. Maternal protein restriction before pregnancy affects vital organs of offspring in wistar rats. Metabolism. 52: 13-18.
26. Castro, G. S. F., Mialich, M. S., Anjos, M., Almeida, L. P., Arroyo, P. F., Portar, G. V. and Jordao Jr, A. A. 2009. Caracterização da esteatose hepática não alcoólica induzida por dieta hipoprotéica em ratos. Medicina (Ribeirão Preto). 42: 48-53.
27. Gonzàlez, R., Ancheta, O., Màrquez, M. and Rodriguez, S. 1994. Hepatoprotective effects of diethylcarbamazine in acute liver damage induced by carbon tetrachloride in rats. Zhongguo Yao Li Xue Bao. 15: 495-497.
28. Brooks, S. E., Goldon, M. H. and Taylor, E. 1992. Hepatic ultrastructure in children with protein-energy malnutrition. West Indian Med. J. 41:139-145.
29. Brooks, S. E., Doherty, J. F. and Golden, M. H. 1994. Peroxisomes and the hepatic pathology of childhood malnutrition. West Indian Med. J. 43:15-17.
30. Reeves, P. G., Nielsen, F. H. and Fahey, G. C. Jr. 1993. AIN-93 purified diets for laboratory rodents: final report of the American Institute of Nutrition ad hoc writing committee on the reformulation of the AIN-76A rodent diet. J. Nutr. 123:1939-1951.