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Hepatoprotective Effects of H20-Hydroxyecdysone in High-Fat/High Carbohydrate-Fed Gerbil

Efectos Hepatoprotectores de la 20-Hidroxiecdisona en Jerbos Alimentados con una Dieta Rica en Grasas y Carbohidratos

Nesrine Semiane[1]; Aicha Mallek[1]; Zineb Bellahreche[1]; Khadidja Mokeddem[2,3]; Ouahiba Sihali-Beloui[1] & Yasmina Dahmani[1]

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SUMMARY: The aim of this study was to determine the impact of an obesogenic diet on biochemical and histopathological changes in the liver of gerbils, and to evaluate the potential curative effects of 20-hydroxyecdysone (20E) in the same animal. Eighteen male gerbils were divided into three groups: a control group (C) fed a control diet (carrots and lettuce), obese group fed a high-calorie diet (HC) consists of a mixture of butter (20 %), barley flour (30 %) and dry dates (50 %) for 3 months and a third group (HC-20E) receiving the same obesogenic diet but supplemented with 20-hydroxyecdysone at a dose of 50 mg/kg body weight starting from the 8th week and continuing for 4 weeks. Compared to control animals, HC-fed gerbils showed metabolic disorders accompanied by a remodeling of liver tissue characterized by the development of steatosis, inflammation, fibrosis and enhanced oxidative stress, which are key contributors to NASH development. The present study revealed that 20E supplementation to the diet is responsible for hepatoprotective effects through abolished liver steatosis, markers of fibrosis and inflammation and through the inhibition of ROS and activation of antioxidant mechanisms. Our research suggests that 20E may represent a suitable natural compound that prevents progression to non-alcoholic steatohepatitis.

KEY WORDS: High-fat/high-carbohydrate diet; Gerbil; Non-alcoholic steatohepatitis; 20-Hydroxyecdysone.

INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) or metabolic-dysfunction- associated fatty liver disease (MAFLD), as it is now called, is one of the most common chronic liver diseases worldwide (Eslam *et al*., 2020). It is characterized by simple steatosis which can lead to steatohepatitis (with inflammatory infiltration) and progressive fibrosis and can progress to cirrhosis and ultimately to hepatocellular carcinoma (HCC) (Anstee *et al*., 2019). The development of NAFLD is fueled by a sedentary lifestyle, low frequency of physical activity, unhealthy diet and excessive calorie intake (Inoue *et al*., 2018). At present, several drug therapies have been recommended for the management of NAFLD, but none has exhibited sufficient efficacy on the entire scope of liver damage and their prolonged use may induce unfavorable effects (Petroni *et al*., 2021). Accordingly, thus, NAFLD medication discovery and development should preferably be shifted toward natural sources without side effects.

Ecdysteroids are polyhydroxylated steroids present in invertebrates and plants. 20-Hydroxyecdysone (20E) is the most common and the main biologically active compound of ecdysteroids. A number of studies have revealed many potential beneficial health effects of 20E in mammals, including the wound-healing, immunoprotective and anti-osteoporosis effects (Dinan & Lafont, 2006). Furthermore, it has been reported that 20E is involved in metabolic effects in cell-based and animal-based studies (Seidlova-Wuttke *et al*., 2010). The hypoglycemic (Chen *et al*., 2006) and anti-obesity effects (Kumar *et al*., 2013) of 20E have been demonstrated in HepG2 cells and obese rodents, respectively. However, relatively scarce literature is available on the properties of 20E as a treatment for multiple phenotypic characteristics of NASH. For this study, we used a nocturnal desert rodent, *Gerbillus gerbillus* a valuable natural model for human diet-induced metabolic disorders and NAFLD. The specific aim of this current study was to investigate the

[1] LBPO/ Nutrition & metabolism, Department of Biology and Physiology of Organisms, Faculty of Biological Sciences, University of Sciences and Technology Houari Boumediene (USTHB), Algiers, Algeria.
[2] Department of Pathological Anatomy and Cytology and Molecular Biology, Blida-1 University, Algeria.
[3] University of Health Sciences Algiers, Algeria.

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effects of 20E, extracted from Cyanotis vaga, on the main features of obesity induced NASH in *Gerbillus gerbillus* fed a high-fat/ high-carbohydrate diet.

MATERIAL AND METHOD

Animals and diets

Authorization to capture animals in the desert region was given by the Ministry of Higher Education and Scientific Research (Algeria). Capture, accommodation and all experimental procedures were authorized by the Institutional Animal Protection Committee of the Algerian National Administration of Higher Education and Scientific Research (DGRSDT; http://www.dgrsdt.dz). Permits and ethical rules were respected in accordance to the guidelines of the Local Institutional Animal Care Committee of USTHB University with decision number of CEEA-USTHB-2023/11119 and were supported by the Algerian Association of Experimental Animal Sciences (Agreement 2.2. Experimental protocol number 45/DGLPAG/ DVA.SDA.14).

The experiment included eighteen adult male gerbils (*Gerbillus gerbillus*), trapped in the semi-desert region of Béni-Abbès (Algeria). After acclimation to laboratory conditions in animal facilities (22°C, 50 %-relative air humidity and 12:12 hours light and dark cycles) during two weeks, gerbils were divided, individually, into three groups of six animals each:

- Group 1: control group (C) received a control diet (carrots, lettuce and few barley seeds) for 12 weeks, corresponding to a daily calorie intake of 10 kcal/animal
- Group 2: obese group fed a high-calorie diet (HC) consists of a mixture of butter (20 %), barley flour (30 %) and dry dates (50 %) compacted in croquettes corresponding to a daily calorific intake of 19 kcal/animal for 8 weeks to induce obesity
- Group 3: receiving the same obesogenic diet but supplemented with 20-hydroxyecdysone incorporated into the diet (HC-20E) at a dose of 50 mg/kg body weight, starting from the 8th week and continuing for 4 weeks.

The nutritional composition of this diet is described in Table I.

Sample Collection

At the end of the experimental period, blood was collected from the retro-orbital sinus of the eyes using Pasteur pipette. Blood samples were collected into EDTA-containing tubes. The plasma was immediately separated by centrifugation at 3000 rpm for 10 min at 4°C and rapidly stored at -20 °C until analysis. Animals were killed by decapitation; liver and different visceral adipose depots were isolated and weighed. Livers were sliced; one portion was fixed in a neutral formalin buffer solution and embedded in paraffin for histological analysis, and the rest were stored at -80°C for subsequent analysis.

Plasma biochemical parameters

Plasma concentrations of glucose, triglycerides and cholesterol were determined using commercial kits (Cypress Diagnostics, Belgium). Liver function was evaluated in the animals by measuring the plasma concentrations of alanine amino- transferase (ALT) and aspartate aminotransferase (AST) using commercial kits (Cypress Diagnostics, Belgium).

Table I: Composition of diets administered during the study to the different groups.

Nutritional composition g. 100 g^{-1}	High Fat and Carbohydrate diet			Natural diet	
	Barley	Dried date	Butter	Lettuce	Carrots
Carbohydrates	63.3	65	<1	2.3	6.6
Protein	11.2	2	0.7	0.7	0.8
Total fat	2.1	0.5	82.6	0.1	0.2
Water	12.2	20	15.6	95.1	89.4
Fibre	9.8	9	0	1.3	2.1
Energy (kcal)	314	275	717	15.5	36.3

Diet	High Fat and Carbohydrate Diet		Natural diet	
	100 g	Daily energy intake	100 g	Daily energy intake
Energy (kcal)	375	19	25	<10

Determination of TG concentration in liver

The hepatic lipids were extracted using the procedure developed by Folch *et al*. (1957). The concentrations of triglycerides in the hepatic lipid extracts were determined using enzymatic kits (Cypress Diagnostics, Belgium).

Histological analysis

Liver tissues were fixed in 10 % neutral-buffered formalin at room temperature for 24 h. Paraffin sections (5mm thick) were cut and deparaffinized for histologic analysis. Masson's trichrome staining was performed for overall morphology for the liver and Sirius Red staining was performed to visualize and quantify fibrosis. Images were obtained using an Olympus DX51 microscope, a high-resolution microscope camera (MA88–500: Pre-miere1, 5.0 Meg- apixels with a 1280 X 1024 resolution) at 400X magnification and captured using TSView 6.2.4.5 software.

Immunohistochemistry

Total macrophage infiltration and neutrophils were measured using the CD68 cell antigen and myeloperoxidase (MPO), respectively. Paraffin-embedded sections were deparaffinized and rehydrated in decreasing concentrations of alcohol. Antigen retrieval was performed in citrate buffer, pH 6.0 at 95˚C, for 20 min. Subsequently, endogenous peroxidase blocking was performed by immersion in 5 % diluted hydrogen peroxide solution for 10 min. Nonspecific binding was blocked by 30 min incubation with goat serum albumin in PBS (3 %). Slides were then incubated overnight at 4 °C with appropriate primary antibodies. The mouse anti human CD68 antibody (Invitrogen, Thermo Fisher Scientific, USA & Canada) was used in a dilution of 1:50. Rabbit polyclonal anti-myeloperoxidase antibody MPO (R & D Systems, Minneapolis, MN) was diluted 300-fold in PBS. Sections were then incubated for 1 hour at room temperature with secondary biotinylated antibody and, successively, with streptavidin–peroxidase (Dako, Denmark). The primary antibody-secondary antibody antigen complex was detected using DAB. The sections were counterstained with Mayer's hemalum for 10 s. Positively stained cells were counted from 10 -12 fields at 400X using Fiji ImageJ software.

Morphometric study

The diameter of the hepatocytes was performed, using an oil immersion objective, at 1000X magnification and included 300 cells selected from different fields on several slides. We manually delineated the cellular connector of each cell by following this path on Fiji/ImageJ: image deposit → Analysis → Set measurement → check area → Freehand selections to delineate the cell → Analysis → Measure.

Biomarkers of oxidative stress

Lipid peroxidation. The measurement of malondialdehyde (MDA) levels using the thiobarbituric acid reactive substances method was used to estimate lipid peroxidation in liver homogenates (Ohkawa *et al*., 1979).

Reduced glutathione (GSH). The thiol-specific reagent dithionitrobenzoic acid (DTNB) was used to analyze the liver GSH content using Ellman (1959) method. The sulfhydryl group of GSH is reacted with DTNB to create a product that is reduced by glutathione reductase to recycle GSH and generate more thionitro-benzoic acid (TNB) in this process. The rate of TNB production was dependent on GSH levels, which were measured in nanomoles/mg protein.

The evaluation of catalase activity was done using hydrogen peroxide decomposition. The latter was monitored at 240 nm for 10 min (Aebi, 1984). The enzyme activity was calculated as millimoles of H_2O_2 consumed min-1 mg-1 protein.

Superoxide dismutase (SOD). SOD activity was measured by inhibiting pyrogallol auto-oxidation. The rate of decrease in optical density at 420 nm is used to measure enzyme activity. One unit of enzyme was defined as the amount of enzyme required to produce 50 % inhibition of pyrogallol autoxidation under the assay conditions and expressed as U/mg protein (Marklund & Marklund, 1974).

The protein content of hepatic samples was determined according to the Bradford's method (Bradford, 1976), using bovine serum albumin (BSA) solution as a standard.

Statistical analysis

Data were statistically analyzed by performing a nonparametric Mann-Whitney test using Prism software (GraphPad Prism version 6.00) to compare experimental groups. Data were expressed as mean standard error of the mean (SEM) and considered significant at $P < 0.05$.

RESULTS

Effects of obesogenic diet and 20E on body weight, organs weight and metabolic profile

As shown in Table II, the body weight of gerbil that received high calorie (HC) diet increased significantly (p <

Table II. Effects of high calorie (HC) and 20-hydroxyecdysone (50 mg/kg body weight) supplement on body weight, organs weight and metabolic parameters.

	C (n=6)	HC (n=6)	HC-20E (n=6)
Body weight (g)	24,29 ± 1,12	29,63 ± 0,69 **	32,08 ± 2,14
Fat pads (mesenteric, perirenal and epididymal)	0,38 ± 0,08	0,81 ± 0,13	1,06 ± 0,16
Fat to body weight ratio (%)	1,55 ± 0,24	2,76 ± 0,48 *	3,06 ± 0,27
Liver weight (g)	0,87 ± 0,02	1,34 ± 0,10 **	1,17 ± 0,07
Liver to body weight ratio (%)	3,67 ± 0,20	4,51 ± 0,35 **	3,77 ± 0,37
Plasma glucose (mg/dl)	0,85 ± 0,07	1,93 ± 0,40 **	1,58 ± 0,17
Plasma triglycerides (mg/dl)	76,57 ± 4,16	163,69 ± 14,87 **	183,32 ± 14,81
Plasma cholesterol (mg/dl)	95,37 ± 9,49	122,70 ± 15,77	108,24 ± 9,02
AST (UI/L)	52,93 ± 10,69	113,42 ± 8,54 **	79,10 ± 12,86
ALT (UI/L)	18,28 ± 2,66	93,49 ± 16,01**	31,41 ± 5,32 ++

Data are presented as mean ± S.E.M. Values are considered significantly different at *P < 0.05, **P < 0.01 (HC vs control); ++p < 0.01 (HC-20E *vs.* HC). C: control animals, HC: animals submitted to high-calorie diet and HC-20E: animals subjected to high-calorie diet supplemented with 20-hydroxyecdysone (50 mg/kg body weight).

0.01) compared to gerbil fed with control diet (C). We also observed a significant difference in adiposity ($p < 0.05$) and relative weight of liver ($p < 0.01$) in animal fed HC diet when compared to the control animals. However, a slight decrease in the ratio of liver/body weight (- 10 %) was observed in animals receiving 20E (50mg/kg body weight) as compared to those fed HC diet. Furthermore, obesogenic diet-fed gerbil exhibited a significant increase ($p <0.01$) in blood glucose and triglycerides levels ($p <0.01$), without changes in cholesterol levels, as compared to gerbil fed control diet. 4 weeks-feeding of a high calorie diet supplemented with 20E (50mg/kg body weight) reduced slightly the levels of plasma glucose (- 18 %) but did not alter plasma triglyceride and cholesterol concentrations, compared to the non-supplemented high calorie diet.

We measured liver function by examining plasma ALT and AST. AST activities were increased significantly ($p<0.01$) in plasma of HC diet fed gerbils compared to the control gerbils which were normalized by 20E supplementation. ALT activities were also increased significantly in plasma of HC diet fed gerbils ($p<0.01$) compared to the control group of gerbils where the treatment with 20E (50mg/kg body weight) significantly normalized ALT activities (- 66 %).

Effect of obesogenic diet and 20E on liver pathology and immune cell infiltration

Examination of the Mason's trichrome-stained hepatic tissues showed that gerbils on the control diet displayed normal liver architecture (Fig. 1A, a-b), liver sections of HC group exhibited an obvious disarrangement of hepatic cells with steatosis, fibrosis and inflammatory cells infiltration (Fig. 1A, c-d). Liver sections of animals receiving 20E (50 mg/kg) showed reduced disarrangement of hepatic cells with a lack of lipid droplets and hepatocyte degeneration only restricted to cells surrounding the centrilobular vein, indicating a marked hepatotoxicity prevention of 20E (Fig. 1A, e-f). The morphometric analysis revealed a highly significant ($P<0.001$) increase in the mean diameter of the hepatocytes (hypertrophy) in the HC groups compared with in the control group. However, hypertrophy was markedly decreased in 20E (50 mg/kg) treated group compared with in the HC group (Fig. 1B). Likewise, the HC group showed significantly higher hepatic triglyceride content (Fig. 1C), and 20E supplementation significantly reduced this (- 69 %). These results suggest that 20E has an inhibitory effect on hepatic triglyceride accumulation, and therefore that it may protect against obesogenic diet-induced hepatic steatosis. Liver fibrosis was further confirmed by Sirius Red staining (Fig. 2A, a-c). As illustrated in Fig. 2B, HC gerbils had approximately 3-fold increase in Sirius Red–positive area when compared to the control group. As shown in Fig. 2B, treatment animals with 20E at a dose of 50 mg/kg markedly attenuated liver fibrosis, as shown by the reduced Sirius Red–positive area (- 66 %).

In order to confirm immune cell presence in the livers of obese gerbils fed with high calorie diet, immunochemistry staining was performed on tissue sections with a CD68 antibody (Fig. 2A, d-f). The CD68 staining assay showed that expression of CD68 was higher (+ 300 %) by obesogenic diet (Fig. 2C). Immune cell infiltrates appeared in the livers of all obese gerbils fed obesogenic diet. However, in tissues derived from HC-20E group, the CD68 positive staining was lower (- 45 %) than in those from HC animals group (Fig. 2C). To characterize the cells contributing to inflammation, we identified also neutrophils by immuno-histochemical detection of MPO (Fig. 2A, g-i). The percentage of MPO+ cells was markedly increased in obese gerbils compared with control animals (Fig. 2D). Neutrophils infiltrates appeared at the level of the central vein and an increased marking intensity at the sinusoids (Fig. 2A, h). However, in HC-20E

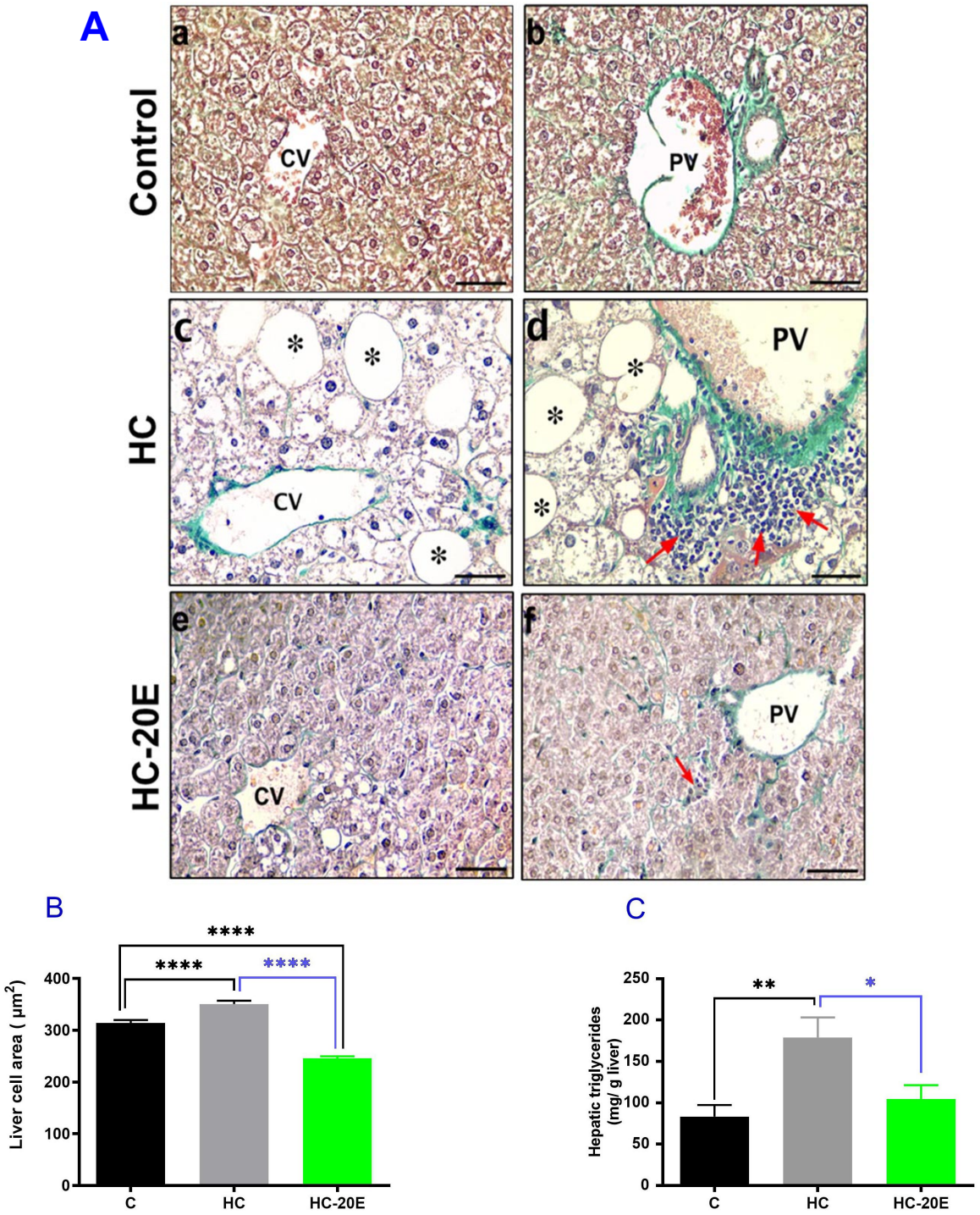

Fig. 1. Effects of high calorie (HC) diet without and with 20-hydroxyecdysone (50 mg/kg body weight) supplement on liver biology. **A**. Representative photomicrographs of livers stained with Masson's trichrome: control group shows normal liver architecture (a and b) with cords of hepatocytes radiating from the central vein (black arrows), whereas the HC group (c and d) demonstrates diffuse macrovesicular steatosis (asterisks), microvesicular steatosis, and inflammatory cells (red arrows). However, 20E (e and f) reduced macrovesicular steatosis (asterisks), microvisicular steatosis, and inflammation. Masson's trichrome staining (×400). Scale bar: 50 µm. **B.** Quantification of cell size (n = 3). **C.** Hepatic triglyceride content. (Data are mean ± SEM for (n= 6/group). *p < 0.05, **p < 0.01 and ****p < 0.0001 between the indicated groups. Abbreviations: PV: portal vein; CV: central vein C: control animals, HC: animals submitted to high-calorie diet and HC-20E: animals subjected to high-calorie diet supplemented with 20-hydroxyecdysone (50 mg/kg body weight).

group, there were marked decreases in the percentage of MPO+ cells (- 144 %) when compared to the HC group (Fig. 2D). These results suggest that 20E may attenuate immune cells infiltration and accordingly the inflammatory response.

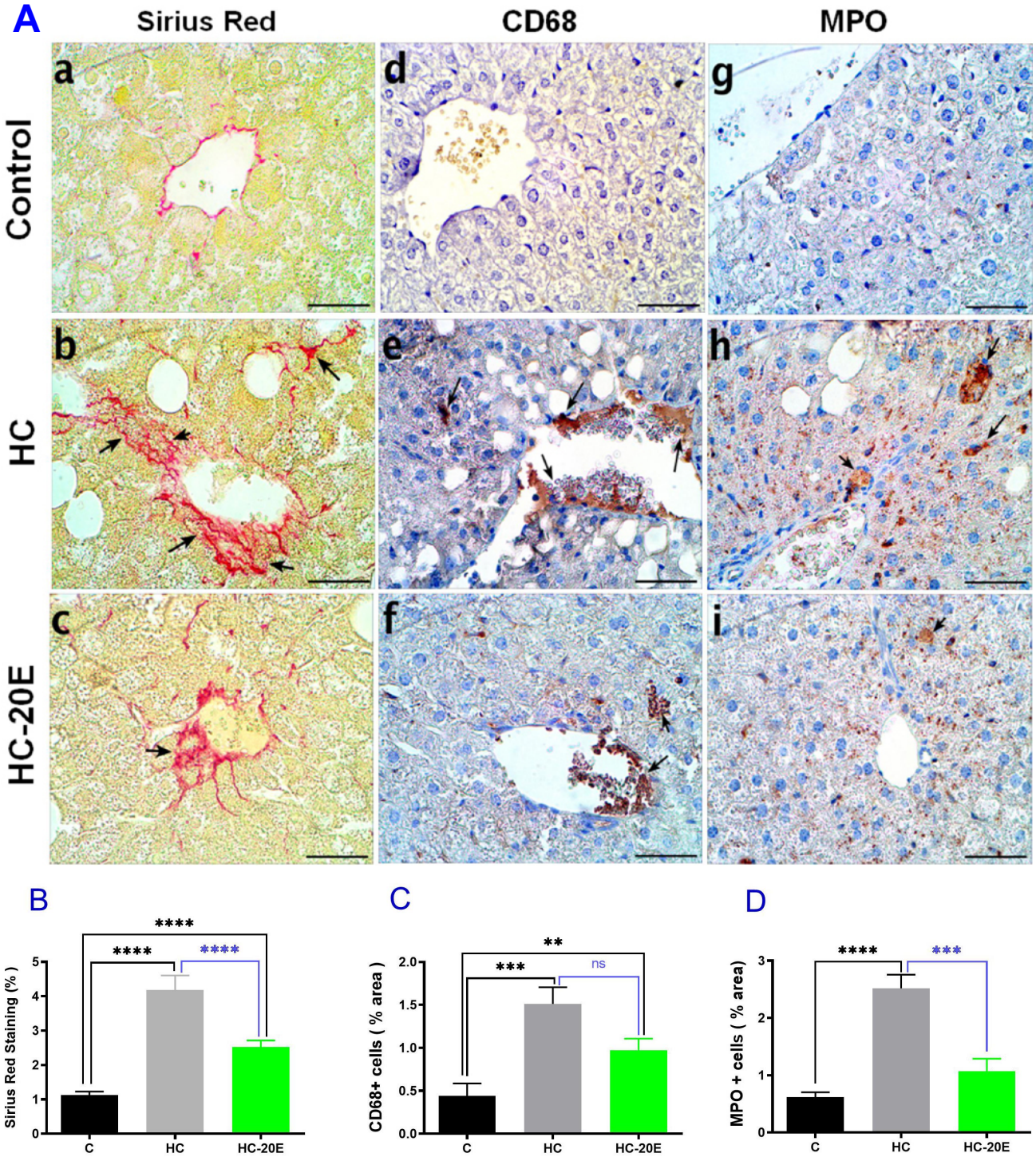

Fig. **2. A, a-c.** Histological sections of liver tissue stained with Picrosirius red. **A, d-f.** Immunohistochemical staining for inflammation marker CD68 in liver tissues. **A, g-i.** Immunohistochemical staining for inflammation marker MPO in liver tissues. Magnification, X400. Scale bar = 50 µm. Black arrows: immunopositive cells. **B.** Area percentage of Sirius Red–stained sections. **C.** Quantification of % area of CD68+ cells. **D.** Quantification of % area of MPO+ cells. (n = 3). Data are presented as mean ± SEM, **p < 0.01, ***p < 0.001 ****p < 0.0001 between the indicated groups. C: control; HC: high calorie diet; HC-20E: high calorie diet supplemented on 20-hydroxyecdysone.

Effect of obesogenic diet and 20E on oxidative stress

Oxidative stress, which may play a role in cellular injury, was measured by examining MDA and antioxidant levels in liver tissue homogenate. The results in all groups were shown in Fig. 3. The levels of lipid peroxidation products (hepatic MDA content) have been significantly increased in HC group (+ 97 %) compared to the control group. However, gerbils fed with obesogenic diet supplemented with 20E (50 mg/kg) showed significant reduction in MDA levels when compared with that of HC group (Fig. 3A). Non-enzymatic antioxidant such as GSH and antioxidant enzymes such as catalase and SOD were decreased significantly in HC group gerbils, compared to that of control group as shown in Figs. 3B-D. The HC-20E group gerbils showed marked increase in the levels of SOD (+ 75 %) but did not show a significant effect on the levels of catalase and GSH levels when compared to HC group animals.

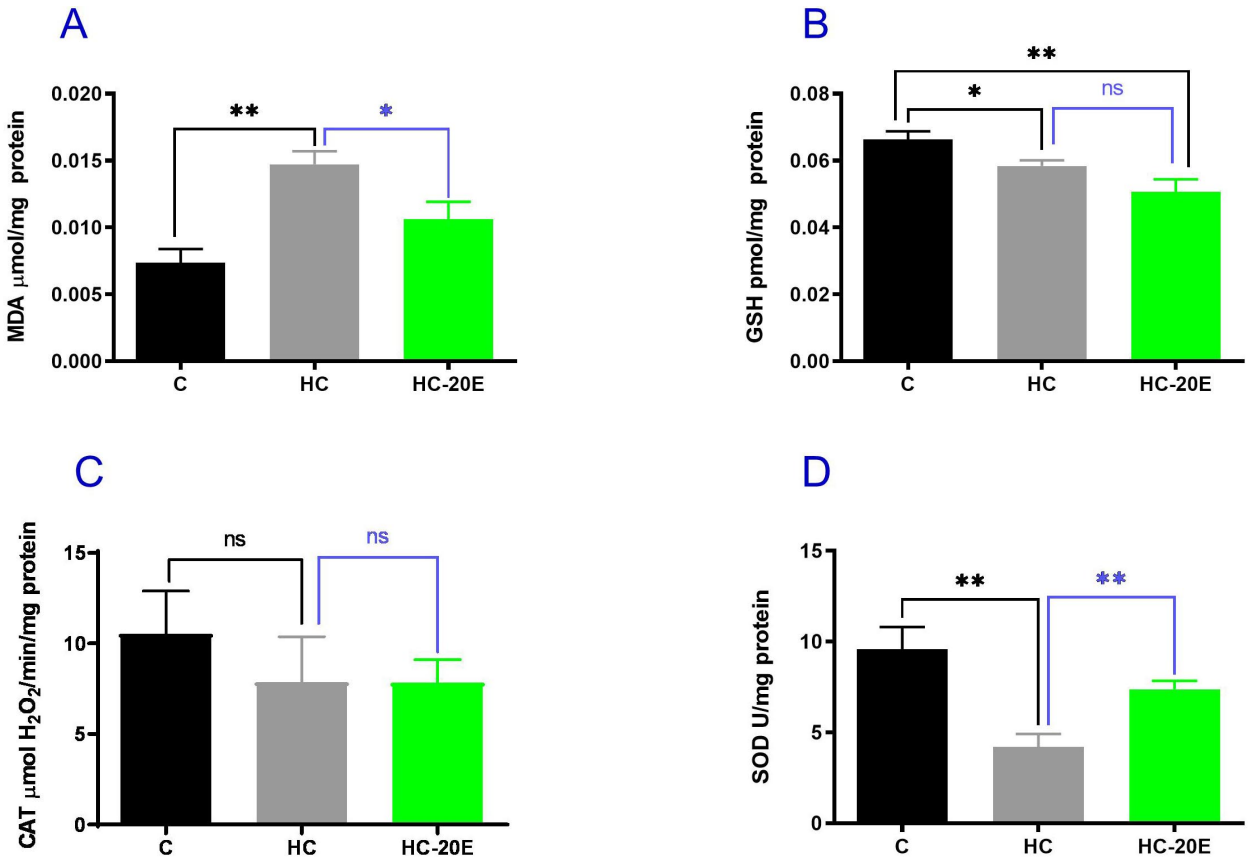

Fig. 3. Effects of high calorie (HC) diet without and with 20-hydroxyecdysone (50 mg/kg body weight) supplement on liver oxidative stress. (A): the levels of malondialdehyde (MDA); (B): the level of reduced glutathione (GSH); (C): the levels of catalase (CAT) and (D): the levels of superoxide dismutase (SOD). Statistically significant comparisons; NS: not significant, values are considered significantly different at * P< 0.05 and **< 0.01. C: control; HC: high calorie diet; HC-20E: high calorie diet supplemented on 20-hydroxyecdysone.

DISCUSSION

In this work, we have confirmed that *Gerbillus gerbillus* is a reliable biological support for the study of metabolic disorders such as obesity and obesity-related metabolic diseases (NAFLD), and whose the etiology is similar to its manifestation in humans. Our work shows that the desert gerbil, maintained on high fat/ high carbohydrate diet for 12 weeks, develops increased body weight, adiposity and liver/body weight ratio when compared with the levels in the control gerbils, thus confirming its sensitivity to caloric overload. In the present study, we also found that gerbils submitted to obesogenic diet showed hyperglycemia and significant increase in the

plasma TG level. Furthermore, obese gerbils showed abnormalities in ALT and AST levels compared with control animals. Our findings indicate that the high calorie diet was able to induce obesity-related NAFLD, in our experimental model. These findings are in agreement with several studies, which indicate that consumption of a high-fat, high-sugar (HFHS) diet is a major contributor to the incidence of obesity and related metabolic diseases (Gershuni *et al*., 2018), including non-alcoholic fatty liver disease (NAFLD). To assess the fatty liver disease, as the first step towards NAFLD, we first determined liver weight and hepatic triglyceride content in the livers from C and HC groups. Our findings showed a net increase in liver weight that was accompanied by a significant hepatic overload of triglycerides. The histopathological examination of the liver of these same gerbils allowed us to demonstrate significant micro and macro vacuolar steatosis, thus strengthening the quantitative analysis. Jointly with this fatty infiltration, histological examination of the gerbil liver submitted to obesogenic diet revealed signs of hypertrophy, inflammation and fibrosis in both interstitial and perivascular area. The hepatic fibro-inflammatory lesions observed in our obese gerbils led us to be interested in characterizing the type of inflammatory infiltrate using specific markers, namely CD68 (marks Kupffer cells and macrophages) and MPO (marks the neutrophils) on our liver sections. Our study's results enabled us to demonstrate a clear increase in the number of macrophages and neutrophils within the liver parenchyma of obese gerbil compared to the normal liver which contained very few cells positive for CD68 and for MPO. All these abnormalities were associated with increased fibrosis that has been identified as the most important predictor of prognosis in NAFLD patients, and therefore a main target in experimental pharmacological approaches (Heyens *et al*., 2021). This liver fibrosis is the consequence of an imbalance in the synthesis and degradation of the extracellular matrix (ECM). In the liver, hepatic stellate cells (HSCs), portal fibroblasts, and myofibroblasts cells are the major sources of ECM production (Lemoinne at al., 2013). These cells have been suggested to have pro-fibrogenic properties in the presence of over nutrition (Hoffmann *et al*., 2020).

In addition to liver fibrosis, it is well-established that oxidative stress is very important in the development and progression of NASH. Increased lipid peroxidation has been demonstrated in both animal models of fatty liver and patients with NASH. Free fatty acids (FFAs) are the likely source of oxidative stress within the liver in these patients. The products of FFA oxidation (hydrogen peroxide, superoxide anion, and lipid peroxides) are capable of generating oxidative stress and subsequent lipid peroxidation. It has also been shown that the balance between oxidative stress and antioxidant defense mechanisms may be impaired by depletion of enzymatic antioxidants and increased level of MDA in patients with NASH. For our part, we found that gerbils fed a HC diet exhibit greater liver oxidative stress that was evidenced by a significant increase in MDA (+ 97 %) and a significant reduction in SOD and GSH levels compared to that of control group, probably as a consequence of a greater production of ROS in HC group. SOD, the first line of defense against oxygen derived free radicals, converts superoxide anion into H_2O_2, forming as neutral products O_2 and H_2O. This could explain the depletion of hepatic SOD reserves in our obese gerbils. Reduced glutathione levels have been reported in NASH patients. Therefore, patients with NASH have an impaired ability to produce sufficient antioxidants. This may be related to decrease in three genes involved in ROS sequestration (Cu/Zn superoxide dismutase, glutathione peroxidase, and catalase) in cirrhosis secondary to NASH (Sreekumar *et al*., 2000).

Moreover, recent studies have shown that ferroptosis (a novel form of iron-dependent cell death), is involved in the initiation and progression of NAFLD (Ji *et al*., 2022) through mechanisms involving oxidative stress and inflammatory response. These findings suggest that ferroptosis is a possible mechanism involved in the pathogenesis of NASH in obese gerbil.

Our findings indicate that treatment of animals with 20E at a dose of 50 mg/kg body weight for 4 weeks did not affect body mass and does not exhibit lipid-lowering action as demonstrated by unaltered triglyceride and cholesterol plasmatic concentrations between obese gerbils fed with or without 20E. These results agree with those of (Buniam *et al*., 2020) which reported no effect of daily intragastric administration of different ecdysterone doses (5, 10, and 20 mg/kg body weight) for 8 weeks on serum triglyceride and cholesterol concentrations in female ovariectomised Sprague Dawley rats fed a high-fat/high-fructose diet. Furthermore, 20E administration was accompanied by reduced plasma glucose by 18 %. It has been reported that 20E improves hyperglycaemia by increasing hepatic glucose uptake and reduce hepatic glucose production by down- regulating PEPCK and G6Pase gene expression through the activation of the insulin-stimulated PI3K/Akt pathway (Sundaram *et al*., 2012).

In our study, 20E alleviated NAFLD in the obese gerbils, in fact this molecule significantly lowers liver-originated plasma enzymes (ALT and AST), hepatic

triglycerides level coupled with a marked reduction in liver weight and hepatic steatosis as shown in Trichrome Masson's stained sections. Red Sirius staining of liver sections also revealed that 20E significantly prevented fibrogenesis in HC diet fed animals. The marked reduction in liver weight and the improvement in fat deposition observed in liver tissue in the present study are in accordance with results of (Buniam *et al*., 2023), who have demonstrated that dietary supplementation with 20E increases lipid b-oxidation and reduces lipogenesis in the liver of ovariectomized rats fed a high-fat/high-fructose diet, thereby ameliorating hepatic steatosis in these rodents. According to the literature, the underlying mechanisms of 20E to mitigate hepatic steatosis may involve the activation of fibroblast growth factor 21 (FGF21), a metabolic hormone mainly expressed in the liver, as a metabolic regulator of lipid and energy homeostasis, which contributes to the suppression of de novo lipogenesis (DNL) via AMPK and acetyl-CoA carboxylase (ACC) pathway (Buniam *et al*., 2020). In addition, 20E has beneficial effects against oxidative stress induced by obesogenic diet as indicated by a significant decrease in hepatic MDA content coupled with an increase in activities of SOD. Superoxide Dismutases are key enzymes, which protect cells from oxidative stress by scavenging superoxide radicals through its dismutation into O2 and H2O2. Several Studies have found that 20E exhibits antioxidant properties by scavenging free radicals (Hu *et al*., 2012). It inhibited lipid peroxidation in rat liver and alleviated neurological deficits caused by subarachnoid hemorrhage in rabbits (Hu *et al*., 2010). 20E has also been shown to reduce oxidative stress-induced damage in B35 neuroblastoma cells by blocking reactive oxygen species (ROS) generation and restoring the cellular antioxidant potential (Hu *et al*., 2012).

CONCLUSION

Our study demonstrated that a high-fat/high-carbohydrate diet induces in gerbils, several metabolic disorders accompanied by a remodeling of liver tissue characterized by the development of inflammation, interstitial and perivascular fibrosis and by hepatocyte hypertrophy. These results collectively confirmed *Gerbillus gerbillus* as a good animal model for studies of NAFLD induced by obesogenic diet. The present study revealed that 20E supplementation to the diet is responsible for hepatoprotective effects through abolished liver steatosis, markers of fibrosis and inflammation and through the inhibition of ROS and activation of antioxidant mechanisms. Our research suggests that 20E may represent a suitable natural compound that prevents progression to non-alcoholic steatohepatitis.

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SEMIANE, N.; MALLEK, A.; BELLAHRECHE, Z.; MOKEDDEM, K.; SIHALI-BELOUI, O. & DAHMANI, Y. Efectos hepatoprotectores de la 20-hidroxiecdisona en jerbos alimentados con una dieta rica en grasas y carbohidratos. *Int. J. Morphol., 44(3)*:871-880, 2026.

RESUMEN: El objetivo de este estudio fue determinar el impacto de una dieta obesogénica en los cambios bioquímicos e histopatológicos del hígado de jerbos y evaluar los posibles efectos curativos de la 20-hidroxiecdisona (20E) en estos mismos animales. Dieciocho jerbos machos se dividieron en tres grupos: un grupo control (C) alimentado con una dieta de control (zanahorias y lechuga), un grupo obeso alimentado con una dieta alta en calorías (HC) que consiste en una mezcla de mantequilla (20 %), harina de cebada (30 %) y dátiles secos (50 %) durante 3 meses y un tercer grupo (HC-20E) que recibió la misma dieta obesogénica pero suplementada con 20-hidroxiecdisona a una dosis de 50 mg/kg de peso corporal a partir de la octava semana y continuando durante 4 semanas. En comparación con los animales control, los jerbos alimentados con HC mostraron trastornos metabólicos acompañados de una remodelación del tejido hepático caracterizada por el desarrollo de esteatosis, inflamación, fibrosis y un aumento del estrés oxidativo, que son contribuyentes clave al desarrollo de la EHNA. El presente estudio reveló que la suplementación con 20E en la dieta es responsable de los efectos hepatoprotectores a través de la eliminación de la esteatosis hepática, los marcadores de fibrosis e inflamación y a través de la inhibición de las ROS y la activación de los mecanismos antioxidantes. Nuestra investigación sugiere que la 20E podría ser un compuesto natural adecuado para prevenir la progresión a esteatohepatitis no alcohólica.

PALABRAS CLAVE: Dieta rica en grasas y carbohidratos; Jerbo; Esteatohepatitis no alcohólica; 20-Hidroxiecdisona.

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Corresponding author:
Nesrine Semiane
Department of Biology and Physiology of Organisms
Faculty of Biological Sciences
University of Sciences and Technology Houari Boumediene (USTHB)
BP 32
El Alia Bab Ezzouar 16111
Algiers
ALGERIA

E-mail: semianenesrine@yahoo.fr