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Restoration of liver function in malnourished mice orally administered with *Pleurotus ostreatus* fruiting bodies extract

[Restablecimiento de la función hepática en ratones malnutridos administrados oralmente con un extracto de cuerpos fructíferos de *Pleurotus ostreatus*]

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Abstract

Context: Malnutrition is considered worldwide as an important burden because it provokes serious damage in several physiological and metabolic mechanisms, among them the hepatic function. Currently, natural products are being used to reduce the negative impact of some pathological disorders in liver. *Pleurotus* genus of edible mushrooms has shown a wide spectrum of medicinal effects, but its role in the management of liver complications associated to malnutrition is not clear.

Aims: To evaluate the restoration of hepatic function in BALB/c malnourished mice, orally administered with a crude water extract (CW-P) from *Pleurotus ostreatus* fruiting bodies.

Methods: Animals (8-week old female BALB/c mice) were starved for 3 days and then refed with commercial diet supplemented with or without CW-P (100 mg/kg) for 8 days. Serum ALP, GGT and amylase activities, hepatic enzymes (ALT, AST, ALP, LDH, GGT), and liver histological examination were assayed.

Results: CW-P (34.3 % proteins, 42.6 % carbohydrates and 3.8 g/ 100 g of phenolics) administered to malnourished mice: (i) increased total serum proteins concentration that was correlated with the stimulation in liver protein anabolism, (ii) alleviated hepatic damage markers such as decrease serum ALP levels as well as, in ALP, GGT y AST activities in liver samples, and (iii) improved liver histological architecture similar to control group with decreased lipid accumulation.

Conclusions: CW-P supplementation favored liver restoration in malnourished mice. Nutritional interventions with this mushroom extract may be suggested to prevent liver complications associated to malnutrition.

Keywords: liver restoration; *Pleurotus ostreatus*; protein-energy malnutrition.

Resumen

Contexto: La malnutrición se considera un importante problema al provocar daños en varios mecanismos fisiológicos y metabólicos, entre ellos, la función hepática. Los productos naturales son utilizados para reducir el impacto negativo de ciertas patologías en el hígado. Las setas *Pleurotus* exhiben un amplio espectro de efectos medicinales; sin embargo, no está dilucidado su papel en el tratamiento de trastornos hepáticos asociados a la malnutrición.

Objetivos: Evaluar el restablecimiento de la función hepática en ratones malnutridos y administrados por vía oral con un extracto crudo de cuerpos fructíferos de *Pleurotus ostreatus* (CW-P).

Métodos: Ratones BALB/c hembras de 8 semanas fueron mantenidos en ayuno durante 3 días, y posteriormente alimentados por 8 días con dieta comercial suplementada o no con CW-P (100 mg/kg). Se evaluó la actividad de las enzimas ALP, GGT y amilasa en suero, de ALT, AST, ALP, LDH y GGT en hígado, y se realizó, además, el examen histológico del órgano.

Resultados: CW-P (proteínas: 34.3%, carbohidratos: 42.6% y fenoles totales: 3.8 g/100 g) administrado a ratones malnutridos: (i) incrementó la concentración de proteínas séricas, correlacionado con la estimulación del anabolismo proteico, (ii) redujo el daño hepático, evidenciado por la disminución de actividad ALP en suero, y ALP, GGT y AST en hígado, y (iii) regeneró la arquitectura histológica del hígado, similar al grupo control, con una disminución de la acumulación de lípidos.

Conclusiones: La suplementación con CW-P favoreció la restauración hepática en ratones malnutridos. La intervención nutricional con dicho extracto podría prevenir complicaciones hepáticas asociadas a la malnutrición.

Palabras Clave: Malnutrición proteico-energética; *Pleurotus ostreatus*; recuperación hepática.

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INTRODUCTION

Malnutrition is considered worldwide an important burden in many developing countries (Nabag et al., 2013; Philipson et al., 2014). It is well-documented that malnutrition provokes serious damage in several tissues and organs, thus affecting some important physiological and metabolic processes (Müller and Krawinkel, 2005). Malnutrition impairs both the immune and endocrine system, alters the gut architecture, the nutrients intake and also the liver function (Schaible and Kaufmann, 2007; Galdeano et al., 2011; Cheung et al., 2012; Rojas-Loureiro et al., 2017).

Liver plays an essential role in the synthesis of several serum proteins, antioxidant enzymes, and also it is involved in drugs and xenobiotics metabolism and biotransformation (Singh et al., 2011). In addition, it has been reported that accumulation of oxygen free radicals generated by normal and altered metabolic pathways may disturb the redox balance leading to hepatic failure (Xiang et al., 2018). For that reason, the maintenance of hepatic health highly improves the quality of life in patients with liver complications.

Nowadays, a plethora of studies are being focused to demonstrate the hepatoprotective effect of several biocompounds from plants and edible mushrooms. Many traditional preparations and herb formulations are available for the complementary treatment of liver disorders. A variety of bioactive compounds like polysaccharides, functional peptides and secondary metabolites, e.g. polyphenols, have shown to protect the hepatic function in acute liver injury induced by CCl_4, paracetamol and high-fat emulsion in rodent models (Pipelzadeh, 2013; Soares et al., 2013; Fiaz et al., 2017; Nchouwet et al., 2017).

Pleurotus sp. is an edible and medicinal mushroom with many nutritional and therapeutic effects. Different extracts and bio-substances from *Pleurotus* mushrooms have demonstrated the potential to combat cancer, diabetes and to reduce the oxidative stress (Gregori et al., 2007; Deepalakshmi and Mirunalini, 2014). In addition, both polyphenols enriched, and polysaccharide fractions derived from the oyster mushroom have exhibited hepatoprotective activity in animal models (Kumar et al., 2014; Dandapat et al., 2015; Zhao et al., 2017). Nevertheless, there is no pharmacological research focused to the recovery of liver function in malnourished mice administered with a mushroom fraction.

The present study was aimed to evaluate the restoration of the hepatic function in malnourished mice orally administered with a *Pleurotus ostreatus* fruiting bodies extract. The evidences obtained in this work may support the use of bioproducts from *Pleurotus ostreatus* as palliative of liver disorders associated to the nutritional status, and also to reduce the negative effects of toxicants.

MATERIAL AND METHODS

Pleurotus ostreatus (Jacq:Fr.) Kumm. strain and preparation of CW-P extract

In the study, a commercially cultivated strain of *Pleurotus ostreatus* (Pleurotaceae) (Code number: CCEBI-3024) deposited at the Culture Collection of the Centre of Studies for Industrial Biotechnology (CEBI, Cuba) was used. The strain was maintained on slants with solid medium of potato dextrose agar (PDA) incubated at 5°C. The taxonomic identification and authentication were confirmed by specialists of Eastern Centre of Ecosystems and Biodiversity (BIOECO, Santiago de Cuba, Cuba).

Pleurotus ostreatus (Jacq:Fr.) Kumm cultivation was performed by solid-state fermentation of mushroom spawn on pasteurized coffee pulp used as substrate in plastic bags of 2 kg (30 x 40 cm). The fruiting bodies (250 g) were harvested, carefully sliced into small pieces and were extracted with cold water at 15-20°C (3 mL per g of mushroom) on stirring (150 rpm) during 3 h. The suspension was filtered through sterile gauze and centrifuged at 3000 rpm for 10 minutes (HERMLE, Germany). The resulting extract (CW-P, 1.29% in terms of dried weight) was obtained at a yield of 3.78 g/100 g. Then, CW-P was filtered twice with 0.2 µm bacteriological filters, suspended in endo-

toxin-free ultra-pure water and kept frozen at -20°C until use.

Mycochemical assessment

Total carbohydrates and protein contents were determined by the phenol-sulphuric method (Dubois et al., 1956) and the Folin phenol reagent method (Lowry et al., 1951), respectively. Glucose ($y = 1.563x - 0.0608$, $R^2 = 0.994$) and bovine serum albumin (BSA) ($y = 0.3157x + 0.0053$, $R^2 = 0.999$) were used as standards for the calibration curves. Total polyphenols were estimated by the Folin-Ciocalteu reagent using gallic acid as standard ($y = 0.0098x - 0.1231$, $R^2 = 0.993$) (Slinkard and Singlenton, 1977). In addition, the extract CW-P was analyzed by HPLC on an Agilent 1200 series system with degasser, quaternary pump, automatic injection, thermostatic column compartment and a diode array detector (DAD) (Agilent Technologies, Eindhoven, Netherlands). The system was operated with a C18 Luna column (250 mm × 4.6 mm, 5 µm) from Phenomenex (Utrecht, The Netherlands) and gradient was performed with H_2O + 0.1% formic acid (A) and acetonitrile (B) under the following standard gradient to a flow rate of 1 mL/min: 0 - 5 min 5% B, 45-50 min 100% B, 55-60 min 5% B. The UV detection was carried out at 280 nm. Samples were prepared in 50% methanol at a concentration of 1 mg/mL

Animals and experimental design

BALB/c mice (female 8-week old) weighing 18–21 g, obtained from the Laboratory of Antibodies and Experimental Biomodels, Centre of Molecular Immunology (LABEX-CIM, Santiago de Cuba, Cuba) were randomly divided into four groups (n=6) and housed individually under standard laboratory conditions of 23 ± 2°C and 12 h light/12 h dark cycle. The animals were starved for three days with free access to salted water. A malnourished group (M) was killed after this period and the other animals were refed *ad libitum* for eight days with commercial pelleted diet (M-CD-Group) or with the commercial diet supplemented with CW-P (100 mg/kg of body weight per day, M-CW-P) (Llauradó et al., 2016). A no-malnourished control group (Control) was maintained with standard pelleted food diet (ALYco®, Havana, Cuba) and water *ad libitum*. Animal welfare and experimental procedures were approved by the Institutional Ethical Committee (Centre of Toxicology and Biomedicine, TOXIMED) and have been strictly performed in accordance with Cuban legislation and the EU Directive 2010/63/EU on the protection of animals used for scientific purposes.

Biochemical analysis

All animals were sacrificed for collection of whole blood and liver samples. Serum was prepared from collected blood samples and stored at -20°C until required. Total serum proteins were measured by the Biuret colorimetric assay, using bovine serum albumin as a standard.

Liver tissue was removed, immediately weighed and then homogenized in ice-cold 0.01 mol/L phosphate buffer saline pH 7.4 (1:3 w/v). Hepatic total protein content was determined by Lowry´s method (Lowry et al., 1951).

The serum (ALP, GGT and amylase) and hepatic (ALT, AST, ALP, LDH, GGT) enzyme activities were determined using an automatic biochemical analyzer (HITACHI COBAS C 311, Japan) with the standard enzymatic colorimetric tests according to the manufacturer's instructions (Roche Diagnostics, Indianapolis, IN).

Histological analysis

Histological studies were performed by fixing the hepatic tissue in 10% formalin and embedded in paraffin. Afterward, the samples were cut into slices and taken for staining with the hematoxylin-eosin coloration (0.5% hematoxylin and 1% eosin in ethanol 95%) and then visualized in an optical microscope (NOVEL, China) (x 400). Digital micrographs were taken by a coupled camera Canon, 8 MP (Japan).

Statistical analysis

All the results were statistically analyzed and expressed as the arithmetic means ± standard deviation (SD). The Kruskal-Wallis rank ANOVA test followed by the Student-Newman-Keuls test was applied to determine the significance of dif-

ferences between treatments. Differences at $p<0.05$ were accepted as significant. The software Statgraphics Centurion XV-I (Statistical Graphics Corporation, 2006) was used in all the analysis.

RESULTS

Mycochemical assessment

Pleurotus crude extract showed the presence of carbohydrates (42.6 %) and proteins (34.3%) as the main biochemical constituents. In addition, the level of total polyphenols in CW-P was 3.8 ± 0.49 g/100g.

A representative HPLC profile of the CW-P extract is showed in Fig. 1. A total of 8 peaks were eluted between 3 and 20 min (retention times) at 280 nm. However, only the peak with retention time of 15.21 min would be typical of phenolic compounds found in mushrooms, such as homogentisic acid (Hoan et al., 2014).

Body weight and biochemical serum determinations

After the starvation period statistically significant differences in body weight were found among groups. The animals lost about the 25% of the initial body weight from 20.6 ± 1.1 g up to 14.3 ± 0.8 g. Afterward, the groups identified as M-CD and M-CW-P, increasingly recovered the corporal mass during 8 days of repletion (20.0 ± 1.8 and 19.9 ± 0.9 g, respectively) similarly with control group (18.7 ± 1.2 g) ($p<0.05$). Previously, we demonstrated that no relevant differences on average daily and food consumptions were found between the groups after the re-feeding.

In general, the animals orally administered with CW-P extract exhibited higher values of serum proteins with respect to M-CD and control groups ($p<0.05$) (Table 1). The malnourished group showed a significant decreasing of serum total protein at the end of the starvation period ($p<0.05$) (Table 1).

The M group evidenced pathological signs with high levels of gamma-glutamyltransferase, a well-established serum marker of liver disorders. The serum values of GGT revealed no statistical differences between the animals refed with standard food diet and mushroom extract ($p<0.05$) (Table 1).

BALB/c mice administered with CW-P mushroom fraction displayed significant differences compared with the M-CD group and control animals in ALP activity ($p<0.05$) (Table 1). By the contrary, no relevant differences were exhibited in amylase activity.

Biochemical liver assessment

Liver weight showed no statistically significant differences among the experimental groups ($p<0.05$) (Table 2). Nevertheless, mushroom treatment resulted in a significant augment of total liver proteins compared to the animals fed only with the standard diet ($p<0.05$) (Table 2).

In the case of liver alkaline phosphatase, its activity was statically similar between the control and the M-CW-P groups and reduced compared to the M-CD group ($p<0.05$) (Table 2). On the other hand, GGT exhibited the lowest activities in CW-P treated mice ($p<0.05$) (Table 2).

The starvation period increased significantly the liver activities of AST, ALT and LDH in mice (M group) (Table 2) ($p<0.05$). A significant increment of the hepatic activities of these enzymes is often associated with the impairment of this organ. *Pleurotus* extract administered to BALB/c mice significantly restored AST enzyme compared with M-CD group. In the case of LDH and ALT enzymes no differences were found between animals re-nourished with standard diet or mushroom extract ($p<0.05$).

Histological observation

The histological examination of samples from malnourished mice exhibited a whiteness cytoplasm, micro vesicular changes, deterioration with partial necrosis and increment of lipid store in hepatocytes (Fig. 2). The histology assessment of mushroom extract treated mice showed a restoration of the liver architecture similar to that of control group evidenced by a colored cytoplasm and decreasing of lipid depots.

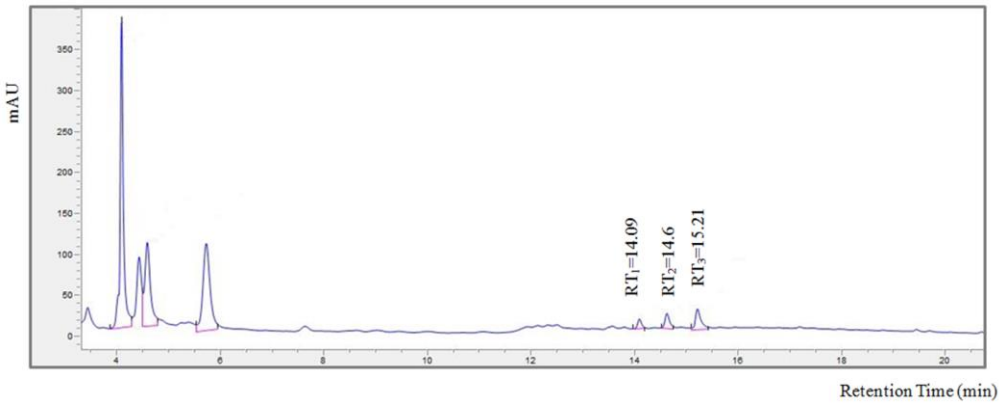

Figure 1. High-Performance Liquid Chromatography-Ultraviolet UV chromatogram of *Pleurotus ostreatus* mushroom extract.
All peaks (RT 1-RT2- RT3) were identified by the retention time.

Table 1. Serum biochemical assessment in malnourished mice treated or not with CW-P extract

Parameter	Control	M	M-CD	M-CW-P
Total serum proteins (g/dL)	5.9 ± 0.4^{b}	3.9 ± 0.2^{c}	6.2 ± 0.2^{b}	6.7 ± 0.1^{a}
GGT (U/L)	15.8 ± 6.2^{b}	41.5 ± 11.9^{a}	22.8 ± 1.7^{b}	23.1 ± 5.0^{b}
ALP (U/L)	148.2 ± 32.0^{b}	523.2 ± 24^{a}	133.8 ± 10.0^{b}	106.9 ± 8.0^{c}
Amylase (mmol/L)^{ns}	428.2 ± 9.0	522.8 ± 79.0	499 ± 44.0	477.0 ± 46.0

All values are expressed as the arithmetic mean ± SD (n= 6). (ns) No significant differences. Different letters in the same row indicate statistically significant differences according to the Kruskal–Wallis rank test followed by the Student–Newman–Keuls test ($p<0.05$).

Table 2. Liver biochemical assessment in malnourished mice treated or not with CW-P extract

Parameter	Control	M	M-CD	M-CW-P
Liver mass index (mg/100 g)	55.0 ± 7.0^{b}	46.5± 0.4^{a}	56.7 ± 6.6^{b}	55.2 ± 4.17^{b}
liver protein (mg/g)	74.0 ± 11.0^{b}	19.0 ± 3.0^{c}	78.0 ± 8.0^{b}	95.0 ± 14.0^{a}
ALP (U/L)	5.6 ± 1.5^{c}	160.4 ± 18.0^{a}	7.1 ± 0.5^{b}	5.1 ± 0.8^{c}
GGT (U/L)	12.1 ± 1.7^{bc}	16.7 ± 4.2^{a}	15.6 ± 3.4^{ab}	9.1 ± 3.4^{c}
AST (U/L)	4.9 ± 0.4^{b}	6.3 ± 1.1^{a}	7.4 ± 1.2^{a}	3.8 ± 0.6^{b}
ALT (U/L)	2.5 ± 0.7^{b}	5.7 ± 0.3^{a}	2.1 ± 0.4^{b}	2.3 ± 0.1^{b}
LDH (U/L)	4.1 ± 1.3^{c}	16.6 ± 3.4^{a}	7.3 ± 0.6^{b}	6.9 ± 0.6^{b}

All values are expressed as the arithmetic mean ± SD (n= 6). Different letters in the same row indicate statistically significant differences according to the Kruskal–Wallis rank test followed by the Student–Newman–Keuls test ($p<0.05$).

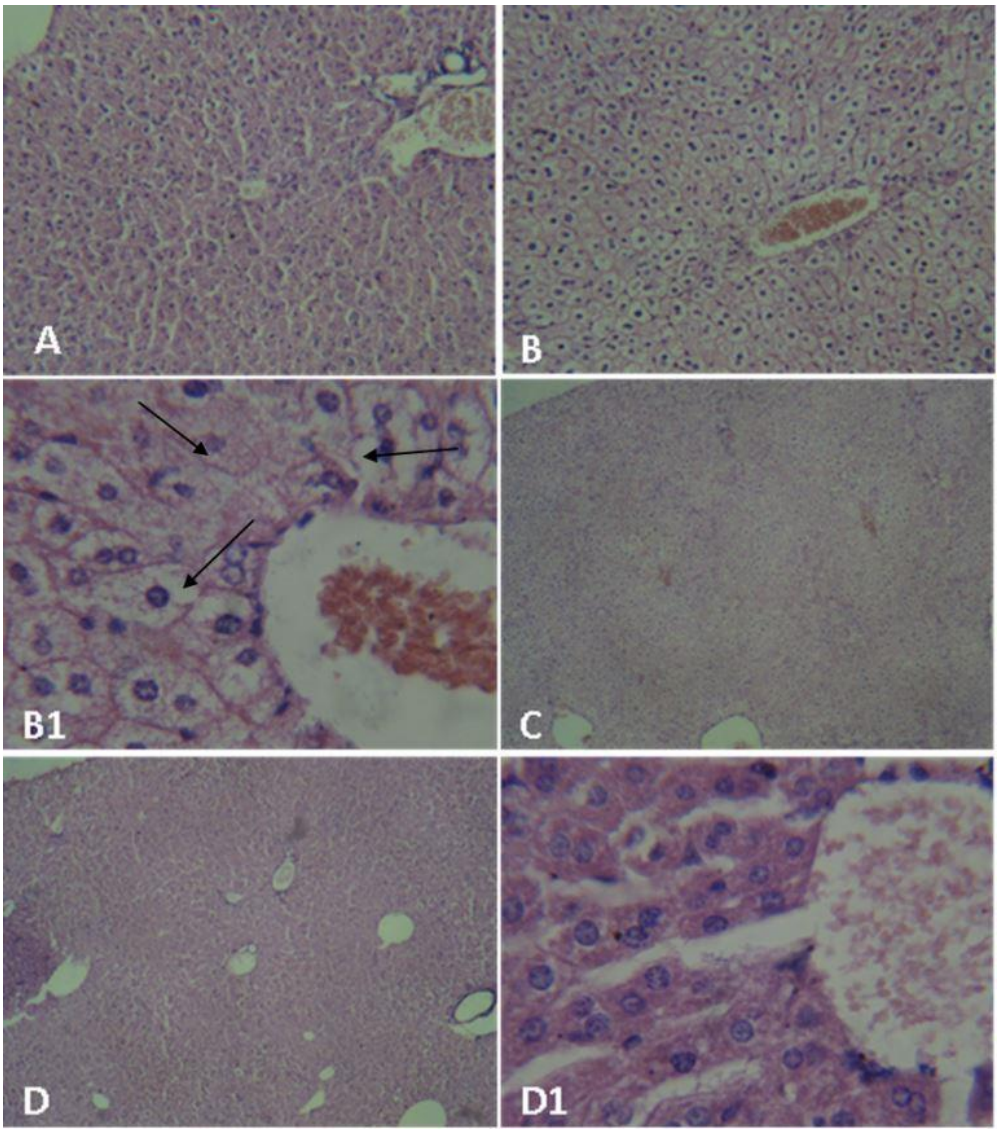

Figure 2. Optical micrographs of liver sections of malnourished BALB/c mice treated or not with CW-P.

Histological studies were performed fixing the hepatic tissue in 10% formalin, embedded in paraffin and staining with the hematoxylin-eosin coloration. Liver sections from mice in the control group (**A**, 40x) showing a normal coloration and architecture; malnutrition group (**B**, 40x; **B1**, 400x) showing with black arrows the whiteness cytoplasm, the micro vesicular changes in hepatocytes and increment of lipid store; M-CD group (**C**, 40x) shows a partial recovery of cell coloration; and mice orally treated with 100 mg/kg body weight CW-P (**D**, 40x; **D1**, 400x) exhibit a retrieving of cell shape, a colored cytoplasm and a diminished lipids store.

DISCUSSION

The ethno-mycological use of mushrooms to prevent and combat several diseases has a worldwide extended knowledge, mainly in oriental medicine (Wasser et al., 2014; Rathore et al., 2017). Many biopreparations and isolated compounds from mushrooms have demonstrated a wide spectrum of pharmacological effects focused for promoting human health, among them the hepatoprotective action (Soares et al., 2013). In this sense, several studies have validated the protective ef-

fects of mushrooms against experimentally induced liver damage. However, a little is known regarding the restoration of hepatic function in mice orally administered with a mushroom fraction after a starvation regime.

The most important therapeutic effects of mushrooms are generally attributed to natural polysaccharides, bioactive peptides and secondary metabolites like polyphenols (Rathore et al., 2017). These active substances have been investigated because of their stimulating effect on liver functions. Both polyphenols and polysaccharide-enriched alcoholic and aqueous fractions, derived from mycelium and fruiting bodies of mushrooms, have demonstrated to protect liver metabolism (Soares et al., 2013, Dandapat et al., 2015).

Pleurotus mushroom is considered an important source of high-quality proteins and healthy polysaccharides (Patel et al., 2012; Deepalakshmi and Mirunalini, 2014). In this work, CW-P showed similar amounts of total protein and polysaccharides compared to other study with *Pleurotus ostreatus* mushroom (protein and polysaccharides ranging between 17-42% and 37-48 %, respectively) (Khan, 2010).

El et al. (2009) and Jayakumar et al. (2007) studied the main components of *Pleurotus ostreatus* mushroom extracts with hepatoprotective potential. They finally proposed that β-D-(1→3)-glucans and other carbohydrates, as well as phenolic compounds and bioactive peptides may exert liver protection in carbon tetrachloride-induced toxicity.

Here, the polyphenol levels differ with a previous study (3.8 g/100g *vs* 5.49 g/100g) done by Jayakumar et al. (2011). However, a correlation between higher concentrations of polyphenols and the biological activity, e.g. antioxidant effect has not always found (Unekwu et al., 2014). These authors showed that *Lactarus deliciousus* mushroom exhibited a stronger DPPH scavenger activity with lower levels of polyphenols. Therefore, a detailed study should be conducted to explore the hepatoprotective effect of different concentrations of polyphenol fractions from *Pleurotus* mushroom in malnourished mice.

The results of the HPLC profile were contrasted with other reports and confirmed the presence in the CW-P extract of phenolics compounds. The pattern of water extract observed in the chromatogram was similar to other HPLC studies (Alam et al., 2010; Hoan et al., 2014). The peaks identified were analogous to standard reference compounds and *Pleurotus florida* phenolic compounds (Hoan et al., 2014) using similar chromatography conditions. Specifically, they reported homogentisic acid in the hot water extract from *Pleurotus florida* fruiting bodies, with similar retention time of our experiment. The homogentisic acid has been recently referred as a good candidate for 1-hydroxyethyl radical scavenger and presumable useful for liver protection after ethanol consumption (Medina et al., 2018). Nevertheless, a more comprehensive study is necessary to establish the main chemical constituents of extract from *Pleurotus ostreatus*.

The HPLC profile of CW-P extract can be also considered as a fingerprint spectra analysis for quality control of *Pleurotus* mushroom preparations. The importance of this tool as a platform to identify and corroborate the main or partial chemical composition of herb preparations (e.g. mushrooms bioproducts) has been referred (Liang et al., 2004; Xu et al., 2010; Hua-Bin et al., 2012). Mushrooms are mainly used as raw material for nutritional or functional formulations (e.g. dietary supplements). Key points to guarantee the quality control of dietary supplements derived from medicinal mushrooms, are emphasized, such as, the standardization of protocols for identification of bioactive substances, the production and testing of dietary supplements as well as their safety, regulation, efficacy and the elucidation of the mechanism of action (Wasser, 2014). The spectra obtained in this research matched with other published in different studies.

Malnutrition is a common complication in some chronic and non-chronic liver pathologies like cirrhosis, hepatitis, cancer and also by prolonged exposure to drugs or xenobiotic agents (Singh et al., 2011; Cheung et al., 2012). Several physiological and metabolic pathways are seriously damaged by a malnutrition event, thus justifying new ap-

proaches to protect and improve the hepatic function.

Natural products have emerged as complementary therapy to promote and maintain the liver functioning with great benefits (Pipelzadeh, 2013). In this sense, it has been well demonstrated that *Pleurotus* mushroom possesses a number of bioactive ingredients or substances able to exert nutritional and pharmacological effects in different metabolic processes, including the liver (Patel et al., 2012).

The nutritional assessment of liver functions comprises a thorough of anthropometric measurements and the evaluation of serum and liver markers.

The animals body mass was completely diminished after the starvation period. A gradually recuperation was observed in animals refed during eight days, but the mice treated with *Pleurotus ostreatus* did not show special body weight changes. No body mass variations were also evidenced in Wistar Albino rats supplemented with *Pleurotus florida* extract, orally administered during 30 days, after paracetamol induced liver damage (Sumy et al., 2010). The present study suggests that the most important metabolic changes may be associated to modifications in physiological functions and were not related with corporal variations.

In addition, the mushroom extract did not induce hepatomegaly as judged by the liver index weight. One of the most important criteria to choose hepatoprotective drugs/substances is the lack of toxic modifications in the liver (Ali et al., 2017). Mushrooms-based biopreparations that combat various diseases and also to support the nutritional status have a long ancient practice. In last years, the potential adverse effects of mushrooms formulations have been exhaustively studied through *in vitro* and *in vivo* experiments, and also by clinical trials (Valverde et al., 2015). Despite the available complete experimental data corroborating the nontoxic effects, some studies remain still uncompleted.

It is well known that a pathological change in total serum proteins is an underlying indicator of malnutrition status and usually implicated with liver impairment (Mandato et al., 2018). The increment of serum proteins by the administration of CW-P may suggest the restoration of liver functions and the protein biosynthesis in this organ. This finding was also corroborated by the augment of total hepatic protein, thus suggesting that protein synthesis was stimulated in animals supplemented with mushroom extract.

Other markers are useful to diagnose and monitoring the hepatotherapy in liver diseases (Sumanth et al., 2018). The evaluation of different serum and liver enzymes after hepatocellular injury may provide valuable information regarding the cell membrane permeability in protein-energy malnutrition models. The levels of serum alkaline phosphatase were reverted after administration of *Pleurotus* extract. The enzyme alkaline phosphatase is considered a significant serum analyte and its abnormal increment in serum and liver is often correlated with hepatic failure (Sharma et al., 2014).

By the contrary, non-effect was found in amylase enzyme activity in animals re-nourished with oyster mushroom extract. Amylase is secreted mainly by the salivary and pancreas, but a little amount is produced in liver and its assessment in serum has been recently reported as biomarker of liver pathological disorder (Afsartala et al., 2016). Nevertheless, further studies should be aimed to demonstrate the role of this marker in a malnutrition status.

In the case of GGT, the supplementation of CW-P to malnourished mice did not restore the serum levels of gamma-glutamyltransferase. Conversely, liver GGT concentration was re-established in BALB/c mice administered with CW-P extract. Here, a plausible explanation can be linked to the release of this enzyme and the timing of the starvation and re-nutrition periods. Although GGT is a sensitive marker of liver disease, there is a little information documented in mushrooms treated animals concerning this parameter. On the other hand, it has been referred that its efficacy as biomarker is limited due to lack of specificity (Sumanth et al., 2018).

Additionally, liver alkaline phosphatase was reduced in CW-P group after the repletion time.

AST and ALT activities are among the most important indicators to assess the liver function. Liver ailments are the most important cause of augmented transaminase activity (Singh et al., 2011).

The mycochemical components contained in *Pleurotus ostreatus* extract could exert an important reestablishment of hepatocyte functions. In this sense, CW-P restored the AST levels compared with the control groups. This finding is partially in accordance with some reports that referred the stabilization of cell membrane in hepatocytes associated with a lower serum ALT and AST activities. Major bioactive compounds like flavonoids, tannins and polysaccharides from *Pleurotus tuber-regium* and the wild mushroom *Termitomyces albuminosus* can exert the hepatoprotective effect (Dandapat et al., 2015, Zhao et al., 2017).

Although no effect was found in ALT activity in mice orally administered with *Pleurotus* extract, it has been described that alterations of ALT activity can persist longer that aspartate aminotransferase enzyme (Sumanth et al., 2018). This outcome justifies further studies to elucidate the behavior of aminotransferases activity in this model of protein-energy malnutrition.

Muthulingam et al. (2010) examined the levels of AST, ALT and ALP in a rodent model treated with Thioacetamide and orally administered with *Pleurotus florida*. They reported the hepatic protection conferred by mushroom and propose the synergic action of some mycochemical substances.

According to the results, we hypothesized that some phenolic compounds together with active polysaccharides may contribute to the repairing of hepatic tissue, thus having healing effect in liver dysfunction induced by malnutrition. This conclusion is supported by the histopathological examination that showed a recovery of liver cell architecture in mice treated with *Pleurotus* mushroom. The extract potentially reduced the disruption of hepatocytes and increased its regeneration evidenced by a color recovery and the normal shape of liver cells.

The therapeutic effect of edible and medicinal mushroom has been examined in several studies, mainly using animal models supplemented with the whole mushroom or with semi-purified and crude extracts derived from mycelium and fruiting bodies. The hepatoprotective actions have been mainly attributed to various classes of mycochemicals, among them, functional polysaccharides, bioactive peptides and especially low-molecular-weight compounds like triterpenoids and flavonoids (Soares et al., 2013). In most of the studies, the synergic effect of some mushroom compounds appears to be essential for the liver protection and for the reduction of oxygen free-radicals accumulation.

CONCLUSIONS

Pleurotus aqueous extract exhibited stimulating effects in some hepatic functions in animals seriously affected by a nutritional restriction period. The re-feeding with the mushroom formulation restored the liver protein biosynthesis and the enzyme activities, as well as the hepatic architecture. *Pleurotus ostreatus* bioproducts derived from fruiting bodies may represent a promising alternative as complementary intervention for liver diseases, together with an appropriate dietary or nutritional assessment. Mushroom-derived formulations open new possibilities to develop specific food/nutritional supplements and nutraceuticals with hepatoprotective effects.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Contribution	Llauradó G	Beltrán Y	Morris HJ	Marcos E	Díaz U	Marcos J	García J	Disotuar D	Cos P
Concepts or ideas	x	x	x						x
Design	x	x	x						x
Definition of intellectual content	x	x	x						
Literature search	x	x		x	x			x	
Experimental studies	x	x		x	x	x	x	x	
Data acquisition	x	x		x	x	x	x	x	
Data analysis	x	x		x		x		x	
Statistical analysis	x	x		x					
Manuscript preparation	x		x						
Manuscript editing	x		x						
Manuscript review	x	x	x	x	x	x	x	x	x

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