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Hepatoprotective Effects of Crude Phenolic-rich Extract from Oyster Mushroom (*Pleurotus ostreatus*)

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IN this study the hepatoprotective activity of the crude phenolic rich extract (CPRE) isolated from oyster mushrooms on carbon tetrachloride (CCl_4)-induced oxidative stress was investigated in albino rats. The hepatoprotective activity was examined through various biochemical parameters. Administration of CCl_4 for 28 days exhibited a significant increase (P<0.05) in serum markers of liver damage, i.e., alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, lactate dehydrogenase, urea, creatinine, total lipids and triglycerides. Whereas, the serum markers were significantly decreased (P<0.05) by CPRE administration. CCl_4 exposure significantly decreased (P<0.05) the hepatic antioxidant enzyme activities such as superoxide dismutase and glutathione peroxidase. Contrarily, the CPRE treatments showed significant increases (P<0.05) in these hepatic activities. The results obtained from this study clearly validated the potential antioxidant activity of CPRE isolated from oyster mushrooms against several oxidation systems *in-vivo*, which contributed to its hepatoprotective effects in CCl_4-induced liver injury in male albino rats. Finally, oyster mushrooms could be added as an additional nutrient to food products as it constitutes a new potential source of natural antioxidant and antibacterial agents.

Keywords: Oyster mushrooms, *Pleurotus ostreatus,* Phenolic compounds, CCl_4, Hepatoprotective, Oxidative stress.

Introduction

Free radicals like reactive halogenated hydrocarbons and oxygen species, etc., have been shown to adjust biological molecules, that might affect in several pathological conditions (Gupta et al., 1992 and Bhattacharya, 2015). Thus, the intake of antioxidants and other natural products as protective measures is being proposed. The studies on hepatoprotective experiential models have noted that CCl_4 intercede hepatotoxicity fundamentally acts on liver out of free radical-mediated practicability (Achuthan et al., 2003 and Jawad et al., 2017). Lately, precedent drugs have been used to treat chronic liver troubles, these drugs have often much side effects (Mahmoud et al., 2012). As it is major to search for appropriate normal drugs that could replace artificial drugs. The deficiency of effective new drugs for therapy chronic and acute liver injury (Vuda et al., 2012) has navigable the research into the hepatoprotective activity of many natural origins like medicinal plants (Taha and Osman, 2015), natural colorants (Ou et al., 2010) and simple or conjugated proteins (YU et al., 2012; Osman et al., 2019) using different experiential models. Lately, the expansion of new suitable antioxidant molecules is winning much concern since they play fundamental roles in prohibition or attenuating hepatotoxicity. Edible mushrooms are beneficial sanitary foods, having a wealthy source of vitamins, proteins, and minerals,

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especially in potassium and phosphorus. They are also low in calories and fats (León-Guzmán et al., 1997 and Öztürk et al., 2011). Oyster mushrooms (Pleurotus ostreatus) is regularly applied as raw active ingredients in the making of various dishes. Mushrooms have shown to have good health advantages (Lindequist et al., 2005) and they have proven to be efficient as antimicrobial, antitumor, antioxidant, anti-inflammatory, and antiviral articles (Dore et al., 2007; Chen et al., 2009 and Garcia-Lafuentea et al., 2010). Lately, they have been increasingly enchanting as functional foods due to their probability beneficial effects on human health. One of the main ingredients of oyster mushrooms is phenolic and flavonoid compounds (Reis et al., 2012). Phenolic compounds possess characteristic as antioxidants (Puttaraju et al., 2006). Hence, in the present study, the prospect hepatoprotective effects of crude phenolic-rich extract (CPRE) from Oyster Mushroom against CCl4-induced damage male albino rats was estimated.

Materials and Methods

Mushrooms

Oyster mushroom (*Pleurotus ostreatus*) was obtained from Horticulture Department, Faculty of Agriculture, Zagazig University, Egypt.

Crude phenolic-rich extract (CPRE) preparation

The Oyster mushroom (Pleurotus ostreatus) sample was lyophilized. Then, the lyophilized material was defatted by soaking in petroleum ether (10 % w/v) for overnight (Kavishree et al., 2008) and petroleum ether was removed from the sample by rotary evaporator under vacuum (BüCHI-water bath-B-480). A hundred grams mushrooms flour were extracted with methyl alcohol (1000 ml) using magnetic stirrer at 25 °C ±3 °C for 2 h, followed by filtration by filter paper Whatman No.1. Methyl alcohol was removed from the sample by rotary evaporator under vacuum. To eliminate methyl alcohol fully, the sample was re-dissolved in deionized water and filtered through a 0.20 µm filter followed by freeze-drying (Thermo- electron Corporation–Heto power dry LL 300 Freeze dryer). The freeze-dried CPRE was incubated in a -20 C to more analysis.

Total phenolic compounds estimation

The total phenolic compounds for the CPRE from oyster mushroom (10 mg in 10 ml distilled water) were evaluated by Foline-Ciocalteu reagent as observed in (Singleton et al., 1999). Gallic acid was used as standard at several concentration (20, 40, 80 120, 160 and 200 µg/ml) to prepare standard curve. The absorbance of sample and at standard curve was recorded at 765 nm.

Total flavonoids estimation

Total flavonoids for the CPRE from oyster mushroom (10 mg in 10 ml distilled water) were evaluated as described in (Ordonez et al., 2006). Quercetin was used as standard at several concentration (20, 40, 80 120, 160 and 200 µg/ml) to prepare standard curve. The absorbance of sample and at standard curve was recorded at 420 nm.

Antioxidants activity (DPPH-assay)

The antioxidant activity of CPRE was estimated by using DPPH (2, 2-diphenyl-1-picrylhydrazyl) assay according to (Hatano et al., 1988; Ramadan et al., 2008). 500 µl of each extract at different concentrations (100, 250, 500, 1000, 1500 and 2000 µg extract/1ml solvent) were added to 2500 µl of 0.1 mM DPPH dissolved in methanol. After incubation period of 30 min at 27 °C ± 3 °C, the absorbance was recorded with the control at 517 nm. The antioxidant potential of DPPH radicals (%) was studied as follow:

Inhibition (%) = [(Abs control-Abs sample)/Abs control] x 100

Where Abs. control is the absorbance of the control and Abs. sample is the absorbance in the presence of mushroom extract.

Animals and biological experimental design

The proceedings of the biological experience got the concent of the institutional Animal Care and Use Committee of Zagazig University (ZU-IACUC). Twenty male Wistar albino rats (140-160 ±10 g body weight) were used in the current study. The rats were gained from the Faculty of Veterinary Medicine, Zagazig Univerisity (Zagazig, Egypt) and stay in plastic cages under 25 ±1 °C with alternating periods of lighting and dark of 12 h period (El-Saadany et al., 1991 and Sitohy et al., 2013). The animals were fed on essential feed as described in AIN-93 guidelines (Reeves et al., 1993) and were provided with water ad libitum through the experiential time. The rats were at random split into four groups (5 rats/group) as described following:

- Group 1: normal control (NC).
- Group 2 was received intraperitoneal (IP) injection with single dose of 0.5 ml/kg body weight (50 % CCl_4/corn oil) and kept as positive control (PC).

- Groups 3 and 4 received intraperitoneal (IP) injection with single dose of 0.5 ml/kg body weight (50 % CCl_4/corn oil) + 200 or 400 mg/Kg body weight, respectively.

All groups were kept for 28 days.

Biochemical parameters estimation

Blood samples were obtained at the end of the experiment. Serum was isolated by centrifugation at 3000 xg for 10 min. Serum was applied to explore the biochemical parameters of inclusive liver and kidney functions and serum lipid profile. Serum AST, ALT, ALP and LDH (aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, and lactate dehydrogenase). Also, urea, creatinine, total proteins, albumin, total lipids and triglycerides were estimated assessed according to the manufacturer's protocol from Diagnostic kits.

Antioxidant enzymes

Immediately, the liver was washed with frosted saline to take off surplus blood. Potassium phosphate saline (0.1 M, pH 7.4) was used to the homogenization of liver tissue (1:9 w/v). Then, the suspension was centrifuged at 3000 xg for 15 min at 4 ℃. Then, the gained supernatant was estimated for antioxidant biomarkers such as SOD, CAT, MDA, and GSH (superoxide dismutase, catalase, malondialdehyde, and glutathione) were estimated as described in the manufacturer's protocol from Diagnostic kits.

Statistical analyses

Data were subjected to ANOVA and statistical analyses using the statistical software SPSS 11.0 (SPSS Ltd., Surrey, UK

Results and Discussion

Crude phenolic rich extract characterization

Total phenolic and total flavonoid compounds for crude phenolic rich extract isolated from oyster mushrooms were recorded 56 ±1.5 mg GAE/g extract and 23 mg QE/g extract, respectively (data not shown).

Antioxidants activity (% inhibition) for CPRE isolated from Oyster Mushroom using the DPPH assay is presented in Table 1. Oyster Mushroom was the species that presented the highest radical scavenging activity. These results compatible with our results recorded in total phenolic compounds. It can be noted that the antioxidant activity of CPRE isolated from Oyster Mushroom increased gradually with increasing concentration of TPCs and TFCs. These results are in agreement with results obtained by Chirinang and Intarapichet, 2009; Tsai et al., 2009 and Oke and Aslim, 2011).

TABLE 1. Antioxidants activity (inhibition %) for CPRE from Oyster Mushroom using DPPH assay.

Sample	Antioxidants activity (inhibition %) / Concentration (µg/ml)				
	100	500	1000	1500	2000
CPRE	27 ±1.4	36 ±2.0	40 ±2.0	55 ±1.7	76 ±2.8

Effects of CPRE on blood serum enzymes

The effect of CPRE administration on serum enzymes; ALT, AST, ALP, and LDH, is recorded in Table 2. The serum levels of ALT, AST, ALP and LDH in the positive control group was significantly ($P<0.05$) increased compared to the normal control group . The levels of ALT, AST, ALP, and LDH in PC were recorded 116 ±8.3, 122 ±6.3, 185 ±7.2and 568 ±12U/L, respectively. Whereas, these values decreased by almost more than twice in the NC (32 ±5.3, 55 ±4.5, 78 ±4.1 and 189 ±8.7U/L, respectively). In comparison with the PC, CPRE groups (200 and 400 mg/Kg body weight) exhibited a significant ($P<0.05$) reduction in ALT, AST, ALP and LDH levels (68 ±4.2, 65 ±3.9, 112 ±6.2and 265 ±10U/L in group was recevied 200 mg/Kg body weight; 47 ±3.2, 49 ±2.5, 79 ±4.8and 215 ±8U/L in group was recevied 200 mg/Kg body weight, respectively). Serum hepatobiliary enzymes such as AST, ALT, ALP and LDH are present in high concentrations in the liver under normal conditions. When there is hepatocyte necrosis or membrane damage, these enzymes will be released into the circulation, as indicated by elevated serum enzyme levels (Drotman and Lawhorn, 1978). This increase in the serum AST, ALT and ALP enzyme levels in CCl_4-treated animals indicates hepatic cell damage (Wolf, 1999). CPRE admisntration signific-ntly reduced the AST, ALT, ALP, and LDH activities in the blood serum, indicating that BSMRH has protection effects against CCl_4 induced acute liver injury.

TABLE 2. Effect of crude phenolic rich extract (CPRE) isolated from oyster mushrooms at different levels (200 and 400 mg / Kg body weight/day) on the activities of ALT, AST, ALP and LDH.

Groups	Serum enzymes activities (U/L)			
	ALT	AST	ALP	LDH
NC*	32 ±5.3[d]	55 ±4.5[c]	78 ±4.1[c]	189 ±8.7[c]
PC**	116 ±8.3[a]	122 ±6.3[a]	185 ±7.2[a]	568 ±12[a]
CPRE-200	68 ±4.2[b]	65 ±3.9[b]	112 ±6.2[b]	265 ±10[b]
CPRE-400	47 ±3.2[c]	49 ±2.5[c]	79 ±4.8[c]	215±8[c]

* Negative control (NC), **Positive control (PC) (CCl_4-treated rats).

Effects of CPRE on kidney function

The data in Table 3 represent the changes in levels of urea and creatinine. The serum levels of urea and creatinine in the PC was significantly (P<0.05) increased compared to the NC. The levels of urea and creatinin in the NC group recorded 44 ±1.3, and 0.39 ±0.004 mg/dL, respectively, whereas it increased by almost 3 times in the PC to reach values of 96 ±4.6, and 0.89 ±0.006 mg/dL, respectively. The CPRE treatments (200 and 400 mg/kg body weight) showed significant (P<0.05) lower levels of urea and creatinin than those of the PC. The reducing effect of CPRE on urea and cratinine contents, as well as on proteins and lipids profiles is in accordance with El-Hadary (El-Hadary and Ramadan Hassanien, 2016).

Effect of CPRE on protein and lipids profiles

The comparison between four expermintal rat groups in total protein and albumin levels in blood serum is shownin Table 4. The values of total protein and albumin in PC group were significantly (P<0.05) decreased compared to the NC group (group 1). The levels of total protein and albumin in the PC group were recorded 6.2 ±0.11 and 3.8 ±0.08 g/dL, respectively. It is worth noting that the total protein and albumin values in CPRE groups were signicantly (P<0.05) higher than those of PC group and exhibited no significance (P<0.05) in total protein content with that of the NC group.

With respect to the lipid profile, it is clear from the data shown in Table 5 that the levels of total lipids and triglycerides in PC group were significantly (P<0.05) higher than those of other expermintal groups. The values of total lipids and triglycerides in PC group were 711 ±20, and 288 ±10 mg/dL, respectively, whereas, in NC group it recorded 497 ±13, and 180 ±8 mg/dL, respectively. In this context, it should be noted that the groups of CPRE admistration showed low values of the total lipid and triglycerides and exhibited no significance (P>0.05) with those of the NC group.

TABLE 3. Effect of crude phenolic rich extract (CPRE) isolated from oyster mushrooms at different levels (200 and 400 mg / Kg body weight/day) on levels of urea and creatinine.

Groups	Urea (mg/dl)	Creatinine (mg/dl)
NC*	44 ±1.3[d]	0.39 ±0.004[c]
PC**	96 ±4.6[a]	0.89 ±0.006[a]
CPRE-200	55 ±3.8[b]	0.64 ±0.004[b]
CPRE-400	47 ±2.1[c]	0.39 ±0.005[c]

TABLE 4. Effect of crude phenolic rich extract (CPRE) isolated from oyster mushrooms at different levels (200 and 400 mg / Kg body weight/day) on levels of total protein and albumin.

Groups	Concentration (g/dl)	
	Total protein	Albumin
NC*	7.4 ±0.11 [a]	4.7 ±0.10 [b]
PC**	6.2 ±0.11 [b]	3.8 ±0.0.8 [c]
CPRE-200	6.9 ±0.12 [a]	4.6 ±0.09 [b]
CPRE-400	6.8 ±0.14 [a]	5.1 ±0.11 [a]

.(Negative control (NC), **Positive control (PC) (CCl_4-treated rats *

TABLE 5. Effect of crude phenolic rich extract (CPRE) isolated from oyster mushrooms at different levels (200 and 400 mg / Kg body weight/day) on levels of total lipids and triglycerides.

Groups	Concentration (mg/dl)	
	Total lipids	Triglycerides
NC*	497 ±13 [b]	180 ±8 [b]
PC**	711 ±20 [a]	288 ±10 [a]
CPRE-200	509 ±16 [b]	193 ±9 [b]
CPRE-400	479 ±21 [b]	185 ±5 [b]

* Negative control (NC), **Positive control (PC) (CCl_4-treated rats).

Effect of CPRE on antioxidant biomarkers

The data in Table 6 represent the changes in levels of some oxidative stress parameters in liver of male albino rats. As can be seen in Table 6, the NC group showed a significant (P<0.05) higher values of SOD and CAT (13 ±0.12 and 14 ±0.3 U/ mg protein, respectively) that were almost twice compared to those in PC group (6 ±0.14 and 6.6 ±0.5 U/ mg protein, respectively. In addition, CPRE groups exhibited a significant (P<0.05) increase in SOD and CAT values (10 ±0.13 and 10 ±0.5 U/ mg protein for group recevied 200 mg; 15 ±0.15 and 13 ±0.2 U/ mg protein for group recevied 400 mg) coared to the PC group. The MDA levels, determined as the main degradation product of lipid peroxidation in liver tissues, were significantly (P< 0.05) higher in PC rats compared to those of the NC group. The treatment with both levels of CPRE showed a significant (P< 0.05) reduction in MDA level. It is worth noting that CPRE (400 mg) exhibited no significancy (P> 0.05) with the MDA of NC group. Reduced glutathione (GSH), a natural antioxidant in liver tissue, showed similar trend of MDA result. The PC group exhibited the lowest GSH values among the expermintal groups. Among the CPRE groups, which exhibited significanlty (P< 0.05) higher GSH than that in NC group, group was recevied 400 mg CPRE obtained the highest GSH value and showed no sifnicanlty different from NC group. The level of MDA is an indicator of oxidative damage and cell injuries, and one of the principal products of lipid peroxidation (Lee et al., 2004). Free radical scavenging is one of the main antioxidation mechanisms inhibiting the chain reaction of lipid peroxidation (Vuda et al., 2012). In the current study, BSMRH admisntration significantly reduced the lipid peroxidation by decreasing the MDA levels, confirming the free radical scavenging activity of BSMRH in-vivo conditions as reported in our prvious study (Abdel-Hamid et al., 2017). GSH is a critical biomarker for tissue susceptibility to oxidative damage. The treatment with both levels of BSMRH increased GSH to levels like negative control group, which could be attributed to the antioxidants activity of BSMRH. Enzymatic antioxidant including SOD, CAT, and a non-enzymatic antioxidant GSH protected a balanced of redox status (Athmouni et al., 2018). SOD can convert superoxide anions into hydrogen peroxide; CAT can catalyze the breakdown of hydrogen peroxide to generate nontoxic molecular oxygen and water (Liu et al., 2015). GSH combine with glutathione-s-transferase in scavenging free radicals and/or detoxifying enzyme glutathione peroxidase at expense of reduced glutathione (Messaoudi et al., 2010; Bargougui et al., 2019).

TABLE 6. Effect of crude phenolic rich extract (CPRE) isolated from oyster mushrooms at different levels (200 and 400 mg / Kg body weight/day) on some oxidative stress parameters in liver of male albino rats such as SOD, CAT, MDA and GSH.

Groups	SOD (U/mg protein)	CAT (U/mg protein)	MDA (nmol/mg protein)	GSH (mg/mg protein)
NC*	13 ±0.12 [a]	14 ±0.3 [a]	4.2 ±0.23 [c]	7.4 ±0.2 [a]
PC**	6 ±0.14 [d]	6.6 ±0.5 [d]	12.12 ±0.32 [a]	4.35 ±0.4 [c]
CPRE-200	10 ±0.13 [c]	10 ±0.5 [c]	6.8 ±0.35 [b]	5.6 ±0.3 [b]
CPRE-400	15 ±0.15 [b]	13 ±0.2 [b]	4.6 ±0.28 [c]	6.7±0.4 [a]

* Negative control (NC), **Positive control (PC) (CCl_4-treated rats).

Conclusions

The results obtained from this study clearly validated the potential antioxidant activity of CPRE isolated from oyster mushrooms against several oxidation systems *in-vivo*, which contributed to its hepatoprotective effects of CPRE in CCl_4-induced liver injury in male albino rats. The crude phenolic rich extract isolated from oyster mushrooms can be recommended for food and health applications aiming at reduction prospect oxidative stress.

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تأثر نشاط الكبد بالمستخلص الفينولى لفطر عيش الغراب المحارى

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فى الدراسة الحالية تم تقييم تأثير مستخلص عيش الغراب المحارى الغنى بالمركبات الفينولية على الاجهاد التأكسدى فى فئران التجارب نتيجة تجريعها برابع كلوريد الكربون (مدة التجربة ٨٢ يوم). تم تتبع التأثير على نشاط الكبد فى المعاملات من خلال قياس نشاط الانزيمات واليوريا والكرياتينينوكذلك الليبيدات الكلية والجليسريدات الثلاثية. أظهرت المعامله برابع كلوريد الكربون زيادة معنوية فى نشاط بعض الانزيمات (الألانين أمينو ترانسفيريز، وأسبارتات أمينو ترانسفيريز، والفوسفاتيز القلوى، واللاكتات ديهيدروجينيز) بالاضافة الى اليوريا، والكرياتينين، والليبيدات الكلية، والجليسريدات الثلاثية. أدى تجريع الفئران بمستخلص عيش الغراب المحارى الغنى بالمركبات الفينولية الى خفض نشاط تلك الانزيمات واليوريا والكرياتينين والليبيدات الكلية والجليسريدات الثلاثية الى المستوى الطبيعى وذلك بالمقارنة بمجموعة الكنترول. على الجانب الأخر أظهرت المعامله برابع كلوريد الكربون انخفاض معنوى فى نشاط بعض الانزيمات (السوبراوكسيد ديسموتيز والجلوتاثيون بيروكسيديز). وعلى العكس فإن تجريع الفئران بمستخلص عيش الغراب المحارى الغنى بالمركبات الفينولية قد أظهرت زيادة ملحوظةفى نشاط تلك الانزيمات الى المستوى الطبيعى وذلك بالمقارنة بمجموعة الكنترول. تبرهن هذه الدراسة على أهمية النشاط التأكسدى لمستخلص عيش الغراب المحارى الغنى بالمركبات الفينولية ضد الاجهاد التأكسدى الذى تعرضت له الفئران نتيجه تجريعها برابع كلوريد الكربون ولذلك ينصح باستخدام فطر عيش الغراب المحارى كاضافات للمنتجات الغذائية لمحتواها العالى من المركبات الفينولية ذات التأثير المضاد للأكسدة والمضاد للبكتيريا.