Egypt. J. Chem. Environ. Health, 6 (1):23-35 (2020)
On line ISSN: 2536-9164

Hepato Protective Effect of Dried MUSHROOM against CCL4 Induced Hepato Toxicity in Liver and Cytogenicity Change in Rats

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ABSTRACT

The present study was carried out to elucidate the Hepato protective effect of dried mushroom against carbon tetrachloride (CCl_4) induced hepato toxicity in liver and cytogenicity change in rats .A total of thirty adult male albino rats (150-200 g) were used in this study.Rats were divided into 6 groups each of 5 animals .Group one was kept as acontrol –ve and fed on basal ration only, while the two other groups were fed on basal ration mixed with grind dried mushroom at concentration 5 and 10 % for 30 successive days. Other three groups were subcutaneous injected of with CCl_4 (0.1 ml/100 g b.wt. for two weeks) to induced liver fibrosis and cytogenicity changes .One of these group was left as a control +ve (subcutaneous injection of CCl4), whereas the other two groups were fed on basal ration mixed with grind dried mushroom at concentration 5 and 10 % for 30 successive days.

At the end of experimental period blood samples were collected from each rat for biochemical analysis and cytogenicity. Subcutaneous injection of CCl4 caused significant increase in serum levels of AST,ALT,ALP,creatinine,urea, triglyceride,glucose and total cholesterol LDL. Subcutaneous injection of CCl4 caused significantly increased in micronucleated polychromatic erythrocytes (MPCEs) and ratio of polychromatic to normochromatic erythrocytes (PCE / NCC).Feeding on ration mixed with dried mushroom and injected CCl4 caused significant decreases of MPCEs if compared with control +ve group and decrease of AST and ALT when compared with control +ve group and directed toward normal. The result showed that feeding on dried mushroom showed statistical decrease in micro nucleated polychromatic erythrocytes (MPCEs) and significantly increased in normo chromatic erythrocytes (NCE) compared with control –ve group.

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INTRODUCTION

Mushrooms are edible fungus that can provide several important nutrients. The many kinds of mushroom have varying compositions and nutritional profiles. **(Barbie Cervoni 2020**) One cup of raw mushrooms contains only 15 calories and 2.3 grams of carbohydrate. Mushrooms are also a good source of fiber particularly the soluble fiber beta-glucan and provide a small amount of protein: 2.2 grams per cup. This is only 4% of your daily need. (**Nakashima et al 2018**) Mushrooms are full of micronutrients they are a good source of copper, niacin ,vitamin B3, vitamin B5,potassium, phosphorus and iron. In addition to the many vitamins and minerals mushrooms contain they have also been found to have high levels of some antioxidant compounds. All of these compounds can be beneficial to health. **(Chaturvedi et al 2018)**. Medicinal mushrooms have a long history of use in traditional oriental therapies and fungal metabolites are increasingly being used to treat a wide range of diseases . Moreover edible mushrooms rich in bioactive compound and many substances could have some biological activity. The long list includes polysaccharides, phenolics, proteins , small peptides and amino acids, nucleotides and nucleosides. (**Preeti et al ., 2012)** The antioxidant content in atherosclerosis, hypercholesterolemia mushrooms may help prevention of a range of disease, including cancer, hepatitis, , diabetes, dermatitis and have various health benefits according to the National Cancer Institute. **(Katherine Marengo., 2019)** Studies have demonstrated that the regular consumption of mushrooms or the consumption of isolated bioactive constituents present in mushrooms is beneficial to health. They are usually considered functional foods or nutra ceutical products **.(Anderia et al ., 2013)**

MATERIALS AND METHODS

Materials:

-Mushroom white button mushroom (shiitake "*Lentinus-edodes*):

Mushroom obtained from Local - market as dry grind before mixed with ration and kept in dry clean plastic bags.

- Carbon tetrachloride (CCl_4):

Carbon tetrachloride (99.9 purity) was purchased from Sigma Chemical Company. It used in (50ml Ccl_4/50 ml propylene glycol) twice/week, subcutaneously injection according to ***Borah et al.*, (2004)**.

-Animals:

Thirty mature white Albino rats (Sprague Dawley strain) of an average body weight 150-200 g were obtained from the laboratory of animal colony, Ministry of

Health and population, Helwan, Cairo, Egypt. Rats were fed on standard ration supplying the essential vitamins, trace elements and water supply was given adlibitum.

-Ration composition:

As outlined in the material and methods section, the ration was analyzed for their dietary values by determining the various parameters. Ration was free of aflatoxin.

- Methods:

Experimental design:

The experiment was done in the Pharmacology unit, Biochemistry, Toxicology and Food Deficiency Department, Animal Health Research Institute. Rates were housed in wire cages in a room temperature maintained at $25^{o}C\pm 2$ and kept under normal healthy conditions. All rats and food consumption weight every week for determination the body weight gain and food intake.

The rats were divided into (6) Groups of 5rats each as follow:

Group 1: Kept as control negative and fed on basal ration.

Group 2: Kept as control positive, fed on basal ration and was injected subcutaneously by (0.1 ml/100 g b.wt.) CCl_4 S/C twice week for two weeks

Group 3: Feed on basal ration mixed with grind dried mushroom at concentration 5% for 30 successive days.

Group 4: Feed on basal ration mixed with 10% grind dried mushroom for 30 successive days.

Group 5: Feed on basal ration mixed with grind dried mushroom at concentration 5% for 30 successive days and at same time injected subcutaneously by (0.1 ml/100 g b.wt.) CCL4 S/C twice week for two weeks.

Group 6: Feed on basal ration mixed with grind dried mushroom at concentration 10% for 30 successive days and at same time injected subcutaneously by (0.1 ml/100 g b.wt.) CCL_4 S/C twice week for two weeks.

Table (1) shows the result of ration analysis which used in feeding of rats. Ration was free of aflatoxin.

Composition of ration (g/100g)

Constituents	Ratio %
Crude protein	14 .6
Crude fiber	3 .6
Moisture	2.18
Ash	10.7
Calcium	0.99
Phosphorus	0.75
Magnesium	0.17
Aflatoxin	None
Acid number of fat	7.2

Chemical composition of mushroom:

Moisture content, crude protein and ash content were determined according to the described by **AO**AC (**1995),** the fat content according to **Horwitz (1980),** crude protein **Less (1975),** and vitamin E according to published procedure Egyption **pharmacopeia (1984).**

Samples:

1- Serum samples: Blood samples was obtained from the orbital plexuses of each animals and received into dry clean tube. Samples were left to clot at room temperature for about 2 hours, stored overnight in a refrigerator at 4 °C and centrifuged at 3000r.p.m.for 15 min . Serum samples were drawn in dry clean capped bottles and kept in a deep freeze for serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) (**Reitman and Frankel1957),**alkalinephosphatase(ALP) **(Roy1970);** total protein (**Sonnenwirth and Jaret 1980);** albumin **(Young, 1995**); creatinine (**Faulkner and King 1976**);triglycerides (**Knight et al. 1972**); total cholesterol **(Fredrikson et al. 1967);**serumHDL- Cholesterol(**N.C.E.P.1995**); serum glucose **(trinder, 1969**).Serum LDL- Cholesterol concentration was calculated according to **Friedewadet al. (1972**) formula. Serum Globulin was calculated by subtracting serum albumin from serum protein level.

Determination of cytogenicity:

The micronucleus test was performed to detect chromosomal damage associated with the treatment. Micronuclei were identified as dark blue staining bodies in the cytoplasm of the polychromatic erythrocytes (PCEs) according to the protocol mentioned by ***Salamon et al ., (198)*** ; bone marrow cells of male rats were extruded with a pin into clean dry glass slide, homogenized with two drops of fetal calf serum. Cells were smeared on slide, air dried, fixed in absolute methanol and stained with Giemsa 5% in phosphate buffer pH6.8. The polychromatic erythrocytes (PCEs, 1000 per animal) were screened for micronuclei, and the changes in the mitotic activity **(*Hart and Engberg, 1983& Al- Bekairi et al., 1991*)** were assessed on the basis of the ratio of polychromatic to normchromatic erythrocytes (PCE/NCE ratio).

Statistical analysis:

Parametric data were statistically analyzed by using Analysis Of Variance (ANOVA) test and comparative of means were performed according to least significant differences test (LSD) according to ***(Snedecor, 1969)*****using SPSS 14 (2006)** . Results were presented as (Mean ± S.E.).

RESULTS AND DISCUSSION

The present study was carried out to elucidate the Hepato protective effect of dried mushroom against carbon tetrachloride (CCl_4) induced hepato toxicity in liver and cytogenicity change in rats using two concentrations of dried mushroom for 30 days in male albino rats. The tested parameters were chemical constituents, some biochemical parameters and cytogenicity in normal and treated rats. Their effects and constituents are registered in tables .It is generally believed that carbon tetrachloride (CCl4) has hepatotoxicity results from the bioactivation of the CCl4 molecules to the trichloromethyl which is toxic free radical induced by certain isozymes of cytochrome P450 (CYP – 450). Once the tri choromethyl radical is formed it reacts with molecular oxygen to form the highly toxic peroxy radical which then attacks call membrane lipids to propagate a chain reaction leading to initiation of lipid peroxidation and break down of membrane structure **(Wong et al., 1998 and Youssef, 2000).**The chemical constituents of dried mushroom recorded in table (2). The record results are in agreement with those found by **(Yang et al., (2006) and (Barbie Cervoni 2020)**

Effects on liver function and biochemical analysis

The obtained results showed that Subcutaneous injection of CCL_4 at dose of 0.1 ml/ 100 g b.wt., significantly increased AST , ALT and ALP It was clear from table (3) .Feeding on ration mixed with dried mushroom (5 and 10 g / 100 g ration) with injected CCl4 produced significant decrease of AST ,ALT and Alp when compared with control +ve group and presence of antioxidants of mushroom which had

important beneficial effects on the liver regeneration . These results are agreement with **Abeer et al ., (2012) ; (Anderia et al ., (2013) (TiQiang et al .,2018) and (Kennethlo and Orish 2020)** found that mushroom could reduce CCl4-induced toxicity, particularly hepatotoxicity, by suppressing ALT and AST activities, and increasing antioxidant enzyme activity and protect the liver against CCl4-induced oxidative damage in rats.

Bohi et al ., (2009) found **that** Pretreatment with PC mushroom extract significantly prevented the increased serum enzyme activities of alanine and aspartate aminotransferases in a dose-dependent manner, and suppressed the expression of CYP2E1. PC mushroom extract also protected hepatocytes from the damage effects of CCl4 as remarked by histological and electromicroscopical findings. daily doses of aqueous extracts of PC mushroom reduced the toxic effects exerted by CCl4 on the liver.

Feeding on ration with Injection of CCl_4 Subcutaneously caused significantly decreased in serum total protein, albumin and A : G ratio. Rats feed on ration mixed with dried mushroom and injected CCl_4 showed significant increase in total protein, albumin and A: G ratio directed toward normal value. The obtained data are agreement with **Hesx et al.,(2006) and (TiQiang et al .,2018) .**Serum urea and creatinine concentration were statistically higher in the serum of rats injected with CCl_4 alon l it was clear from table (4). Rats feeding dried mushroom with injected CCL4 showed significantly decrease in urea concentration but it was higher than control –ve group. Serum creatinine concentration was lower in the serum of rats given dried mushroom with injected CCl_4 than the control +ve group and toward the normal. Our results are agreement with **Ogeturk et al., (2004**) and (**Kenneth et al ., (2018)** who found that urea and creatinine were significantly higher in CCl_4 treated rats than in the controls while urea and creatinine were significantly lower in melatonin administration group.

Triglyceride , cholesterol and glucose concentrations did not induce changes in the serum of rats given dried mushroom than the control –ve group. Our results are in agreement with **Hossain et al., (2003); Hong et al., (2007) and Khatun et al., (2007),** concerning the changes in triglyceride and cholesterol concentration a significant decrease were recorded in hypercholesterolaemic of mushroom. Triglyceride and cholesterol concentration showed significantly increased in serum of rats injected CCl_4**. Abdel-Hamid (2006)** observed the increase in the concentrations of triglyceride and cholesterol in the serum of rats injected with CCl_4. The effect of mushroom may be investigated in a large sample for a longer duration to evaluate its efficacy and toxicity. **Aline et al ., (2014)** conclude that in our experimental model and in the concentration use mushroom was effective in improving the lipid profile of the animals.

Effects on cytogenicity:

The present investigation revealed that feeding on ration mixed with dried mushroom (5 and 10%) showed statistical decreased in micronucleated poly-chromatic erythrocytes (MPCEs) and significantly increased in normochromatic erythrocytes (NCE) compared with control –vegroup.Our results are agreement with **(Abdulahad et al ., 2018) founf that** The extract of mushroom contained a rich composition of bioactive compounds including phenolics (protocatechuic acid and p-hydroxybenzoic acid), volatile compounds (benzaldehyde, palmitic acid and linoleic acid suggests that A. arvensis might be used for food industries in order to obtain nutritional products.) and mineral compounds (K, Si, Mg and Na). Data obtained within this study **Lee et al., (2003)** showed that mushroom *Paxilluspannoides* inhibit lipid peroxidation and H_2O_2 neurotoxicity and so inhibiting DNA single strand breaks. These results suggest that the neuro-protective action of mushroom is depending on their ability to chelate iron. Subcutaneous injection of CCl_4 caused significantly increased in micronucleated polychromatic to normochromatic erythrocytes (PCE / NCE) and feeding on ration mixed with dried mushroom and injected ccl4 caused significant decreases of MPCEs if compared with control +ve group and directed toward normal. Our data are in agreement with **Wan et al ., (2010)** who found that oral administration of CCL4 a non-genotoxichepatocarcinogen, at a dose of 1600 mg/kg b.wt. did not induce chromosome aberrations, SCEs or micronuclei within 4-72 hr.The present method of in vivo cytogenetic assay using rats without partial hepatoctomy or mitogen treatment in vivo showed is useful for evaluating the tumor –initiating activities of hepatocarcinogens. **Bohi et al., (2009)** found that the significant increase in PCE / NCE ratio and enhancement of mitotic activity of bone marrow cells could be considered as a sign of toxicity and / or damage of some organs of the body. Ccl_4 did not induce chromosomal aberrations in the mouse bone marrow cells under these experimental conditions.**Almeida et al., (2005)** found that cytogenetic monitoring of individuals occupationally exposed to chemical and biological hazards increased frequencies of cells with chromosomal aberrations. **Guoxiao et al., (2019)** who recorded that aqueous chaga mushroom extract for their potential for protecting against oxidative damage to DNA human lymphocytes. Cells pretreated with chaga extract showed over 40% reduction in DNA fragmentation compared with the positive control. It is widely believed that the oxidative DNA damage over the human life span contributes significantly to the age related development of major cancer such as those of the colon prostate and breast. The result refer to mushroom treatment affords cellular protection against endogenous DNA damage produced by H_2O_2.

Table (2): Chemical composition of mushroom (100g) diet mushroom)

Nutrients	Nutritional value
Energy	24 .00 k cal
Protein	2.784 g
Fat	0.317 g
Ash	0.864 g
Fiber	1.152 g
Vit. C	2.21 mg
Calcium	4.8 mg
Phosphrous	99.84 mg
Iron	0.998 mg

Table (3): Mean activities of transaminases and alkaline phosphatase in the serum of rats fed diets containing mushroom without and with CCl_4 (n = 5).

	Dose (g/100g food)	AST(u/l)	ALT(u/l)	Alkaline Phosphatase (u/l)
Control(-ve)		25.26 ± 0.327^{de}	11.896 ± 0.414^{d}	41.78 ± 1.816^{d}
CCl_4	0.1	49.43 ± 0.232^{a}	25.645 ± 0.186^{a}	84.748 ± 2.53^{a}
Mushroom	5	24.4 ± 0.203^{d}	11.441 ± 0.089^{d}	40.76 ± 2.68^{d}
Mushroom	10	26.19 ± 0.37^{e}	10.64 ± 0.119^{d}	40.55 ± 1.197^{d}
M+ Ccl_4	5+0.1ml	33.008 ± 0.36^{b}	16.42 ± 0.446^{b}	72.839 ± 2.5^{b}
M + Ccl_4	10+01ml.	31.27 ± 0.579^{c}	14.22 ± 0.962^{c}	51.25 ± 1.64^{c}
F-calculated		657.463#	142.115#	79.37#
LSD		1.067	1.383	6.2096

Significant at $P < 0.05$ using ANOVA test.a, b, c, d, e, insignificantly different between two comparison groups within the same litter and column using Least Significant Different at $P < 0.05$.

Egypt. J. Chem. Environ. Health, 6 (1):23-35 (2020)
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Table (4): Mean values of total serum protein, albumin, globulin and albumin: globulin ratio (A: G ratio) in rats fed diets containing mushroom without and with CCl_4 (n = 5).

	Dose (g/100g food)	T. Protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)	A:G ratio
Control (-ve)		a7.47 ± 0.396	3.76± 0.33 ^{ac}	3.64 ± 0.098 ^{ac}	1.126± 0.096 ^{ac}
Ccl_4	0.1 ml	5.21 ± 0.43 ^{b}	2.21 ± 0.15 ^{b}	2.757 ± 0.199^{b}	0.764 ± 0.064^{b}
Mushroom	5	7.12 ± 0.59 ^{a}	3.995 ± 0.16 ^{a}	3.32 ± 0.17^{a}	1.363 ± 0.13^{ac}
Mushroom	10	7.41 ± 0.5 ^{a}	3.83 ± 0.28 ^{ad}	3.438 ± 0.13^{a}	1.09 ±0.087^{abc}
M+ Ccl_4	5+ 0.1 ml	6.77 ± 0.33 ^{a}	3.195 ± 0.22^{cd}	3.593 ± 0.12^{a}	0.89 ± 0.138^{ac}
M+ Ccl_4	10+ 0.1 ml	7.16 ± 0.66 ^{a}	3.43 ± 0.2 ^{a}	3.773 ± 0.13^{c}	.954 ± 0.058^{ab}
F-clculated		2.885#	7.889#	6.147#	2.986#
LSD		1.447	0.678	0.424	0.339

Significant at P < 0.05 using ANOVA test.a, b, c, d, e, insignificantly different between two comparison groups within the same litter and column using Least Significant Different at P < 0.05.

Table (5): Mean values of serum urea, creatinine, triglycerides, glucose and cholesterol in rats fed diets containing mushroom without and with Ccl_4 (n = 5).

	Dose (g/100g food)	Urea (mg/dl)	Creatinine (mg/dl)	Triglycerides (mmol/L)	Glucose (mg/dl)	Cholesterol (mg/dl)
Control (-ve)		17.01 ±0.78^{a}	0.78 ±0.008^{ad}	4.38±0.38^{a}	113.52 ±5.66^{a}	136.00 ±5.05^{a}
Ccl_4	0.1 ml	69.89 ±5.35^{b}	1.1 ±0.06^{b}	6.41 ±0.38^{b}	148.16 ±2.95^{b}	241.15 ±8.369^{b}
Mushroom	5	17.76 ±0.85^{a}	0.7 ±0.013^{a}	4.29 ±0.22^{a}	111.2 ±4.37^{a}	132.88 ±1.227^{a}
Mushroom	10	17.595 ±0.74^{a}	0.81 ±0.026^{dc}	4.31 ±0.31^{a}	113.42 ±6.39^{a}	146.53 ±10.44^{a}
M +Ccl_4	5+ 0.1 ml	48.7 ±1.26^{c}	0.95 ±0.021^{e}	5.36 ±0.27^{cd}	130.04 ±6.7^{c}	192.93 ±11.06^{c}
M + Ccl_4	10+ 0.1 ml	31.73 ±2.46^{d}	0.85 ±0.033^{dc}	4.64 ±0.207^{ad}	104.48 ±5.2^{a}	148.88 ±15.09^{a}
F-calculated		58.37#	21.02#	7.74#	8.949#	19.396#
LSD		8.169	0.0899	0.883	15.632	28.129

Significant at P < 0.05 using ANOVA test.a, b, c, d, e, insignificantly different between two comparison groups within the same litter and column using Least Significant Different at P < 0.05

Table (6): Effect of mushroom without and with CCl_4 on percentage of MPCEs and PCE / NCE ratio in rats. (n = 5)

	Dose (g/100g food)	PCE	MPCE / 1000	NCE	PCE / NCE
Control (-ve)		5000	5.42 ± 0.107^{a}	2175.6 ± 33.42^{a}	2.32 ± 0.13^{a}
CCl_4	0.1 ml	5000	13.3 ± 0.24^{b}	740 ± 17.32^{b}	6.88 ± 0.116^{b}
Mushroom	5 10	5000	5.23 ± 0.12^{a} 4.97 ± 0.08^{a}	1821 ± 14.7^{c} 1914 ± 12.186^{d}	2.75 ± 0.08^{c} 2.53 ± 0.08^{ac}
M+ Ccl_4	5+ 0.1 ml	5000	8.0 ± 0.179^{c}	1075 ± 27.66^{e}	4.97 ± 0.16^{d}
M+ Ccl_4	10+ 0.1 ml		9.13 ± 0.146^{d}	927.2 ± 10.39^{f}	6.29 ± 0.103^{e}
F-calculated			431.52#	812.465#	307.928#
LSD			0.453	61.275	0.336

Significant at $P < 0.05$ using ANOVA test.a, b, c, d, insignificantly different between two comparison groups within the same litter and column using Least Significant Different at $P < 0.05$.

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