Protection of the Mushroom (shiitake "*Lentinus-edodes*) against Carbon-Tetrachloride-Induced Renal Injury in Rats

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Abstract: Background and objectives: Exposure to carbon tetrachloride (CCl4) induces acute and chronic renal injuries as well as oxidative stress in rats. Mushroom characteristically contains much different biological activities as various degrees of immunity, lowering antitumor and other beneficial or therapeutic health effect without toxicity. The present study was carried out to investigate the protection effect of mushroom against carbon tetrachloride intoxication in rats. Dried mushroom (shiitake "*Lentinus-edodes*) grind mushroom at concentrations (5 and 10 %) showed significant protection activity against carbon tetrachloride induced kidney in rats by normalizing the levels of serum urea and creatinine. Dried mushroom improved the Serum minerals, calcium, iron and phosphorus content levels in a dose dependent manner. Gross necropsy and histopathological examination further confirmed the protection effects of mushroom (shiitake "*Lentinus-edodes*). This is the first report on protection effects of mushroom (shiitake "*Lentinus-edodes*). The present study showed that mushroom (shiitake "*Lentinus-edodes*) was able to prevent or reduce the severity of carbon tetrachloride -induced renal dysfunction.
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1. Introduction:

The pathogenesis of Carbon tetrachloride (CCl_4)-induced renal dysfunction is not completely known. It may be due to the functional state of liver, or renal injury may develop independently to hepatic events **(Hamed *et al*., 2012).** CCl_4 induces oxidative stress in many settings **(Kalava and Menon, 2012)**; therefore, it might be expected to contribute to nephrotoxicity. CCl4 does not occur naturally, it is a clear liquid with sweet smell that can be detected at low levels **(Adaramoye, 2009).** Exposure to various compounds including a number of environmental pollutants and drugs can cause cellular damages through metabolic activation of those compounds to a highly reactive oxygen species (ROS). Free radical induced lipid peroxidation is believed to be one of the major causes of cell membrane damage leading to a number of pathological situations **(Chen *et al*., 2012)**. CCl_4 was formerly used for metal degreasing and as dry cleaning, fabric-spotting, and fire extinguisher fluids, grain fumigant and reaction medium. Because of its harmful effects, these uses are now banned and it is only used in some industrial applications. The primary routes of potential human exposure to CCl4 are inhalation, ingestion, and dermal contact. High exposure to CCl_4 can cause liver, kidney and central nervous system damage, and liver is especially sensitive to CCl_4 because of its role as the body's principal site of metabolism **(Adewole *et al*., 2007).** Reports from our laboratory and other investigators have established that CCl_4 is a potent environmental hepatotoxin **(Acharya *et al*., 2012).** A number of reports clearly demonstrated that in addition to hepatic toxicity, CCl4 also causes disorders in kidneys, lungs, and testis as well as in blood by generating free radicals **(Adewole *et al.*, 2010).** Findings by **Ogeturk *et al*., (2005)** suggested that exposure to this solvent causes acute and chronic renal injuries. In addition, report on various documented case studies established that CCl4 produces renal diseases in human **(Adewole *et al*., 2007).** A number of endogenous and exogenous nephropathy risk factors generate oxygen free radicals *in vivo*. Therefore the role of oxygen-derived free radicals and lipid peroxidation has attracted considerable attention **(Azlina *et al*., 2011)**. Volatile organic compounds such as CCl_4 are a class of solvents to which many people are exposed occupationally and environmentally. Early studies of dichloromethane, CCl_4 and 1, 1-dichloroethylene revealed susceptibility of rats to liver and kidney damage by these chemicals **(Adewole *et al*., 2007)**. It has been found that metabolism of CCl4 involves in the production of free radicals through its activation by drug metabolizing enzymes located in the endoplasmic reticulum **(Adaramoye, 2009).** Therefore, it has become a task to prevent nephropathy and hepatic damage by eliminating free

radicals and prevent lipid peroxidation through the use of terminal antioxidant like mushrooms **(Preeti *et al*., 2012).**

It was the aim of this work to explore the protection effect of Mushroom (shiitake "*Lentinus-edodes*) against Carbon-Tetrachloride-Induced Renal Dysfunction.

2. Materials and Methods:

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 male Albino rats of sparague Dawley strain of an average body weight 150 ± 10g were obtained from the laboratory of animal King Fahd Center for Medical Research, Jeddah, Saudi Arabia. Rats were fed on standard ration supplying the essential vitamins, trace elements and water supply was given ad libitum.

Experimental design:

Six equal groups each of five rates were housed in wire cages in a room temperature maintained at 25^{o}C± 2 and kept under normal healthy conditions. All rats and food consumption weight every week for determination the body weight gain and food intake. Rats of first group (GI) kept as control negative (normal control) and fed on basal ration. Rats of the second group (G2) were used as positive control, 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. Rats of the third group (G3) fed on basal ration mixed with grind dried mushroom at concentration 5% for 30 successive days. The fourth Group (G4) fed on basal ration mixed with 10% grind dried mushroom for 30 successive days. The fifth Group (G5) were fed 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.) CCl_4 S/C twice week for two weeks. Rats of sixth group (G6) were fed 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.

Blood samples:

At the end of the experimental period blood samples were collected from the animal eye plexuses. Each sample was collected into both heparinized tubes to obtain the plasma and into a free coagulation dry clean centrifuge glass tube to prepare serum. Blood samples were left for15 min at room temperature, and then the tubes were centrifugated for 15 min at 3000 r.p.m for 10 minute. Serum samples were separated and used for determination of different biochemical parameters. After collection of the blood samples, all rats were sacrificed to collect the liver for histopathological.

The gain in the body weights was calculated by the body weight gain = initial weight- final weight.

Biochemical parameters:

Serum kidney functions, urea and creatinine activities were assayed by the method of **Reises *et al.*, (1965)** and **Faulkner and King (1976),** respectively. Serum minerals, calcium, iron and phosphorus were assayed by the method of **Tietz, 1970, Fairbanks and Klee, 1987** and **Itani and Tsang, 2003,** respectively.

Pathological studies:

Kidney tissue specimens were collected from all groups at the end of experiments (30 days), 'immediately after sacrifice of rats and fixed in 10% neutral buffered formalin dehydrated in alcohol, cleared in xylol and embedded in paraffin sections of 4µ thickness were prepared, stained by Hematoxylene and Eosin (H&E) **(Yoon *et al*., 2001).**

Statistical analysis:

Results are expressed as mean ± standard deviation (SD). Differences between means in different groups were tested for significance using a one-way analysis of variance (ANOVA) followed by Duncan's test and P value of 0.05 or less was considered significant. Comparative of means were performed according to least significant differences test (LSD) according to **(Snedecor, 1969)** using **SPSS 14 (2006).**

3. Results:

The present study was carried out to elucidate the nutritional and protective effect of dried mushroom (shiitake "*Lentinus edodes*) in normal and on carbon tetrachloride (Ccl_4) induced cytogenicity and renal fibrosis, using two concentrations of dried mushroom for 30 days in male albino rats. The tested parameters were chemical constituents, some biochemical parameters, cytogenicity and histopathological examination of kidney in normal and treated rats. Their effects and constituents are registered in tables and Photograph.

Effects of injected with CCl_4 on food intake and body weight gain:

Effect of feeding on ration mixed with dried mushroom (5 and 10 g / 100 gm ration) for 30 successive days with or without subcutaneous injection of carbon tetra-chloride (twice / week for two weeks) on food intake and body weight gain are recorded in table (1). Food intake and body weight gain was significantly increased in rats feed on ration mixed with dried mushroom (5 and 10 g / 100 g) when compared with other groups, while the group injected Ccl_4 showed significantly decreased. The groups feed on dried mushroom at both concentration with injection of CCl_4 improved the body weight gain and food intake and showed significantly increased when compared with group subcutaneous injection of CCl_4.

Effects of injected with CCl_4 on relative organ weight of kidney organ:

There was no differences in relative organ weight in rats feed on ration mixed with dried mushroom (5 and 10 %), and rats injected subcutaneously Ccl_4 compared with control + ve group (Table1).

Table (1): Food intake (g), body weight gain (g) and relative kidney weight of rats fed diets containing mushroom without and with CCl_4 (n = 5).

	Dose (g/100g food)	Food intake (g)	b.wt. gain (g)	Kidney %
Control (-ve)		3504.8 ± 70.8[a]	46.24 ± 1.615[a]	0.71 ± 0.02 [a]
Ccl_4 (+ve)	0.1 ml	2630 ± 75.17[b]	28.94 ± 1.71[b]	0.843 ± 0.011 [b]
Mushroom	5	3876 ± 78.78[ce]	74.76 ± 1.835[c]	0.58 ± 0.003 [c]
Mushroom	10	4454 ± 71.87[d]	95.54 ± 2.01[d]	0.59 ± 0.012 [c]
Mushroom + CCl_4	5+ 0.1	3772 ± 69.531[e]	53.08 ± 2.505[e]	0.6 ± 0.016 [c]
Mushroom + CCl_4	10+ 0.1	3960 ± 69.64[e]	63.7 ± 3.23[f]	0.523 ± 0.031 [d]
F-calculated		70.5 #	109.033 #	44.228#
LSD		211.352	6.478	0.0512

Significant at $P < 0.05$ using ANOVA test.

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

Effects on serum urea and creatinine: It was obvious from table (2) that serum urea and creatinine concentrations did not induce changes in groups feed on dried mushroom when compared with control –ve group. Serum urea and creatinine concentrations were significantly higher in serum of rats injected with CCl_4 (0.1 ml / 100 g.b.wt.).Rats feeding on ration mixed with dried mushroom (5 and 10 %) with injected Ccl_4 showed significantly decreased 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 directed toward the normal (c –ve).

Table (2): Mean values of serum urea and creatinine in rats fed diets containing mushroom without and with Ccl_4 (n = 5).

	Dose (g/100g food)	Urea (mg/dl)	Creatinine (mg/dl)
Control (-ve)		17.01 ±0.78[a]	0.78± 0.008[ad]
Ccl_4 (+ve)	0.1 ml	69.89 ±5.35[b]	1.1 ± 0.06[b]
Mushroom	5	17.76 ±0.85[a]	0.7 ± 0.013[a]
Mushroom	10	17.595 ±0.74[a]	0.81 ±0.026[dc]
Mushroom + CCl_4	5+ 0.1	48.7 ± 1.26[c]	0.95 ±0.021[e]
Mushroom + CCl_4	10+ 0.1	31.73 ±2.46[d]	0.85 ±0.033[dc]
F-calculated		58.37#	21.02#
LSD		8.169	0.0899

Significant at $P < 0.05$ using ANOVA test.

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

Effects on some serum minerals: Feeding on ration mixed with dried mushroom (10 %) showed significant increase in calcium concentration when compared with control –ve group. Significantly increased in calcium concentration in serum of rats given dried mushroom with injection of Ccl_4 when compared with control +ve groups (Table 3). Feeding on ration mixed with dried mushroom (5 and 10 %)

did not induce changes in serum iron concentration when compared with control –ve group. Subcutaneous injection of CCl_4 caused a significant decrease in serum iron concentration when compared with other groups, but feeding on ration mixed with dried mushroom showed a significant increase in concentration of iron in serum when compared with control +ve group (Table 3).On the other hand, phosphorus level in the serum of rats feed on ration mixed with dried mushroom (5 and 10%) showed significantly increased, but was significantly decreased in the serum of rats injected with Ccl_4 when compared with control –ve group. Rats feed ration mixed with dried mushroom and injected Ccl_4 showed significantly increased in phosphorus level when compared with control +ve group (Table 3).

Table (3): Mean values of serum minerals in rats fed diets containing mushroom without and with Ccl_4 (n = 5).

	Dose (g/100g food)	Ca (mg/dl)	Iron (mg/dl)	Phosphorus (mg/dl)
Control (-ve)		7.08 ± 0.11^{a}	3.02 ± 0.25^{a}	6.998 ± 0.217^{a}
Ccl_4 (+ve)	0.1 ml	7.279 ± 0.087^{a}	2.00 ± 0.12^{b}	4.987 ± 0.19^{b}
Mushroom	5	7.56 ± 0.126^{a}	3.16 ± 0.15^{a}	8.03 ± 0.3^{c}
Mushroom	10	8.49 ± 0.24^{b}	3.36 ± 0.24^{a}	9.10 ± 0.3^{d}
Mushroom + Ccl_4	5+ 0.1	7.69 ± 0.19^{a}	2.79 ± 0.24^{a}	5.28 ± 0.27^{b}
Mushroom + Ccl_4	10+ 0.1	8.61 ± 0.4^{b}	3.18 ± 0.20^{a}	7.33 ± 0.35^{ac}
F-calculated		4.7998#	5.559#	32.801#
LSD		0.639	0.603	0.807

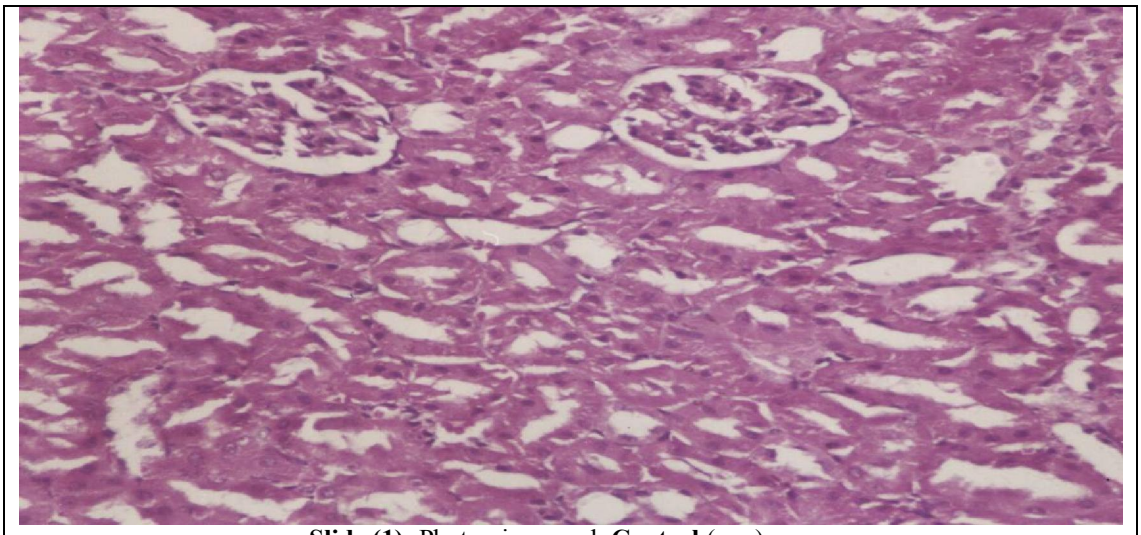
Significant at $P < 0.05$ using ANOVA test.

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

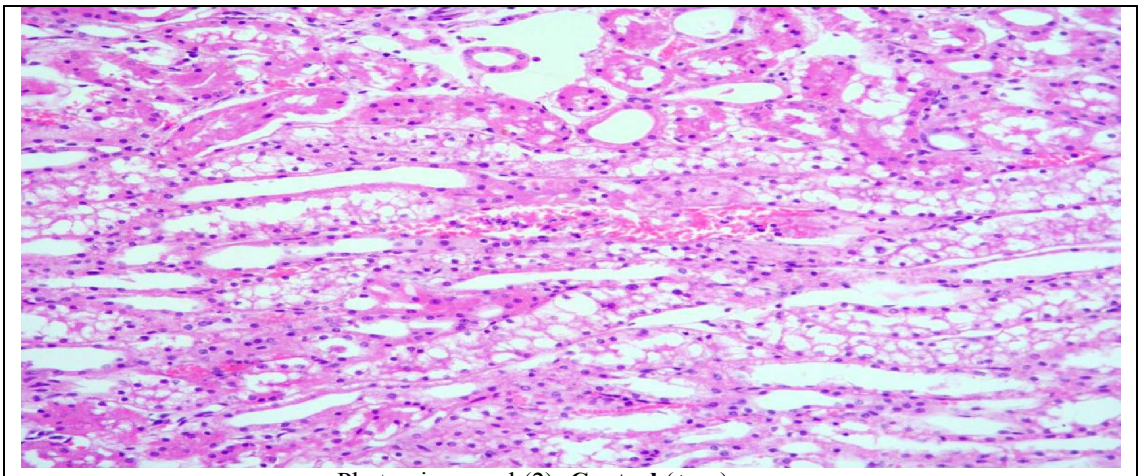
Pathological effects of kidney organ: Photomicrograph (1) showed the microscopic estimation of the kidney of the tested rat groups. Control, (- ve) group revealed a normal histological structure (Slide 1). Meanwhile, kidney was characterized by diffuse vacuolar degeneration of the living tubular epithelium with congestion in group injected Ccl_4 control (+ ve) group (Slide 2). Whereas kidney of rat from all groups mushroom + Ccl_4 showed, slight vacuolations of epithelial lining few renal tubules (Slide 3). In addition, kidney of rat from all groups mushroom showed no changes with apparent normal kidney (Photomicrograph 1).

4. Discussion:

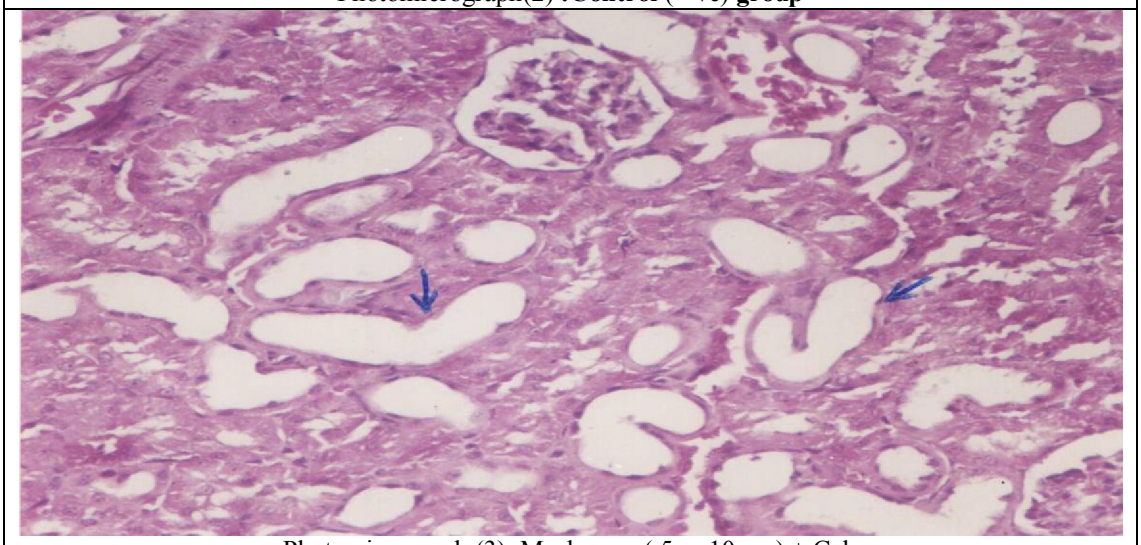
Mushroom (*Lentinus edodes*) has long been known to be endowed with beneficial and diverse activity. The present study was carried out to elucidate the hepatoprotective effect of Mushroom (shiitake "*Lentinus-edodes*) against Carbon-Tetrachloride-Induced Liver Injury in Rats.



Slide (1): Photomicrograph **Control** (- ve)**group**



Photomicrograph(2) **:Control** (+ ve) **group**



Photomicrograph (3) :Mushroom (5 or 10 gm) + Ccl_4

Photomicrograph (1): Histopathological changes in tissue sections kidney.

It is generally believed that carbon tetrachloride (Ccl_4) hepatotoxicity results from the bioactivation of the Ccl_4 molecules to the trichloromethyl toxic free radical by certain isozymes of cytochrome P450 (CYP – 450). Once the trichoromethyl radical is formed, it reacts with molecular oxygen to form the highly toxic peroxy radical which then attacks cell membrane lipids to propagate a chain reaction leading to initiation of lipid peroxidation and break down of membrane structure **(Youssef, 2000 and Kalava and Menon, 2012).**

Effects of injected with Ccl_4 on food intake and body weight gain: Feeding of dried mushroom at both concentrations for 30 successive day's significant increase in body weight gain and food intake. These findings correlated with those obtained by **Hush *et al.*, (2006),** but our results were disagree with the results of **Fukushima *et al.*, (2001)** who reported that there did not change in body weight gain and **Hong *et al.*, (2007**) who recorded that feed on mushroom decrease body weight in mice and rats. Subcutaneous injection of Ccl_4 caused a significant decrease in body weight gain and food intake. These results are in agreement with **Lee *et al.*, (2007)** who reported that injection of Ccl_4 was significantly decreased body weight gain and food intake. Feeding on dried mushroom with injection Ccl_4 seemed to alleviate the inhibitory effect of Ccl_4 on body weight gain and foods intake. Moreover, the beneficial effect of antioxidant administration against Ccl_4 poisoning with respect to body weight observed in the present study confirms previous results obtained by **Aneja *et al.*, (2005**) who concluded that feeding rats with antioxidants could play an important role as a prophylactic against the toxic effects of Ccl_4. **Hesx *et al.*, (2006)** found that mushroom contain several compound such as polysaccharide, polysaccharide – peptide complex and phenolic components proposed and flavonoids to be responsible for this antioxidant effect.

Effects of injected with Ccl_4 on relative weight of kidney organ: There was no difference in relative organ weight in rats feed on ration mixed with dried mushroom (5% and 10%), while rats injected subcutaneously CCl_4 showed significantly increased in relative organ weight kidney. The obtained data are agreements with; **Lin and Lin (2006); Lin *et al.,* (2006) and Fang *et al.,* (2007),** which reported that injection of CCl_4 in rats to induce liver fibrosis lead to increase the weight of kidney. **Lee *et al.,* (2007)** who reported that relative organs weight were significantly increased after injection with Ccl_4. Feeding on ration mixed with dried mushroom and injected Ccl_4 showed significantly decreased in relative organs weight of kidney when compared with control +ve group and tended toward the normal value.

Effects of injected with Ccl_4 on serum urea and creatinine: It was obvious that no significant changes in serum urea and creatinine concentrations in rat groups feed with dried mushroom (5% and 10%) in comparison to the control - ve group. Our results disagree with **Kuroiwa *et al.,* (2005)** who reported that feeding of *Agracius blazeimurill* (an edible mushroom) for 90 days showed slightly increase serum urea and reduce the creatinine level. He suggest that the increase of blood urea nitrogen due to little toxicological significance. Serum urea and creatinine concentration were statistically higher in the serum of rats injected with Ccl_4 +ve group. Rats feeding dried mushroom with injected Ccl_4 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**) 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. The mechanism of elevated serum levels of urea and creatinine were explained by **Ogeturk *et al.,* (2005),** who found that kidney malondialdehyde (MDA) levels were increased significantly following injected Ccl_4 exposure. Also we in agreement with **Hong *et al.,* (2007)** and **Jayakumar *et al.,* (2007)** who found that the feed on mushroom with Ccl_4 elevated urea level in plasma higher than normal urea were reflecting dietary protein intake **Kuroiwa *et al.,* (2005).**

Effects of injected with Ccl_4 on some serum minerals: Feeding of dried mushroom (5% and 10%) showed significant increase in calcium concentration when compared with control –ve group. Significantly increased in serum calcium concentration of rats given mushroom with injection of CCl_4 compared with control +ve group.

Our results are agreement with **Wong *et al.,* (2006**) which demonstrated that the feed on three dietary fibers prepared from mushroom namely *Pleurotus tuber regium*, *Poly porus rhinocerus* and *Wolfiporia cocos* on calcium and magnesium absorption was evaluated in overictomized rats feed on last diet for 14 days. The animals in the *W. cocos* DF diet group possessed significantly higher levels of cecal total short chain fatty acids and had an increased the concentration of cecal soluble Ca^+ and Mg^+. Besides the apparent Ca^+ and Mg^+ absorptions of the *W. cocos* DF group were also significantly enhanced (Ca^+ and Mg^+) together with significantly higher serum Ca^+ and Mg^+ levels when compared with those of the cellulose control group. These results suggest the ingestion of *W. cocos* of DF could improve the over all Ca^+ and Mg^+ absorption of the rats feed a low Ca^+ diet. The administration of Ccl_4 caused a remarkable elevation of calcium content in serum, the liver tissues and the nuclei of rats. The results demonstrated that calcium transport system in the liver nuclei is impaired by liver injury with Ccl_4 administration in rats (**Katsumata *et al.,* 1998).** Our results disagree with him, but this due to relationship between dose of Ccl_4 injected and degree of liver injury which affect on calcium level in serum.

The present investigation revealed that subcutaneous injection of Ccl_4 caused a significant decrease in serum iron concentration when compared with other group. Feeding of mushroom showed a significant increase in serum iron concentration when compared with the control +ve (Ccl_4) group. Our results are in agreement with **Jasinghe *et al.,* (2005)** who reported that mushroom is good source of many minerals. On other hand **Suzuki *et al.,* (1997)** disagree with us because he reported that from chronic hepatitis to liver cirrhosis concentrations decreased serum iron. We are disagreement with **Fang *et al.,* (2007)** who found that CCl_4 exposure produced serum iron concentration a higher rate in serum than in the normal. Acute hepatocellular injury is followed by increased hepatic iron uptake **(Batey and Johnston, 1993).**The mechanism of trace element abnormalities may reflect such pathological conditions as liver dysfunction, cholestasis, hepatic fibrosis or liver regeneration **Suzuki et al., (1997)** and changes in liver cell pathology compounded by functional impairment may alter the metabolism of trace metals, in particular zinc and copper. The possible relationship of these changes to the pathogenesis of chronic liver disease was reported by **Pramoolsinsap *et al.,* (1994).** On the other hand, phosphorus level in the serum of rats feed on ration mixed with dried mushroom (5% and 10%) showed

significantly increased, but was significantly decreased in the serum of rats injected with Ccl_4 when compared with control –ve group. Rats feed on ration mixed with dried mushroom and injected Ccl_4 showed significantly increased in phosphorus level when compared with control +ve group. Mushroom had the highest levels of phosphorus, calcium, potassium, zinc, copper and iron. The nutritional data suggested that mushroom is the best vegetable, which is a good source of many minerals (**Jasinghe *et al.*, 2005**).

Pathological effects of different organs:

There were different pathological effects on internal organs as follows: rats injected with CCl_4 (control +ve). Kidneys was characterized by diffuse vacuolar degeneration of the lining tubular epithelium with congestion of the vasculature in group injected with Ccl_4 (+ve group), while, in groups feed on dried mushroom (5 and 10%) and injected Ccl_4, kidney revealed to histopathological changes of the epithelial lining of the most of the renal tubules, but the hemorrhage still present.

Adewole *et al.*, (2007) who found that the histo- pathological sequence of administration of CCl_4 and in the kidney, vacuolation of epithelial cells in the proximal tubules disappeared rapidly, but glomerular lesions progressed even after cessation of CCl_4 administration, and marked glomerulosclerosis developed.

Ogeturk *et al.*, (2005) and Hamed *et al.*, (2012) found that Ccl_4 administration alone also caused histopathologically prominent damage in the kidney compared to the control group. Glomerular and tubular degeneration, interstitial mononuclear cell infiltration and fibrosis, and vascular congestion in the peritubular blood vessels were observed in the renal cortex.

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