Biochemical and histological studies on the effect of the Patulin mycotoxin on male rats' liver and treatment by crude venom extracted from jelly fish

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Abstract: Patulin mycotoxin on some biochemical parameters and histological changes on male rats' liver and effect of crude venom extracted from jelly fish Cassiopea Andromeda as a treatment. 50 Inbreeding weanling white male wistar lewis rats were divided randomly into 5 groups. Control group was gavage fed daily with distilled water; three treated groups were gavage fed daily dose with Patulin (0.2 mg/kg b.w.) for one, two and three weeks respectively. The last group was treated by Patulin for one week then injected intraperitoneally with single dose of crude venom (1.78 mg/20 g b.w.) for 24 hours according to LD_{50}. Level of (AST) and (GGT) were increased significantly in serum of all treated groups compared with control group but level of (ALT) was increased significantly in treated group after one week only. Although the concentration of (TNF-α) was increased significantly and gradually in all treated groups, the concentration of ferritin was decreased significantly in treated three after three weeks only. Histopathological changes of rat liver coincided with biochemical changes. In conclusion, oral exposures of Patulin indicate that hepatic alteration was produced in manner related to dose duration and crude venom may used as new therapeutic approach to detoxify hepatocytes from Patulin.
[Nagwa M. El-Sawi, Hanaa M. Gashlan, Sabry H. H. Younes, Rehab F. Al-Massabi and S. Shaker. **Biochemical and histological studies on the effect of the Patulin mycotoxin on male rats' liver and treatment by crude venom extracted from jelly fish.** *Life Sci J* 2012;9(4):1143-1153]. (ISSN: 1097-8135). http://www.lifesciencesite.com. 171

Keywords: mycotoxin, patulin, liver, rat, Jelly fish crude venom.

1. Introduction

Huge quantities of food are wasted every year because they are invaded by toxic fungi or contaminated by fungal metabolic products. Over the past 15-20 years, toxic mold exposure has become hazardous and frequent.[1] Mycotoxins are secondary metabolites produced by certain filamentous fungi, which can be produced in foods as a result of fungal growth.[2] Fruits and vegetables are an important part of a balanced diet, but they may be colonized by moulds that produce mycotoxins[3]. In fruit juice, Patulin is considered to be the most important and is produced by several species of fungi including *Penicillium expansum*.[4]

Many experimental studies have been carried out using crude venom extracted from jelly fish for the treatment of animal disease. Venoms are complex mixtures of many different components, whereas a toxin is a single pure compound. Toxins found within venoms often have novel, highly specific activities that have the way for the design of therapeutically useful molecules based upon the structural information obtained.[5] The toxins of coelenterates have been detected to include bradykinnin and related polypeptides which have a kinin like action.[6] BK have effective role in liver regeneration[7,8] and enhanced prostaglandin synthesis[9] which in turn improved hepatic function.[10]

The work plan involves testing experimental animals for the influence of patulin mycotoxin on liver enzymes, some tumor markers, carbohydrate metabolism as reflected on liver function and structure. Additional attentions are given to crude venom extracted from jelly fish as treatment.

2. Materials and methods

Fifty white male Wistar Lewis rats each weighs (55-65 g) were obtained from the animal facility of King Fahd Medical Research Center, King Abdul-Aziz University, Jeddah, Saudi Arabia. The animals were conditioned at room temperature and commercial balanced diet and tap water was provided throughout the experiment. Animals were divided randomly into five groups (10 rats each in two cages) and were subjected to the following schedule of treatments: Control group was gavage fed daily with distilled water; three other treated groups were gavage fed daily dose with Patulin (0.2 mg/kg b. w.) for one, two and three weeks respectively. The last fifth group was treated by Patulin as treated one for one week then injected intraperitoneally with a single dose of crude venom from jelly fish (1.78 mg/ 20 g b.w.) for 24 hours according to LD_{50}. Blood samples

were collected from the orbital sinus and stored at -20°C until analysis was performed. Sera were used for the measurement of AST, ALT, GGT, TNF, ferritin and glucose. The animal was decapitated and the abdomen was opened. The liver was excised and washed in sterile saline solution. Small parts were taken for histological studies and other parts of liver were stored at -20°C for glycogen analysis.

3. Results

The biochemical and histological changes were studied in blood and liver of male Wistar Lewis rats as a result of administration of a daily dose of Patulin (0.2 mg/kg b. w.) and observed over a period of one (T1), two (T2) and three (T3) weeks compared to the control group. In addition to treated four (T4) using single dose intraperitoneally of crude venom extracted from jelly fish (1.78 mg/ 20 g b. w.) for 24 hours after treated with Patulin for one week (Tables 1, 2).

Table 1: Effect of Patulin (0.2 mg/Kg b. w.) on Some Biochemical Parameters of Male Rats.

Groups / Parameters	Control	T1	T2	T3
AST U/L	35.146 ± 0.42	70.656±0.79**	72.404±0.87**	65.596±0.41**
ALT U/L	41.073 ± 0.68	45.341 ± 0.97**	39.393 ± 0.71 N.S	40.528 ± 0.99 N.S
GGT U/L	8.203 ± 0.21	9.834 ± 0.41**	9.539 ± 0.25**	9.899 ± 0.0.22**
TNF Pg/ml	38.027 ± 0.97	53.925 ± 0.81**	60.680 ± 0.35 **	93.588 ± 0.98 **
Ferritin µg/ml	1.617 ± 0.03	1.605 ± 0.06 N.S	1.539 ± 0.08 N.S	1.048 ± 0.06 **
Glucose mmol/l	3.495 ± 0.11	3.356 ± 0.14 N.S	4.127 ± 0.06 **	3.974 ± 0.08 **

Data are expressed as mean ± SE. Number of sample in each group is 10. T1, T2, T3 = Treated group with Patulin
Significant change in comparison between groups:
** Highly significant ($P \leq 0.01$) * Significant ($0.01 \pm P \leq 0.05$) N.S Non significant ($P > 0.05$)

Table 2: Effect of Crude Venom (1.78 mg/ 20 g b. w.) on Some Biochemical Parameters in Male Rats after Patulin (0.2 mg/kg b. w.) Hepatotoxicity

Parameter	Control	T 1	T 4
AST U/L	35.146 ± 0.42	70.656±0.79	51.253±0.71
			**
		●●	●●
ALT U/L	41.073 ± 0.68	45.341 ± 0.97	41.232 ± 0.84
			**
		●●	N.S
GGT U/L	8.203 ± 0.21	9.834 ± 0.41	8.819 ± 0.24
			*
		●●	N.S
TNF Pg/ml	38.027 ± 0.97	53.925 ± 0.81	46.445 ± 0.67
			**
		●●	●●
Ferritin µg/ml	1.617 ± 0.03	1.605 ± 0.06	1.607 ± 0.03
			N.S
		N.S	N.S
Glucose mmol/l	3.495 ± 0.11	3.356 ± 0.14	3.217 ± 0.08
			N.S
		N.S	N.S
Glycogen µg/µl	0.052 ± 0.01	0.038 ± 0.004	0.037 ± 0.003
			N.S
		N.S	**N.S**

Data are expressed as mean ± SE. Number of sample in each group is 10.
T1 Treated group with Patulin T4 Treated group with Patulin + crude venom of jelly fish
Significant change in comparison between groups:
**, ●● Highly significant ($P \leq 0.01$) * Significant ($0.01 < P \leq 0.05$) N.S Non significant ($P > 0.05$)
First significant change as compared with T1, Second significant change as compared with control.

Histological results:

The effect of Patulin on liver parenchyma varies in different weeks. In animal group of one

week (T1) (**Fig. 2)**, there was evident hepatocytes vaculation and damage of cellular membrane (necrosis), while in group of two weeks (T2) (**Fig. 3**) the histological changes were less compared to those of one week (T1). Instead of vacuolation, scattered apoptotic cells were observed in groups of two weeks (T2) and three weeks (T3) (**Fig. 4**). The proliferation of bile ducts in group of three weeks (T3) was described to be associated with hepatic metabolic disturbance or toxic exposure.[11] The nuclei showed marked variation in size, Pyknosis (small dark nuclei), karyomegally (large sized) and karyolysis (degeneration) were observed. The enlarged nuclei was observed mainly in animals given Patulin for two weeks (T2) and three weeks (T3) and also in jelly fish treated group (T4) (**Fig. 5**) which may as a result of hepatocytes toxicity.

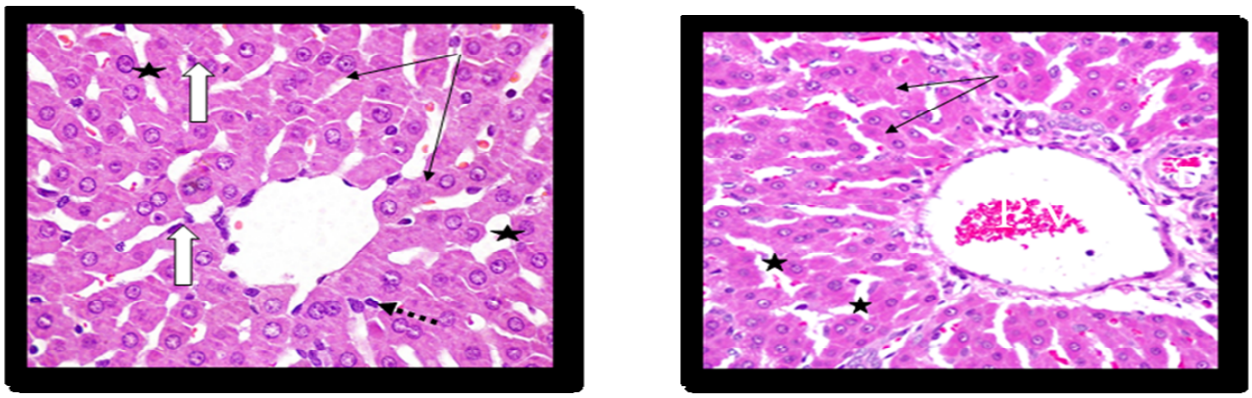

Fig. 1: Normal Hepatocytes

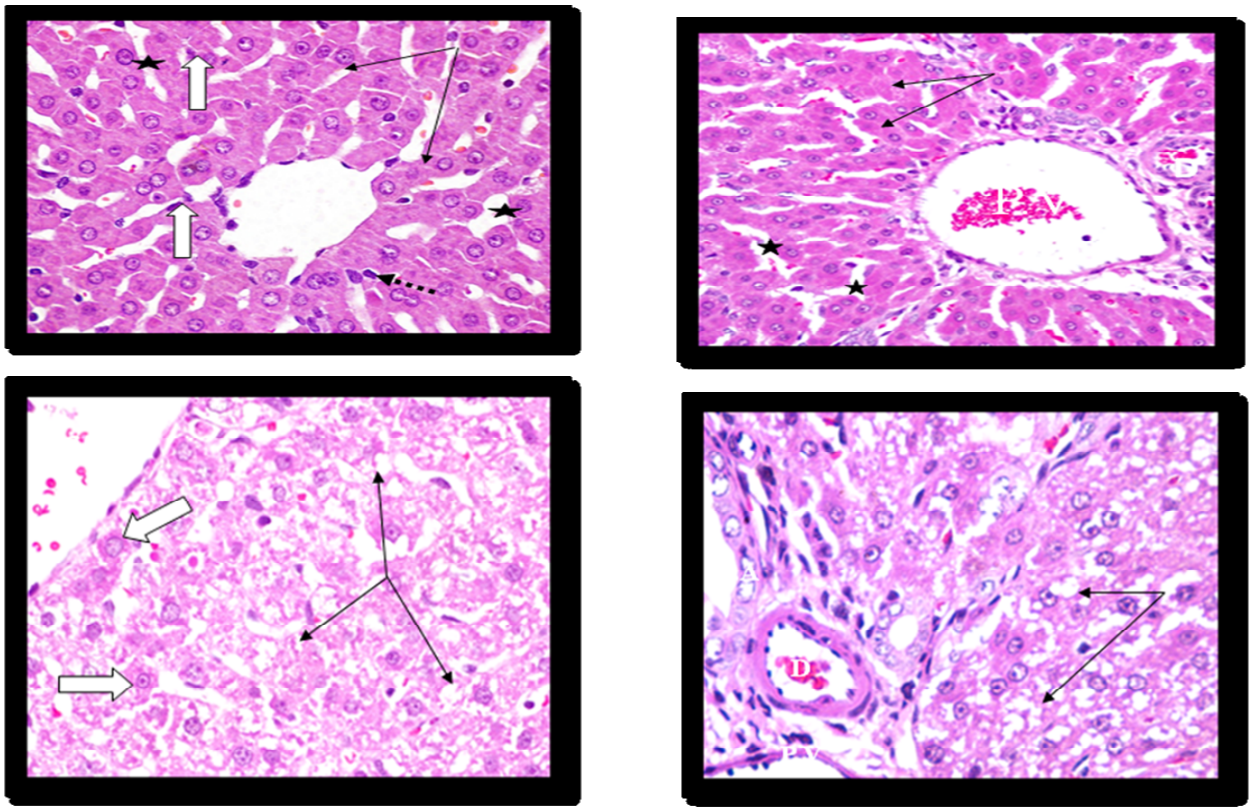

Fig. 2: Normal Hepatocytes & The effect of Patulin on liver parenchyma after one week (T1 Hepatocytes)

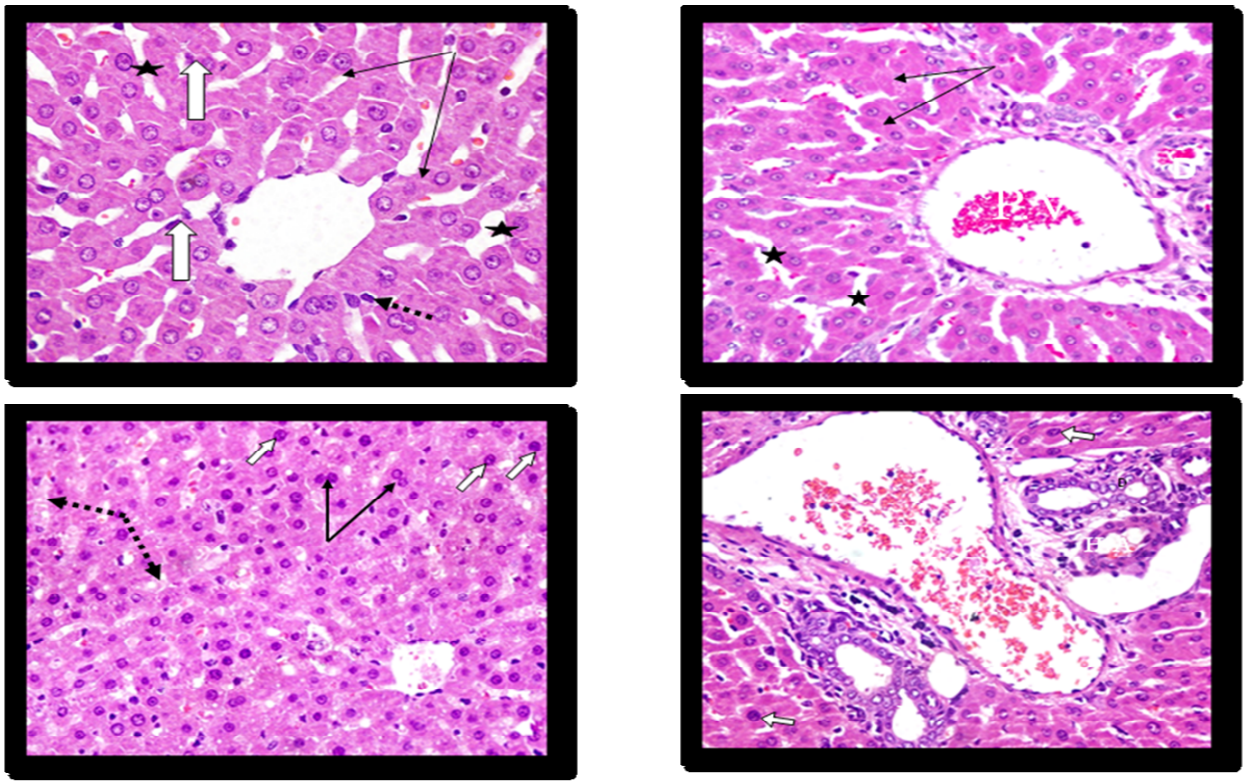

Fig. 3: Normal Hepatocytes & The effect of Patulin on liver parenchyma after two weeks (T2 Hepatocytes)

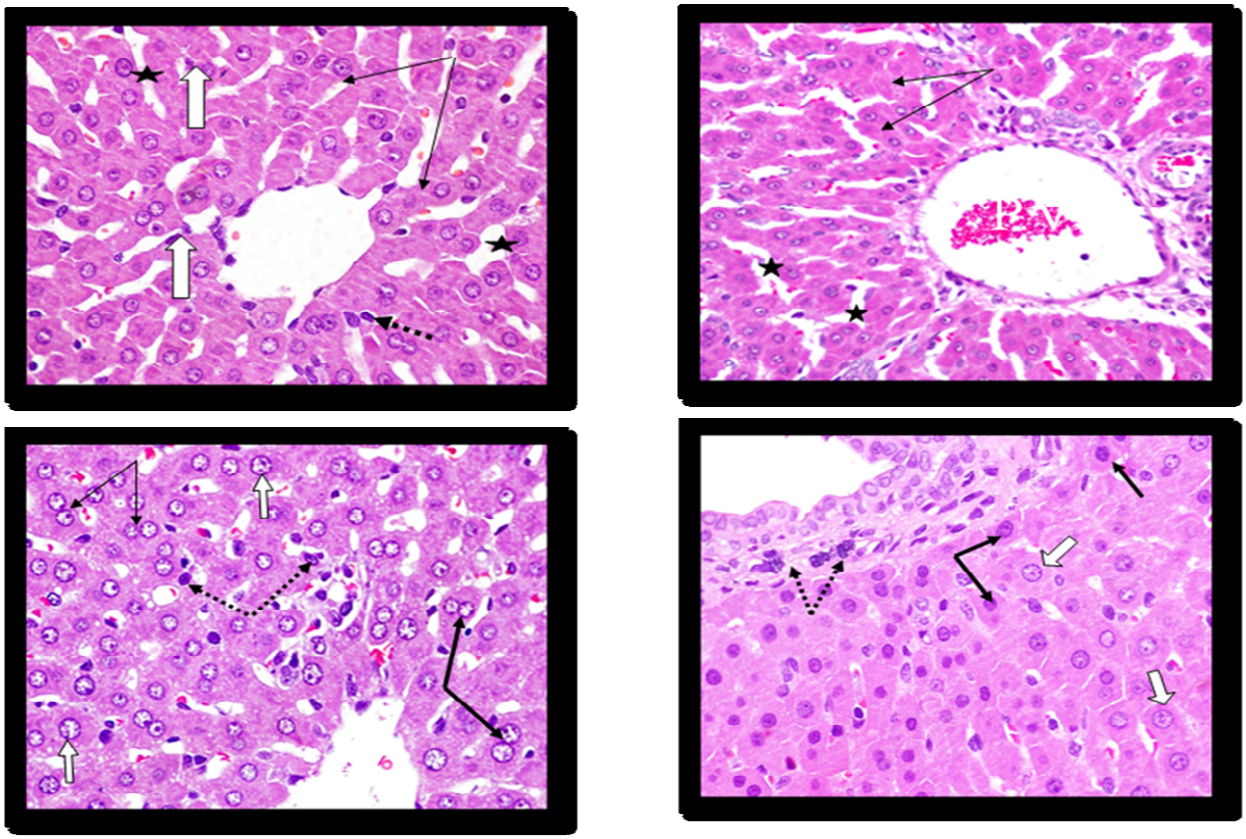

Fig. 4: Normal Hepatocytes & The effect of Patulin on liver parenchyma after three weeks (T3 Hepatocytes)

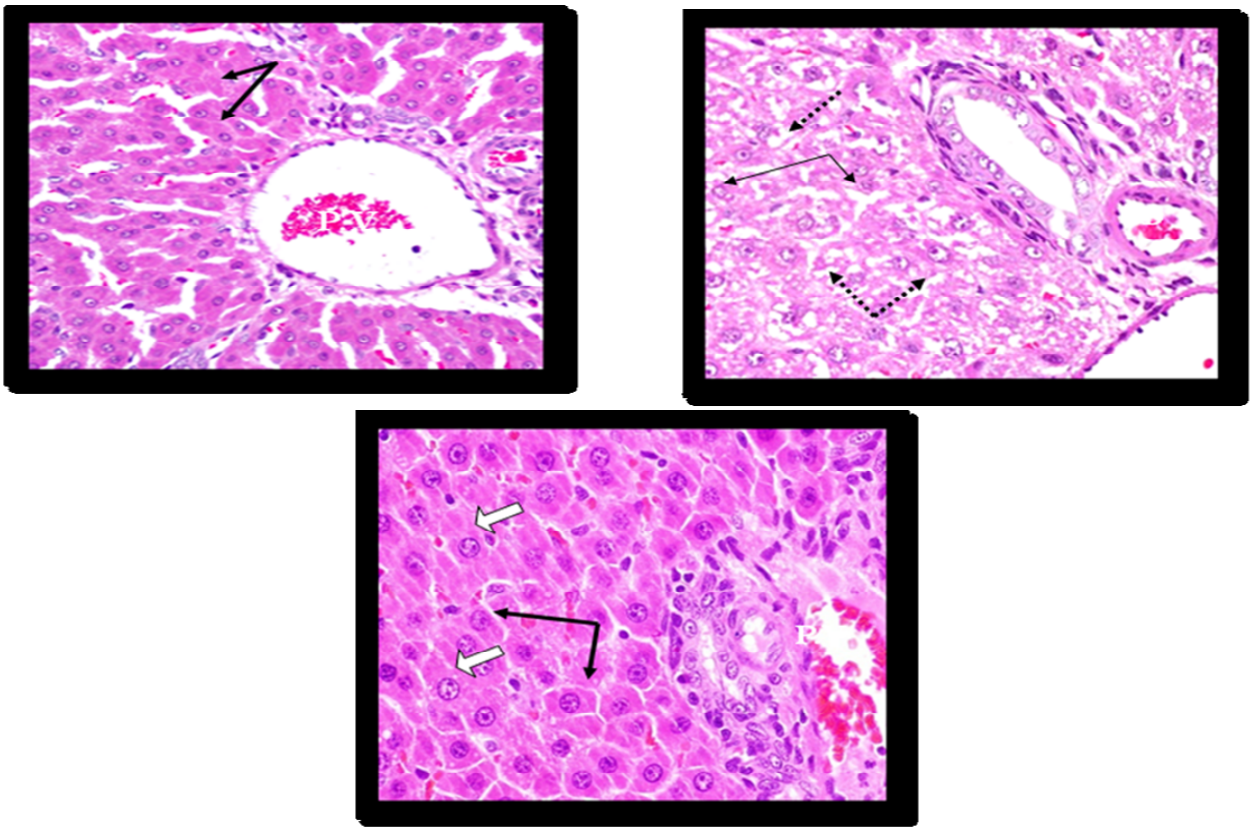

Fig. 5: Normal Hepatocytes & The effect of Patulin on liver parenchyma after one week (T1 Hepatocytes) & The effect of Patulin and crude venom on liver parenchyma after one week (T4 Hepatocytes)

Discussion

From the data in table (**3**), the level of AST and ALT were increased than the control group after administration of Patulin for one week (T1), serum transaminases levels in normal subjects are low and liver tissues is rich in both transaminases. So, the leakage of them into the serum is an indicative of hepatotoxicity (Westhuizen et al., 2001). El-Sawi et al., 2000 reported that the presence of nonfunctional plasma enzymes at levels elevated above normal values suggested an increase rate of tissue destruction.

Table 3: Effect of Patulin (0.2 mg/Kg B.W) on Some Biochemical Parameters of Male Rats.

Parameters \ Groups	Control	T1	T2	T3
AST U/L	35.146 ± 0.42	70.656±0.79 **	72.404±0.87 **	65.596±0.41 **
ALT U/L	41.073 ± 0.68	45.341 ± 0.97 **	39.393 ± 0.71 N.S	40.528 ± 0.99 N.S
GGT U/L	8.203 ± 0.21	9.834 ± 0.41 **	9.539 ± 0.25 **	9.899 ± 0.0.22 **
TNF-α Pg/ml	38.027 ± 0.97	53.925 ± 0.81 **	60.680 ± 0.35 **	93.588 ± 0.98 **
Ferritin µg/ml	1.617 ± 0.03	1.605 ± 0.06 N.S	1.539 ± 0.08 N.S	1.048 ± 0.06 **
Glucose mmol/l	3.495 ± 0.11	3.356 ± 0.14 N.S	4.127 ± 0.06 **	3.974 ± 0.08 **
Glycogen µg/µl	0.052 ± 0.01	0.038 ± 0.004 N.S	0.038 ± 0.005 N.S	0.038 ± 0.004 N.S

Data are expressed as mean ± SE. Number of samples in each group is 10.
T1, T2, T3 = Treated group with Patulin Significant change in comparison between groups:
** = Highly significant ($P \leq 0.01$) * = Significant ($0.01 < P \leq 0.05$)
N.S = Non significant ($P > 0.05$)

The level of AST remained elevated in two weeks (T2) group compared to that of ALT level. From the previous data a highly significant decline of AST level was found in three weeks (T3) group as

compared to two weeks (T2) group, but the level of ALT showed highly significant decrease in two weeks (T2) group compared with one week (T1) group which means gradual recovery of hepatocytes. This means that falling aminotransferase suggests a decrease in hepatocellular damage (Marshall and Bangert, 2004) which was proved in this study by improvement of histological changes in hepatocytes. However, the decrease in Patulin hepatotoxicity in appeared in (T2) and (T3) groups could be explained either by the possibility of metabolic conversion of Patulin into less cytotoxic compound or due to the adaptation of hepatocytes via detoxification process. Another explanation based on its excretion via urine or faces (Moss, 2002; Speijers, 2004).

Otherwise, GGT is a key enzyme implicated in the homeostasis of intracellular reduced glutathione (GSH) and hence in the regulation of the cellular redox state. Besides, the extracellular cleavage of GSH by GGT leads to reactive oxygen species (ROS) production (Accaoui et al., 2000). Moreover, it is known that oxidative conditions occurring during inflammation that evoked by tissue damage can induce the expression of GGT (Zhang et al., 2006).

The results of TNF-α concentration in serum rats summarized in table (**3**), pointed that the concentration was gradually increased between groups in manner related to the duration of treatment. These results are in agreement with those obtained by AL-Anati et al. (2005). TNF-α was reported to induce both necrosis and apoptosis of hepatocytes and the increased concentrations of TNF-α in serum indicated that the production of this cytokine may also be induced (Decicco et al., 1998). These findings indicated that several mycotoxins are able to modulate the production of cytokines in different organs and cell types (Bondy and Pestka, 2000; Oswald and Come´ra, 1998) which signified that Patulin treatment results in the ROS production in mammalian cells and ROS partially contributes to PAT- induced cytotoxicity (Liu et al., 2007).

Ferritin is used for assessment of malignant disease and liver disorders (Sirus and Yusuf, 2006). It is a major iron-storage molecule in the human body cells and has thus been used for decades as a diagnostic indicator of iron concentration in liver and other tissues (Finch et al., 1986; Hasan et al., 2006). Intracellular iron can participate in the formation of free radicals, leading to liver cell damage. This may be prevented by the ability of ferritin to oxidize and store iron (Hagen et al., 2002). However, in liver diseases, redox is increased thereby damaging the hepatic tissue (Muriel, 2009).

The data in the present work, table (**3**) revealed that Patulin induced highly significant decrease in the mean value of ferritin concentration in three weeks group (T3) only. While a non significant decrease in the mean value of ferritin concentrations were noticed after one and two weeks groups (T1, T2) which indicate that the interaction of free radicals, as a result of Patulin accumulation in (T3) group. Ferritin is probably also involved in the pathogenesis of some inflammatory diseases including hepatitis (Biemond et al., 1988). The decrease of ferritin level may be attributed to the induction of oxidative stress by Patulin (Liu et al., 2007) leading to the distruption in iron homeostasis (Erikson et al., 2006) which resulting from impaired liver function (Ganz, 2009).

Concentrations of ferritin in serum increase in a variety of conditions that include cancer, liver disease, inflammation, iron overload, or iron treatment (Tran et al., 1997). Hann et al. (2006) supported that increased serum ferritin levels are associated with an increased risk of primary hepatocellular carcinoma (PHC) which contradict with this work results.

Carbohydrates serve numerous functions in the body, but the bulk of the carbohydrate ingested is used to derive energy for metabolism. The level of glucose in blood of normal animals, and consequently the amounts available to body organs, is closely regulated through the action of several hormones. When carbohydrate intake is high, excess glucose is converted to a glycogen, a glucose polymer, for storage, but the amount of glycogen storage is limited (Donaldson, 2001).

Our results in table (**3**) revealed that the mean value of glucose concentration was not changed in Patulin treated group for one week (T1) but highly significant increased in treated groups for two (T2) and three (T3) weeks compared to the control group. These results were confirmed by Devaraj et al. (1983) in which Patulin is found to interfere with the carbohydrate metabolism. Otherwise, Sakithisekaran et al. (1989) and Wouters and Speijers (1996) demonstrated that via the gluconeogenesis which is stimulated by increase glucose-6-phosphatase and fructose-1, 6-diphosphatase activity. Patulin was proved to be diabetogenic by Devaraj et al. (1986) who explained the elevation of fasting blood glucose level through glucose tolerance test which revealed an elevated glucose curve and reduced insulin production. Also, Speijers (2004) reported that Patulin increase blood glucose level by 60 %. By looking to table (**3**), the mean value of liver glycogen concentration was slightly decreased but not significant in treated groups compared to control group. These results were in accord with the study by Suzuki et al (1974) who found depletion of glycogen in liver after citrinin toxicity. Also, Rastogi et al

(2000) reported that single doses of aflatoxin B1 decreased liver glycogen.

In addition, the present work was done to examine the protecting effect of crude venom extracted from jelly fish Cassiopea Andromeda against Patulin hepatotoxicity through bradykinnin content that was the effector peptide of kallikrein-kinin system.From the obtained data, table (**4**) the serum level of AST, ALT and GGT enzymes showed highly significant decrease in (T4) group as compared to (T1) group and as previously reported in table (**3**), these transaminases and GGT were highly significant increased in (T1) group compared to control group. Crude venom extracted from jelly fish, Eutonina indicans was proved by Abdel-Rehim et al. (1996) to have no effect on liver enzymes activities which indicate its safty use in lab animals. So, decreased aminotransferases was indicator to reduced liver damage (Sanchro-Bru et al., 2007). This means that extracted crude venom of jelly fish Cassiopea Andromeda ameliorate the hepatotoxic effect of Patulin administrated for one week.

On the other hand, only the level of AST was still highly significant increase in (T4) group compared with control group. This result was coincident with the studies done by Omran and Abdel-Rahman (1992); Assi and Nasser (1999); Pipelzadeh et al. (2006) who using crude venoms of different sources. This increase may due to the presence of AST in organs other than the liver. Thus AST level in rats has a limited value as specific endpoint in toxicological studies (Bondy et al., 2000).

Table 4: Effect of Crude Venom (1.78 mg/ 20 g b.w) on Some Biochemical Parameters in Male Rats after Patulin (0.2mg/kg b.w.) Hepatotoxicity.

Parameter	Control	T 1	T 4
AST U/L	35.146 ± 0.42	70.656±0.79	51.253±0.71
			**
		●●	●●
ALT U/L	41.073 ± 0.68	45.341 ± 0.97	41.232 ± 0.84
			**
		●●	N.S
GGT U/L	8.203 ± 0.21	9.834 ± 0.41	8.819 ± 0.24
			*
		●●	N.S
TNF Pg/ml	38.027 ± 0.97	53.925 ± 0.81	46.445 ± 0.67
			**
		●●	●●
Ferritin µg/ml	1.617 ± 0.03	1.605 ± 0.06	1.607 ± 0.03
			N.S
		N.S	N.S
Glucose mmol/l	3.495 ± 0.11	3.356 ± 0.14	3.217 ± 0.08
			N.S
		N.S	N.S
Glycogen µg/µl	0.052 ± 0.01	0.038 ± 0.004	0.037 ± 0.003
			N.S
		N.S	N.S

Data are expressed as mean ± SE. Number of sample in each group is 10.
T1= Treated group with Patulin T4 = Treated group with Patulin + crude venom of jelly fish
Significant change in comparison between groups: **, ●● = Highly significant (P ≤ 0.01)
* = Significant (0.01<P ≤ 0.05) N.S = Non significant (P > 0.05)
First significant change as compared with T1, Second significant change as compared with control.

By the examination of the effect of jelly fish crude venom on Patulin toxicity in rats, the crude venom was found to decrease significantly the concentration of tumor necrosis factor-alpha (TNF-α) in (T4) group compared with (T1) group. This improvement may be attributed to the protective effects of crude venom bradykinnin and related polypeptide (Burnett and Calton, 1977). On the other hand, the bradykinnin was known to stimulate the release of prostaglandins (PGs) in a variety of animal tissues (Levant et al., 2006). PGs in turn was reported to improve the hepatic function and structure (Lkeya et al., 2002; Fouda, 2004; Rincon-Sanchez et al., 2005) and required for liver regeneration and involved in the regulation of a number of cytokines (Rudnick et al., 2001) acting to limit TNF-α. The mechanisms by which prostaglandins limit TNF-α mRNA levels may underlie endogenous regulatory mechanisms that limit inflammation, and may have important implications for understanding chronic

inflammatory disease pathogenesis (Stafford and Marnett, 2007).

Otherwise, the mean value of TNF-α was highly increased in (T4) group compared to control group. Increased serum concentration of TNF-α as one of the small peptide molecules which act as an important mediator in the regulation of the immune and inflammatory responses (Bouhet and Oswald, 2005) indicated that organs or tissue other than the liver could be affected (Decicco et al., 1998).

The obtained data from the present investigation (table **4**) revealed that the mean value of ferritin, glucose and glycogen concentrations were not significantly changed in (T4) group compared to (T1) group and also control group. This phenomenon indicates that the Patulin had no effect on these parameters in (T1) group and parallel to each other on the same results of (T4) group. In the present study, it was observed that administration of Patulin (0.2 mg/kg b.w.) to laboratory rats, affects the histological structure of liver parenchyma. Individual variation regarding sensitivity to toxic effect was observed in different animals and similar observation was reported by El-Sawi and Habib (2004) as a sign of multi drug gene resistance. The effect of Patulin on liver parenchyma varies in different weeks. In animal group of one week (T1), there was evident hepatocytes vaculation and damage of cellular membrane (necrosis), while in group of two weeks (T2) the histological changes were less compared to those of one week (T1). Instead of vacuolation, scattered apoptotic cells were observed in groups of two weeks (T2) and three weeks (T3). The proliferation of bile ducts in group of three weeks (T3) was described to be associated with hepatic metabolic disturbance or toxic exposure (George et al., 2001). The nuclei showed marked variation in size, Pyknosis (small dark nuclei), karyomegally (large sized) and karyolysis (degeneration) were observed. The enlarged nuclei was observed mainly in animals given Patulin for two weeks (T2) and three weeks (T3) and also in jelly fish treated group (T4) which may as a result of hepatocytes toxicity (Fong et al., 2004).

The significant elevation of liver enzymes observed in the present study in treated groups could be explained in view of histological finding which showed cellular membrane damage especially in group of one week (T1) where the specific liver enzyme ALT was high. Its value was returned to normal level in groups of two weeks (T2) and three weeks (T3). This support histological observation where hepatocytes appeared with intact outlines and only focal apoptosis were observed. The focal damage in hepatocytes of two weeks group (T2) could explain the sustained elevation of AST and GGT levels in this group. Similar association between biochemical finding and histological changes was reported in literature (Wu et al., 2007).

TNF-α was a known cytokine secreted by many cell types including kupffer cells, and macrophages (Gonzalez-Amaro et al., 1994). In the present study Von Kupffer cells, a well known fixed phagocyte of liver, were found to be increased. TNF-α was reported by Fong et al. (2004) to induces apoptosis in target cells that express TNF-α receptors on their cell membrane. Apoptotic hepatocytes were observed especially in groups of two weeks (T2) and three weeks (T3) where TNF-α was described to be significantly high in their serum. Apoptosis was reported to be an effective process for the elimination of unneeded, diseased or transformed cells (D'agostini et a.l, 2001). It causes cell death in a way that differs morphologically and biochemically from necrosis. Earlier studies

Apoptosis was reported to be an effective process for the elimination of unneeded, diseased or transformed cells (D'agostini et a.l, 2001). It causes cell death in a way that differs morphologically and biochemically from necrosis. Earlier studies showed that the common core mechanism of apoptosis is a DNA fragmentation and nuclear morphological lesions, such as condensation and fragmentation (Schwartman and Cidlowski, 1993). In this study apoptotic hepatocytes showed dark stained cytoplasm and condensed small nuclei.

The current study showed scattered apoptotic cells in groups of two weeks (T2) and three weeks (T3) which are in accord with what was reported with other mycotoxins such as ochratoxin A (Bokhari and Ali 2006). Also, Atroshi et al. (2000) reported that any given toxins may induce both apoptosis and necrosis, depending on the dose and period of administration and attributed the changes to the increase in reactive oxygen species (ROS).

On the other hand, inflammation plays a role in classical chemical toxicity as follows: initial toxic injury produces focal tissue damage and necrosis in target organ (Luster et al., 2001). Necrosis and cellular vaculation were initially observed in group after one week (T1) of Patulin administration. The same authors reported that as a result of cellular damage (necrosis), tissue fixed macrophages, are activated and secrete inflammatory products including the proinflammatory cytokine tumour necrosis factor TNF-α, which is a central regulator that aids in tissue repair by stimulating apoptosis and cell proliferation as well as exacerbating cell damage by initiating an overly aggressive inflammatory process. The same was observed in the next groups of Patulin administration after two weeks (T2) and three weeks (T3) where apoptosis was dominant over

necrosis. The inability of the system to neutralize the excessive release of reactive oxygen species are responsible for cell damage or activating genes responsible for cell proliferation (Luster et al., 2001).

To our knowledge, there is no detailed information regarding Patulin pharmacokinetics in human (absorption and metabolism) or toxicokinetic behaviour. Future studies were needed to investigate the cumulative chronic exposure to such mycotoxin. The question here, can the crude venom of jelly fish in the present work use as a new therapeutic approach for enhancing hepatocytes detoxification of mycotoxins? The present work recommended that different feedstuffs or fruit juices known to be liable for mycotoxin contamination must be carried out and checked periodically before popular consuming.

4. Conclusions

The present study indicated that the biochemical and histological effect of Patulin was time dependent. Variation in degree of response was observed in different animals in the same group. Also, the results were pointed to the potential protective effect of crude venom extracted from jelly fish Cassiopea Andromeda which can be useful to protect humans or animals against the adverse health effects of this mycotoxin. This study could be considered preliminary as there are no published studies dealing with Patulin hepatotoxicity on studied parameters.

Acknowledgments

To all staff members of the Biochemistry Department, Faculty of Science. Special thanks to Dr. Soad Shaker for her assistant in histology. I would like to express my thanks to King Abdul aziz City for Science and Technology for the financial support of this work and very grateful to my parents, husband, sisters and brothers.

References

1. Jarvis, B. B. and Miller, J. D. Mycotoxins as harmful indoor air contaminants. Appl. Microbiol. Biotechnol. 2005, 66:367-372.
2. Sweeney, M. J. and Dobson A. D. W. Mycotoxin production by Aspergillus, Fusarium and Penicillium species. International Journal of Food Microbiology, 1998, 43: 141–158.
3. Drusch, S. and Ragab, W. J. Mycotoxins in fruits, fruit juices, and dried fruits. Food Prot. 2003, 66, 1514-1527.
4. Frisvad, J. C. and Thrane, U. Mycotoxin production by foodborne fungi. In: introduction to foodborne fungi. 5th edition. Edited by: Samson, R. A., Hokestra, E. S., Frisvad, J. C. and Filtenborg, O. Centraalbureau voor schimmelcuktures: Barn, The Netherlands. 1996, 251-260.
5. Harvey, A. L., Bradley, K. N., Cochran, S. A., Rowan, E. G., Pratt, J. A., Quillfeldt, J. A. and Jerusalinsky, D. A. What can toxins tell us for drug discovery. Toxicon. 1998, 36:1635–1640.
6. Burnett, J. W. and Calton, G. J. The chemistry and toxicology and some venomous pelgic coelenterates. Toxicon. 1977, 15:177-196.
7. Ramalho, F. S., Ramalho, L. N., Castro-e-silva, J. O., Zucolota, S. and Correa, F. M. Effect of angiotension-converting enzyme inhibitors on liver regeneration in rats. Hepatogastroentrology 2002, 49 (47), 1347-1351.
8. Campbell, D. J., Aloxiou, T. and Xiao, H. D. Effect of reduced angiotension-converting enzyme gene expression and angiotension-converting enzyme inhibition on angiotension and bradykinnin peptide level in mice. Hypertension 2004, 43, 854-859.
9. Levant, A., Levy, E., Argaman, M. and Fleisher-Berkovich, S. Kinins and neuro inflammation:dual effect on prostaglandin synthesis. Eur. J. Pharmacol. 2006, 07:06:54.
10. Arend, A., Aunopuu, M., Masso, R. and Selstam, G. Prostaglandin of the E series inhibit connective tissue proliferation in the liver wound of the rat. Ann. Anat. 2005, 187 (1), 57-61.
11. George, J., Rao, K. R., Stern, R. And Chandrakasan, G. .Toxicology 2001, 156 (2-3), 129-138.
12. Westhuizen, L. V., Shephard G. S., Schalkwyk, D. J. (2001) The effect of a single gavage dose of fumonisin B1 on the sphinganine and sphingosine levels in vervet monkeys. Toxicon 39: 27-281.
13. El Sawi, N. M, El.Wessimy, M. M amd Osama, Y. A. (2000) Biochemical studies of verrucarin J in male mice liver. Toxin review. 19: 384.
14. Marshall, W. J. And Bangert, S. K. (2004). Clinical Chemistry. 5th edition. Mosby, London. 85- 89.
15. Moss, M. O. (2002) Mycotoxin review-1. Aspergillus and Penicillium. Mycologist. 16(3):116-119.
16. Speijers, G. J. A. (2004) Patulin. In: Mycotoxins in foods: detection and control. Edited by: agan, N. and Olsen, M. CRC Press. Woodhead publishing limited, Cambridge, England. 340,344.
17. Accaoui, M., Enoiu, M., Mergny, M., Masson, C. Dominici, S., Wellman, M. and Visvikis, A. (2000) Gamma-Glutamyltranspeptidase-Dependent Glutathione Catabolism Results in Activation of NF-Kb. Biochemical and Biophysical Research Communications. 276: 1062–1067
18. Zhang, H., Liu, H., Dickinson, D.A., Liu, R. M., Postlethwait, E. M., Laperche, Y. and Forman, H.J. (2006) Gamma-Glutamyl transpeptidase is induced by 4-hydroxynonenal via EpRE/Nrf2

signaling in rat epithelial type II cells, Free Radic. Biol.Med. 40 :1281–1292.
19. AL-Anati, L., Katz, N. and Petzinger, E. (2005) Interference of arachidonic acid and its metabolites with TNF-α release by ochratoxin A from rat liver. Toxicology 208: 335–346
20. Decicco, L. A., Rikans, L. E., Tutor, C. G., Hornbrook K. R. (1998) Serum and liver concentrations of tumor necrosis factor a and interleukin-1b following administration of carbon tetrachloride to male rats. Toxicology Letters 98: 115–121.
21. Bondy, G.S. and Pestka, J.J. (2000) Immunomodulation by fungal toxins. J. Toxicol. Environ. Health B: Crit. Rev. 3: 109–143.
22. Oswald, I.P. and Come´ra, C. (1998) Immunotoxicity of mycotoxins. Rev. Med. Vet. 149: 585–590.
23. Liu, B. H., Wu, T. S., Yu, F. Y. and Su, C. C. (2007) Induction of oxidative stress response by the mycotoxin Patulin in mammalian cells. Toxicol Sci. 95: 340-7.
24. Sirus, J. S. and Yusuf, B. (2006) Study of alpha-fetoprotein, ferritin levels and liver ultrasonic findings in hemodialysis patients possessing hepatitis C virus antibodies in Tabriz. Pak. J. med. Sci. 22(2):154-157.
25. Finch, C. A., Bellotti, V., Stray, S., Lipschitz, D. A., Cook, J. D. Pippard, M. J. and Huebers, H. A. (1986) Plasma ferritin concentration as a diagnostic tool. West. J. Med. 145(5): 657-663.
26. Hasan, M. R., Koikawa, S., Kotani, S., Miyamoto, S. and Nakagawa, H. (2006). Ferritin forms dynamic oligomers to associate with microtubules in vivo: Implication for the role of microtubules in iron metabolism. Exp. Cell Res. 312(11): 1950-1960.
27. Hagen, K. Eckes, K., Melefors, O and Hultcrantz, R. (2002) Iron Overload Decreases the Protective Effect of Tumour Necrosis Factor-α on Rat Hepatocytes Exposed to Oxidative Stress. Scandinavian Journal of Gastroenterology. 37(6): 725-731.
28. Muriel, P. (2009) Role of free radicals in liver diseases. Hepatol Int. 3(4): 526–536.
29. Biemond, P., Swaak, A. J. G., van Eijk, H. G. and Koster, J. F. (1988) Superoxide dependent iron release from ferritin in inflammatory diseases. Free Radical Biology and Medicine. 4 (3):185-198.
30. Erikson, K. M., Dormal, B. C.,Fitfamakif, V., Lash, L. H. and Afchner, M. (2006). Alteration of oxidative stress biomarker due to outer and neonatal exposures of airborne manganese. Biol. Trase. Lem. Res. III (1-3):199-215.
31. Ganz, T. (2003) Iron homeostasis. Cell metabolism. 7 (4):288-290.
32. Tran, T. N., Eubanks, S. K., Schaffer, K. J., Zhou C. Y. and Linder, M. C. (1997) Secretion of ferritin by Rat Hepatoma Cells and Its Regulation by Inflammatory Cytokines and Iron. Blood. 90(12): 4979-4986
33. Hann, H. L., Kim, C. Y., London, T. and Blumberg, B. S. (2006) Increased serum ferritin in chronic liver disease: A risk factor for primary hepatocellular carcinoma. International Journal of Cancer. 43(3): 376 – 379.
34. Donaldson, W. E. (2001) Nutritional factors. In: introduction to biochemical toxicology. 3 rd edition. Edited by: Hodgson, E. and Smart, R. C. Wiley interscience, New York. 256.
35. Devaraj, H., Shanmugasundaram, K. R. and Shanmugasundaram, E. R. B. (1983). Changes in carbohydrates metabolism during patulin toxicosis studied in chicks. Journal of the Indian Institute of Science. Bangalore. 46C(6):65-71.
36. Wouters, M. F. A. and Speijers, G. J. A. (1996) Toxicological evaluation of certain food additives and contaminants in food, Patulin. WHO Food Additives Series. 35: 377-402.
37. Speijers, G. J. A. (2004) Patulin. In: Mycotoxins in foods: detection and control. Edited by: Magan, N. And Olsen, M. CRC Press. Woodhead publishing limited, Cambridge, England. 340,344.
38. Suzuki, S., Satoh, T. and Yamazaki, M. (1975) Effect of ochratoxin A on carbohydrate metabolism in rat liver. Toxicology and Applied Pharmacology. 32 (1): 116-122.
39. Rastogi, R., Srivastava, A.K., Srivastava, M. and Rastogi, A. K. (2000) Hepatocurative Effect of Picroliv and Silymarin Against Aflatoxin B_1 Induced Hepatotoxicity in Rats. Planta Med. 66(8): 709-713.
40. Abdel-Rehim, S. A., El-Sawi, N. M. and Aboul-Dahab, H. M. (1996) Effect of extracted crude venom from jelly fish *eutonina indicans* on liver function in male mice. Assiut Vet. Med. J. 34 (68):56-67
41. Sancho–Bru, P., Bataller, R., Fernandez–Varo, G., Moreno, M. *et al*. (2007) Bradykinin Attenuates Hepatocellular Damage and Fibrosis in Rats with Chronic Liver Injury. Gastroenterology.133:2019–2028.
42. Omran, M. A. and Abdel-Rahman, M. S. (1992) Effect of scorpion *Leiurus quinquestriatus* (H&E) venom on the clinical chemistry parameters of the rat. Toxicology letters. 61(1): 99-109.
43. Assi, A. and Nasser, H. (1999) An in vitro and in vivo study of some biological and biochemical effects of Sistrurus Malarius Barbouri venom. Toxicology. 137: 81–94.
44. Pipelzadeh, M. H., Dezfulian, A., Jalali, M. T. and Mansouri, A. (2006) In vitro and in vivo studies on some toxic effects of the venom from Hemiscorpious lepturus scorpion. Toxicon. 48: 93–103
45. Bondy, G. S., Armstrong, C.L., Curran, I.H.A., Barker, M.G. and Mehta, R. (2000). Retrospective

evaluation of serum ornithine carbamyltransferase activity as an index of hepatotoxicity in toxicological studies with rats. Toxicology Letters. 114:163–171
46. Burnett, J. W. and Calton, G. J. (1977) The chemistry and toxicology and some venomous pelgic coelenterates. Toxicon. 15:177-196.
47. Levant, A., Levy, E., Argaman, M. and Fleisher-Berkovich, S. (2006) Kinins and neuro inflammation:dual effect on prostaglandin synthesis. Eur. J. Pharmacol. 07:06:54
48. Lkeya, K., Kume, M., Sato, H., kashimoto, S. and Kumazawa, T. (2002). Effect of prostaglandin E1 infusion during and after total hip arthoplasty under hypotensive anesthesia on postoperative liver function and hemorrhage. Masui. 51(4): 377-81.
49. Fouda, D., Siendones, E., Costan, G., and Muntane, J. (2004) Role of NE-Kappa B activation and nitric oxide expression during PGE protection against d-galactosamin- induced cell death in cultured rat hepatocytes. Liver Int. 24(3): 227-236
50. Rincón-Sánchez, A. R., Covarrubias, A., Rivas-Estilla, A. M., Pedraza-Chaverrí, J. et al. (2005) PGE2 alleviates kidney and liver damage, decreases plasma renin activity and acute phase response in cirrhotic rats with acute liver damage. Exp Toxicol Pathol. 56(4-5):291-303.
51. Rudnick, D. A., Perlmutter, D. H. and Muglia, L. J. (2001) Prostaglandins are required for CREB activation and cellular proliferation during liver regeneration. PNAS. 98(15): 8885–8890.
52. Stafford, J. B. and Marnett, L. J. (2008) Prostaglandin E_2 inhibits tumor necrosis factor-alpha RNA through PKA type I. Biochemical and Biophysical Research Communications. 366(1): 104-109.
53. Bouhet, S. and Oswald, I. P. (2005) The effects of mycotoxins, fungal food contaminants, on the intestinal epithelial cell-derived innate immune response. Veterinary Immunology and Immunopathology.108:199–209
54. Decicco, L. A., Rikans, L. E., Tutor, C. G., Hornbrook K. R. (1998) Serum and liver concentrations of tumor necrosis factor a and interleukin-1b following administration of carbon tetrachloride to male rats. Toxicology Letters 98: 115–121.
55. El Sawi, N. M. and Habib, T. N. (2004) The genetic susceptibility of male mice exposed to verrucarin J toxin. Histol. Histochem., 45C: 307-326.
56. George, J., Rao, K. R., Stern, R. and Chandrakasan, G. (2001) Dimethylnitrosamine-induced liver injury in rats: the early deposition of collagen. Toxicology. 156 (2-3): 129-138.
57. Fong, R.N., Gonzalez, B. P., Fuentealba, I.C. and Cherian, M.G. (2004) Role of tumor necrosis factor-α in the development of spontaneous hepatic toxicity in long-evans cinnamon rats. Toxicology and applied pharmacology. 200:121-130.
58. Wu, Y., Li, L., Wen, T. And Li, Y. (2007) Protective effects of echinacoide on carbon tetrachloride induced hepatotoxicity in rats. Toxicology. 232(1-2): 50-56.
59. Gonzalez-Amaro, R., Garcia-Monzon, C., Garcia-Buey, L., Moreno-Otero, R., Alonso, J.L., Yague, E., Pivel, J.P., Lopez-Cabrera, M., Fernandez-Ruiz, E., Sanchez-Madrid, F. (1994) Induction of tumor necrosis factor alpha production by human hepatocytes in chronic viral hepatitis. J. Exp. Med. 179: 841– 848.
60. D'agostini, F., Balansky, R.M., Lzzotti, A., Lubet, R. A., Kelloff, G. J. and De flora, S. (2001) Modulation of apoptosis by cigarette smoke and cancer chemopreventive agents in the respiratory tract of rats. Carcinogenesis. 22:375-380.
61. Schulman, J. D., Goodman, S. I., Mace, J. W., Patrick, A. D., Tietze, F. and Butler, E. .J. (1975) Glutathionuria: inborn error of metabolism due to tissue deficiency of gamma-glutamyl transpeptidase. *Biochem. Biophys. Res. Commun.* 65 (1): 68–74.
62. Bokhari, F and Ali, S. S. (2006) Ochratoxin A (OTA) contaminated Arabian Coffee seeds: Effects on liver and kidney functions and possible protective role of Vitamin C. Assuit university 5th pharmaceutical Sciences Conference Faculty of pharmacy . Assuit March.
63. Atroshi, F., Biese, I. and Saloniemi, H. (2000) Significance of apoptosis and its relationship to antioxidant after ochratoxin a administraton in mice. J pharm pharmaceut Sci. 3(3): 281-291.
64. Luster, M.I., Simeonova, P.P., Gallucci, R.M. Bruccoleri, A., Blazka, M.E. and Yucesoy, B. (2001) Role of inflammation in chemical-induced hepatotoxicity. Toxicology Letters 120:317–321.

9/12/2012