Minufiya J. Agric. Res. Vol.38 No. 3(2): 791-797 (2013) "http://www.mujar.net"

ALBINO HEPATO-RENAL TOXIC EFFECTS OF CHITOSAN ON EXPERIMENTAL MICE, *MUS MUSCULUS*

Amal A. Eisa, G. E. Abo-Elghar, I. M. Ammar and Samah S. Arafa
Pesticides Department, Faculty of Agric., Minufiya University

(Received : Mar. 14 , 2013)

ABSTRACT: *Chitosan possess bioactive properties such as microbial, fungicidal and plant growth regulator. We applied it in 3 different doses (300 mg/kg B.w. as acute dose for the first group, 200 mg/kg followed by 100 mg/kg after one week for the second group, lastly 50 mg/kg weekly for successive 6 weeks as a subchronic treatment) to clear its effect on both kidney and liver functions of albino mice, Mus musculus. These functions included AST and ALT enzymes, total protein and bilirubin as liver functions and creatinine and urea concentrations for kidney functions. Data showed a relationship between chitosan dose and urea since it is increased in the 3 groups of mice, however there was a negative a relationship between given doses and bilirubin. In fact in the treated mice group with repeated doses 50mg/kg AIT increased significantly as indication of chitosan and deleterious effect on liver.*

Key words: *chitosan, kidney, liver, Mus musculus*

INTRODUCTION

Chitosan is the deacetylated form of chitin, an aminopolysaccharide found in the exoskeletons and the fungal cell wall of various arthropods including insects, crabs and shrimp (Muzzarelli,1977). Although it is not derived from plants, it shares the same characteristics as dietary fiber, which is an indigestible polysaccharide by mammalian digestive enzymes (van Bennekum *et al.,* 2005).

Chitosan is used as insecticide, it acts as plant growth regulator, fungicide and antibacterial agent. It was noted on acute dermal oral, inhalation, eye and skin irritation as acute toxicity. Also Chitosan cause loss of fat-soluble vitamins and block absorption of medicines such as birth control pills (Orr, 1999).This study was designed to investigate the hepato-renal toxic effect of Chitosan on experimental mice reported as liver enzymes, bilirubin, total protein, creatinine and urea.

MATERIALS AND METHODS

1-Pesticide Used:

Chitosan Oligomer, is used as antibacterial, antifungal, plant growth regulator and as nematicide. It was obtained as a liquid solution from a private pesticide Company.

2- Chemicals:

All chemicals used in the biochemical analyses were of analytical grade-kits of purity and purchased locally from Vitro and Diamond companies, Egypt.

3-Test Animals and Husbandry:

Swiss albino male, *Mus musculus*, 7-8 weeks old and weighing 22-32 g, obtained from the holding company of biological vaccines (Vacsera), Cairo, were housed in suspended stainless steel cages which had wire-mesh floors. The room was maintained at approximately 28±2 °C, R.H. 60±5 %, 12 h light/darkness) in the Department of Pesticides, Faculty of Agriculture, Minufiya University. The animals were supplied standard pellets feed and tap water *ad libitum*.

4-Experimental design:

The healthy males were reared in the laboratory as mentioned before:

The males were divided into 4 groups (3 treated groups and one severed as a control).

Group 1: The animals were treated orally with a single dose (300 mg/kg body weight). The samples were taken after one week

post-treatment, followed by weekly samples till 5 weeks post-treatment.

Group 2: The animals were treated with 200 mg/kg in the 1st week, then treated with 100 mg/kg body weight in the 2nd week. The samples were taken for five weeks post-treatments.

Group 3: A dose of 50 mg/kg body weight were administered into every males weekly for 6 successive weeks then the blood samples were taken weekly for 6 weeks post- treatments.

5- Biochemical assays:

5.1- Creatinine Assay:

Creatinine concentrations in blood plasma were determined by diamond kits according to Henry, 1974 method.

5.2- Urea assay:

Urea concentrations in blood serum were determined by vitro kits according to Patton and Crouch, 1977 method.

5.3-Alanine Aminotransferase (ALT) and Aspartate Amino Transferase (AST) activities:

They were assayed with the diamond kits according to Reitman and Frankel, 1957 method.

5.4- Bilirubin assay:

The total bilirubin was assayed by the diamond kits according to Jendrassik and Grof ,1938 method.

5.5- Albumin assay:

The albumin was assayed with the diamond kits according to Doumas and Peters, 1997 method.

5.6- Total protein assay:

The total protein was assayed with the diamond kits according to Henry *et al.,* 1964 method.

6- Statistical analysis:

The significance of difference among groups was analyzed using ANOVA followed by the Least Significant Difference test (LSD) of mean. Differences among the groups were considered significant at $P<0.05$. All statistic analyses were made using the Co Stat computer software.

RESULTS :

Data in table (1) cleared that blood urea contents increased significantly after acute treatment with 300 mg / kg Chitosan, While the blood creatinine decreased but not significant.

Also, the liver function was affected. The two enzymes concerned with amino acid metabolism and total protein increased significantly at 1st week comparing with control. The protein increasing is correlated with the treatment of the chitosan. But , the albumin level was still as in the control. The values of the protein content did not differ significantly with consecutive weeks post-treatment.

Table (1): Effect of chitosan at 300 mg/kg b.w administered orally to male mice on liver and kidney functions

Weeks after treatment	Creatinine (mg/dl) ±SD	Urea (mg/dl) ±SD	Total Bilirubin (mg/dl) ±SD	AST (u/ml) ±SD	ALT (u/ml) ±SD	Total protein (mg/dl) ±SD	Albumin (mg/dl) ±SD	Globulin (mg/dl) ±SD	A/G ±SD
1	0.21 ±0.08	10 ±0.50	0.23 ±0.07	19* ±0.25	15 ±0.2	5.8* ±0.52	1.44 ±0.54	4.36* ±0.44	0.33 ±0.11
2	0.22 ±0.11	10 ±0.85	0.12* ±0.10	89 ±0.35	26 ±0.5	5.00 ±0.2	1.34 ±0.5	3.66 ±0.7	0.37 ±0.25
3	0.18 ±0.25	16* ±0.90	0.34 ±0.11	10* ±0.2	10 ±0.1	4.5 ±0.22	1.41 ± 0.42	3.09 ±0.54	0.46 ±0.32
4	0.28 ±0.18	9* ±0.84	0.21 ±0.15	92* ±0.7	15 ±0.54	4.7 ±0.30	1.52 ±0.64	3.18 ±0.52	0.48 ±0.17
5	0.31 ±0.20	17* ±0.92	0.22 ±0.18	78* ±0.27	10 ±0.3	4.3 ±0.48	1.53 ±0.86	2.77 ±0.2	0.55* ±0.22
Cont.	0.25 ±0.10	6.6 ±0.02	0.77 ±0.23	90 ±0.33	17.6 ±0.65	4.36 ±0.42	1.34 ±0.55	3.22 ±0.32	0.45 ±0.11

* Significance levels is based on P < 0.05

When the animals received 300 mg / kg through two times (200 + 100 mg / kg) and the biochemical determination were done as shown in (Table 2). The creatinine value increases only after 1 and 3 weeks post-treatment. There were no significant difference between them. But on the opposite, the urea content increased significantly comparing with control. With treatment of chitosan, both AST and ALT differ from the control either by decrease or increase comparing with the control, also, bilirubin as a biological marker of liver function was affected.

Total bilirubin decreased not significantly after all weeks post-treatment. The enzymes AST and ALT did not increase, this mean that the liver function did not affected as result of chitosan treatment, since, the elevation serum content of these enzymes refer to liver disorders. The total protein content increased significantly at the 1st week comparing with the untreated animals. The treatment with chitosan did not affect the total protein content. All the values of the protein elevated, protein content was the highest at 1st and 2nd week post-treatment than the other weeks far from the treatment.

Table (3) shows effect of acute and sub-acute treatments and exposure to chitosan on kidney and liver functions when the animals were received 300 mg/kg B.w orally through 6 weeks respectively.

There was not significant differences between the creatinine content after all treatments and the control and the same result was obtained with the urea content.

Discussion:

Proteins are the most abundant compounds in the serum. They are the basic components of enzymes, many hormones, antibodies and clotting agent. Proteins also are the building blocks of all cells and body tissues. They form the cellular structural elements, they are biochemical catalysts, and are important regulators of gene expression.

As for the ALT, the most specific enzyme for the liver function, it increased significantly in the third treated group comparing with the control, referring to liver disorders.

Table (2): Effect of 300 mg/kg b.w chitosan administered orally sporadically (200 mg/kg followed by 100 mg/kg after one week) on liver and kidney functions

Weeks after treatment	Creatinine (mg/dl) ±SD	Urea (mg/dl) ±SD	Total Bilirubin (mg/dl) ±SD	AST(u/ml) ±SD	ALT(u/ml) ±SD	Total protein(m g/dl) ±SD	Albumin (mg/dl) ±SD	Globulin (mg/dl) ±SD	A/G ±SD
1	0.31±0.23	22±0.50	0.21±0.11	80±0.15 *	13±0.8	5.8±0.32*	1.6±0.12	4.2±0.48*	0.38±0.22
2	0.22±0.03	9±0.30*	0.34±0.25	78±0.8	12±0.5*	5.0±0.28	1.7±0.16	3.3±0.81	0.52±0.32
3	0.34±0.08	22±0.70	0.13±0.08	89±0.19*	13±0.3	4.5±0.17	1.50±0.13	3±0.73	0.50±0.20
4	0.11±0.10	17±0.10*	0.49±0.34	29±0.12 *	10±0.2	4.7±0.6	1.4±0.51	3.3±0.21	0.42±0.11
5	0.23±0.14	14±0.40*	0.23±0.012	89±0.10	10±0.4	4.6±0.22	1.7±0.07*	2.6±0.25	0.65±0.15
Cont.	0.25±0.10	6.6±0.02	0.77 ±0.23	90±0.33	17.6±0.65	4.36±0.42	1.34±0.55	3.22±0.32	0.45±0.11

* Significance levels is based on P < 0.05

Table (3): Effect of repeated doses of 50 mg/ kg b.W of Chitosan administered orally to males on liver and kidney

Weeks after treatment	Creatinine (mg/dl)±SD	Urea (mg/dl) ±SD	Total Bilirubin (mg/dl)±SD	AST (u/ml) ±SD	ALT (u/ml)±SD	Total protein (mg/dl) ±SD	Albumin (mg/dl) ±SD	Globulin(mg/dl) ±SD	A/G ±SD
1	0.21±0.02	16±0.4	0.33±0.11*	99±0.21*	10±0.2 *	5.1±0.32	1.41±0.07	3.7±0.7	0.38±0.32
2	0.21±0.20	7±0.5	0.21±0.15*	89±0.15 *	12±0.1 *	4.7±0.14	1.5±0.11	3.2±0.5	0.47±0.25
3	0.24±0.11	10±0.2	0.32±0.23*	78±0.8 *	13±0.4	4.6±0.40	2.4±0.23 *	2.2±0.31	1.91±0.55*
4	0.33±0.23	13±0.3	0.51±0.35*	75±0.16 *	10±0.3 *	3.7±0.02*	1.5±0.29	2.2±0.36	0.68±0.36
5	0.34±0.29	17±0.4	0.22±0.08*	59±0.3 *	13±0.2	4.5±0.22	1.6±0.51	2.9±0.18	0.55±0.21
6	0.24±0.18	16±0.2	0.24±0.10 *	44±0.7 *	15±0.1 *	5.3±0.30	1.5±0.12	3.8±0.24	0.39±0.10
Cont.	0.24±0.25	5.5 ± 0.22	0.84± 0.51	90.3± 0.15	17.5± 0.42	4.6± 0.32	1.32± 0.14	3± 0.11	0.44±0.25

* Significance levels is based on $P < 0.05$

Liver plays important roles in metabolism to maintain energy level and structure stability of body (Guyton and Hall, 1996). It is also a site of biotransformation by which a toxic compounds has been transformed in less harmful form to reduce toxicity (Hodgson, 2004)

When the liver is damaged Alanine transaminase (ALT) enzyme, increased in the liver and is released in the blood stream. Aspartate tranaminase (AST) is an enzyme plays a role in the metabolism of the amino acid alanine. An increase of AST levels may indicate liver damage or disease. AST is a mitochondrial enzyme, predominantly found in the liver, skeletal muscles and kidneys. ALT is a cytsolic enzyme, which is more specific for the liver than aspartate transaminase.

Increased levels of these enzymes in blood are the result of treatment and indicative of toxic liver necrosis (Poli and Dianzani, 1987).

The decrease of both ALT and AST in this study didn't agree with that was obtained (Srinivasan and Radhakrishnamurthy , 1977), Srivastava *et al.,* (1989), Rao and Banerji (1990), Rahman *et al*., (1996), Rahman *et al*., (2001) and Sahni and Saxena (2001) in albino rat with hexachlorohexan treatment.

The increase in transaminase activity in the liver is indicative of liver damage that occurs due to formation of reactive oxygen species and reactive intermediates after treatment of pesticides (Bandyopadhyay *et al*., 1999). Increase in transaminase activity leads to cellular damage and releasing the enzyme in sinusoidal spaces to intralobular vein (Rahman *et al.,* 2001). Liver damage is first indication of toxicity as it encounters firstly by any toxic stress.

This results supported the low toxicity of chitosan to the mice. This result may be reflected of low dose applied or short time of treatment.

AST is normally found in a diversity of tissues including liver, heart, muscles, kidney and brain. It is released into serum when any one of these tissues is damaged. ALT is by contrast normally found largely concentrated in liver and is released into blood stream as

a result of liver injury. The increase in AST and ALT is related to intensity of cellular damage due to chemical induced cellular alteration varying from simple increase of metabolism to death of cell (Giray *et al*., 2001).

Elevated serum urea is also correlated with an increased protein catabolism in mammalian body or from more efficient conversion of ammonia to urea because of increased synthesis of enzyme involved in urea production (Murray *et al.*, 1990). Pesticides induced increase in urea level observed in the present study may be due to the effect of pesticides on liver function, as urea is the end product of protein catabolism (Coles 1986). Because the disturbance happened in the mice system as a result of the chitosan application, this compound should be used with caution applying as insecticide, as it could hazardous to domestic animals and human beings as well. Also, further experiments must be carried out in this respect.

Omara *et al.,* 2012 Serum creatinine and urea significantly (elevated in all treatment groups with chitosan in dose related increase, whereas no gender effect was found in these two parameters. While, the treatment with the lower dose of chitosan showed significant increase in creatinine value comparing with male group with the same dose of chitosan. This result showed that chitosan treatment caused nephrotoxicity and these results were supported by histopathological examination of the kidney.

Lower doses for long period may exert hypertrophy of hepatocytes and thus increase the total protein and FAA in serum of rats (Shakoori *et al*.1988). The increased level of protein in human blood is due to flumethrin (Box and Lee 1996).

The changing levels of serum albumin, thus, provide valuable indices of severity, progress, and prognosis in hepatic disease. Decreased albumin in serum indicates hepatocellular origin of liver disease (Sood 2006).

There is significant evidence that the long- term high dose chitosan supplementation can result in malabsorption of some crucial vitamins and minerals including calcium, magnesium, selenium and vitamins A, D. E and K (Koide, 1998 and Yao *et al*., 2010).

REFERNCES

Bandyopadhyay, U., D. Das and K.R. Banerji (1999). Reactive oxygen species: oxidative damage and pathogenesis. Current Science, 5: 658.

Box, S.A. and M.R. Lee1 (1996). A systemic reaction following exposure to a pyrethroid insecticide. Hum. Experi. Toxicol.,15(5) :389-390

Coles, E. H. (1986). Veterinary Clinical Pathology. W.B. Saunders, Philadelphia, USA.

Doumas, B. T. and Peters T, J.R. (1997). Serum and urine albumin: a progress report on their measurement and clinical significance. Clin. Chem. Acta. 258:3-20

Finney, D.J. (1971). Probit Analysis. 3rd ed. Cambridge University Press. Firdaus, Food and Chemical Toxicology 42: 1269-1277.

Giray, B., A. Gurbay and F. Hineal (2001). Cypermethrin-induced oxidative stress in rat brain and liver is prevented by vitamin E or allopurinol. Toxicol. Lett., 118, 139–146.

Guyton, A.C. and J.E. Hall (1996). Text book of Medical Physiology, 9th , ed. Prism Book (Pvt) Ltd. Bangalore, India. Pp XLiii+ 1148.

Henry, R.J. (1974). Determination of Serum Creatinine: Clinical Chemistry Principles and Technics. 2nd Edn., Harper and Row, USA., Page: 525.

Henry, R.J. (1964). Clinical Chemistry, Harper and Row. Puplishers, New York. P 181.

Hodgson, E. (2004). A textbook of modern toxicology. 3rd Edn. John Wiley and Sons, Inc, New Jersey. pp 203-211.

Jendrassik, L. and P. Grof (1938). Vereinfachte photometriache Methoden Zur Bestimmung des Blutbiliruins. Biochem Z,297: 81-89.

Koide, S.S. (1998). Chitin-chitosan: properties, benefits and risks. Nutrition Res 6: 1091-1101.

Murray, R. K., D. K. Granner, P. A. Mayes and V.W. Rodwell (1990). Harpers Biochemistry 22nd Edn., Lange Medical publication.

Muzzarelli, R.A.A. (1977). Enzymatic synthesis of chitin and chitosan. Occurrence of chitin. In: Chitin. Pergamon Press, New York, pp. 5–17.

Omara, A. Enayat, Hanan F. Aly and Somaia A. Nada (2011). Chitosan induced hepato-nephrotoxicity in mice with special reference to gender effect in glycolytic enzymes activities. Regulatory Toxicology and Pharmacology , 62: (1): 29-40.

Orr, A. (1999). Another New Diet Craze? [<http://www.sizeacceptance.org/news/news055.html>].

Patton, C.J. and S.R. Crouch (1977). Spectrophotometric and kinetics investigation of the Berthelot reaction for the determination of ammonia. Anal. Chem.; 49: 464–469.

Poli, G., E. Albano and M. U. Dianzani (1987). The role of lipid peroxidation in liver damage. Chemistry and Physics of Lipids, 45: 117-142.

Rahman, M.F., M. J. Siddiqui and K. Jamil (2001). Effects of vepacide (*Azadirachta indica*) on aspartate and alanine aminotransferase profiles in sub-chronic study with rats. Human and Experimental Toxicology, 20: 243-24.

Rahman, M.F., M. J. Sidduqi and M. Mustafa (1996). Effect of repeated oral administration of vepacide (*Azadirachta indica*) on some haematological and biochemical parameters in rats. Indian Journal of Toxicology, 3: 1-8.

Rao, C.V. and S. A. Banerji (1990). Effect of feeding polychlorinated biphenyl (Aroclor 1260) on hepatic enzymes in rats. Indian Journal on Experimental Biology, 28 : 149-151.

Reitman, S. and S. Frankel (1957). In-vitro determination of transaminase activity in serum, Am. J. Clin. Path. 28: 56-60.

Sahni K. and Y. Saxena (2001). Toxic effects of diefethialone on the liver of Swiss albino mice *Mus musculus*. U.P. Journal of Zoology, 21(1): 47-52.

Shakoori, A.R., S.S. Ali and M.A. Saleem (1988). Effects of six months feeding of cypermethrin on the blood and liver of albino rats. J. Biochem. Toxicol; 3:59-71.

Sood, R. (2006). Medical laboratory technology method and interpretations 5th Edn. Jaypee Brothers Medical Publishers (P) LTD. New Delhi pp723.

Srinivasan, K. and R. Radhakrishnamurthy (1977). Effects of β and γ isomers of hexachlorocyclohexane on some liver and kidney enzymes in albino rats. Curr. Sci., 46(17): 598-600.

Srivastava, A. K., R. Raina and R.K. Chaudhary (1989). Acute toxicity and biochemical alterations in rats after single oral exposure to dichlorvos. Pesticides, 2: 35-49.

Van Bennekum, A.M., D.V. Nguyen, G. Schulthess, H. Hauser and M.C. Phillips (2005). Mechanisms of cholesterol-lowering effects of dietary insoluble fibres: relationships with intestinal and hepatic cholesterol parameters. Br. J. Nutr. 94, 331–337.

Yao, H.T., C.K. Lii, R.H. Chou, J.H. Lin and H.T. Yang (2010). Effect of chitosan on hepatic drug-metabolizing enzymes and oxidative stress in rats fed low- and high-fat diets. Agric. Food Chem. 58 (8), 5187-5193.

التأثير السام للشيتوسان على كلا من الكبد و الكلى على فئران التجارب البيضاء *Mus musculus*

آمال عبد العزيز عيسى، جمال السيد أبو الغار، ابراهيم محمود عمار،

سماح سعيد عرفه

قسم مبيدات الآفات, كلية الزراعة , جامعة المنوفيه

الملخص العربى

يمتلك الشيتوسان صفات بيولوجية فهو يعمل كمضاد ميكروبى و فطرى و ايضا يستخدم كمنظم نمو للنباتات.و قد قمنا بتطبيقه بثلاث جرعات مختلفة (٣٠٠ مليجرام /كجم مرة واحده و بجرعة ٢٠٠ فى الاسبوع الاول ثم يليها جرعة ١٠٠ فى الاسبوع الثانى و بجرعات متكررة تحت مميتة من جرعة ٥٠ لمدة ٦ اسابيع) و ذلك لدراسة تاثير هذه الجرعات على وظائف الكلى و الكبد فى الفئران البيضاء من خلال تقدير انزيم الاسبرتات أمينو ترانسفيرز و الانين امينوترانسفيرز و البيليروبين و البروتين الكلى للدلالة على وظائف الكبد و اليوريا و الكرياتينين للدلالة على وظائف الكلى . وأظهرت النتائج ان هناك علاقة بين جرعات الشيتوسان و اليوريا حيث زادت كمية اليوريا فى الثلاث مجموعات من الفئران , بينما كانت هناك علاقة سلبية بين الجرعات و البيليروبين. و حقيقة فأن الفئران المعالجة بجرعات متكررة من ٥٠ مليجرام/كجم وجد بها زيادة معنوية فى مستوى أنزيم الانين أمينو ترانسفيريز و الذى يعتبر كمؤشر ضار للشيتوسان على الكبد.