Print ISSN: 2073-8854 & Online ISSN: 2311-6544
Magazin of Al-Kufa University for Biology / VOL.6/ NO.1/ Year : 2014

Histological and Physiological Effect of Chitocal On Liver, Some Parameters of Blood And Body Weight In The Male Albino Rats

Abed Hasan Baraaj
University of Baghdad, College of sciences Department of Biology
Correspondence should be sent to:abed.hassan20@yahoo.com

Abstract:
This study was carried out to investigate the effect of the oral administration of chitocal on liver, some parameters of blood and body weight in the male albino rats.
For this purpose sixteen males mature albino rats which were divided into two equal groups, control group (G_1) administrated normal physiological saline (0.9% NaCl) and second group (G_2) administrated orally for 35 days (5 weeks), with 25mg/ kg/ b.w/ day of chitocal. liver and sample of blood was taken from the male rats for the current study. The exposure of rats to chitocal caused histopathological changes in the liver of the male rats were revealed variety to the lesions like accumulation of mononuclear cells (inflammatory cells) around central vein.

The rats exposed to chitocal showed massive necrosis in hepatocyte and hydropic degeneration with loss of architecture of liver tissue, significant decrease ($P<0.05$) in some Parameter of blood such as (PCV, WBC, Hb) and significant increase ($P<0.05$) in enzyme activity level of GPT (Glutamic pyruvic transaminase), GOT (Glutamic oxaloacetic transaminase) and ALP (Alkaline phosphatase). Also it was showed a significant $P<0.05$ decrease in body weight (B.W).

Key words: Chitocal, liver, histopathology, enzymes, blood parameters.

Introduction:

Chitocal is amixture of chitosan, ascorbic acid and gymnema sylvestra. Chitocal is an aminopoly saccharide derived from chitin which makes up the exoskeleton of crustaceans such as shrimp, Lobster and crab[1]. Chitocal with different physico-chemical properties can be prepared under different reaction conditions. The degree of deacetylation (DD) and the viscosity-average molecular weight (Mw) of chitocal are two important characteristics which greatly affect its chemical and physiological properties [2,3,4].

In particular chitocal can decrease the concentration of cholesterol in serum and the liver [5,6]. The strong positive charge carried by the chitocal molecule (aminogroups) causes it to bind negatively charged substances such as lipids [7,8].

Also chitocal can reduced the concentration of lipid and reduced the absorption of some elements such as calcium and iron ions [9,10,11].The liver is a central organ for many physiological and biochemical process necessary for maintenance of life [12], [13]. The morphological alterations that occure in the liver affect many metabolic processes in the organism peroxide formation induced by drug or chemical [14]. Chitocal causes damage in the liver and occurs histopathological changes in the liver. Enzymes such as GOT, GPT and AlP were release into blood. Their increase in the plasma activities of these enzymes was directly proportional to the degree of cellular damage [15].

The purpose of the present study was to evaluate the effects of chitical on rat liver and some Parameters of blood.

2- Materials and Methods

2.1. Experimental animals:

The rats used were of sixteen sexually mature Sprague-Dawley albino rats (*Rattus norvegicus*) of an average body weight of 199.25 ± 4.68gm and 14-15 weeks old. Animals were kept in the department of biology college of science, university of Baghdad under the laboratory conditions (12h Light: 12h dark) Photoperiod with cortrolled room temperature 25-28°C, good ventilation and were feed normal rodent pellets and tap water *ad Libitum*.

2.2 Experimental design:

The rats were randomly divided into two groups each group were kept into four plastic box cages (fuor rats per cage) measuring 40x 25x 15cm. The control group (G_1) was givin normal physiological saline (0.9% NaCl) orally.

The second group (G_2) treated with chitocal (chitosan, ascorbic acid and Gymnema sylvestre) 25mg/ Kg b.w. once dialy orally via intubation directly to the stomach (0.6mm diameter of tube) for a period of 5 weeks.

2.3 Histological preparations:

Histological examination was done by fixing 1cm x1cm of the liver of the rats in 10% Formaline saline and embedded in paraffin wax. Tissue blocks were sectioned 5µm thick and stained with haemotoxyl in and eosin [16].

2-4 Estimation of liver enzyme activity

2.4.1. Estimation of serum activity of glutamic oxalo-acetic trans-aminase (GOT) and glutamic pyruvic transaminase (GPT) were performed using Reitman-Frankel method [17].

2.4.2. Estimation of serum activity of allialine phosphase (AlP) was performed using Kind and King method 1954. [18].

2.4.3. The white blood cell count (WBC), haemoglobin (Hb), and haematocrit (HcT), were determined by automated haematology system analyzer (ADVIA 60 open Tube; Bayer corporation, Tarrytoun, New York, USA. [19].

2.4.4. The body weight was determined for both groups of rats by electron balance through the experimental processes.

2.5. Statistical analysis:

Statistical analysis (standard deviation SD) was carried out according to Fisher [20] LSD (Least significant difference). Test was used to compare the significant differences between means of treatments. [21].

3- Results and Discussion:

3.1. Histopathological of liver

Histological study showed a typical structural organization of the liver in the untreated rats. (Figure-1). While the liver of rats treated with chitocal for 5 weeks showed several types of liver damage such as inflammatory cells accumulation (mononuclear cells) around central vein (Figure 2) with certain of focal distributed of massive necrosis in hepatocytes and hydropic degeneration vacuolation with loss of architecture of liver tissue (Figure-3) abnormality structure was appeared in treated rats with chitocal agrees with was obtained by Steven et al., 2003[22],Who documented that these vaculation were generally divided into two types.

Print ISSN: 2073-8854 & Online ISSN: 2311-6544
Magazin of Al-Kufa University for Biology / VOL.6/ NO.1/ Year : 2014

One may contain water which was then called hydropic change while the other contain lipid. Butterworth *et al*., (1992).[23] suggested that high exposure of drug and chemicals results in cell death or apoptosis. These two factors (drug and chemical) may show either functional or structural side effects as well as the loosing of the liver capacity to stress, but when reaching the threshold, the liver cells will reach the final irreversible stage which was death [24]. Chitocal induce various changes in liver such as inflammation and necrosis in liver of mouse [25].

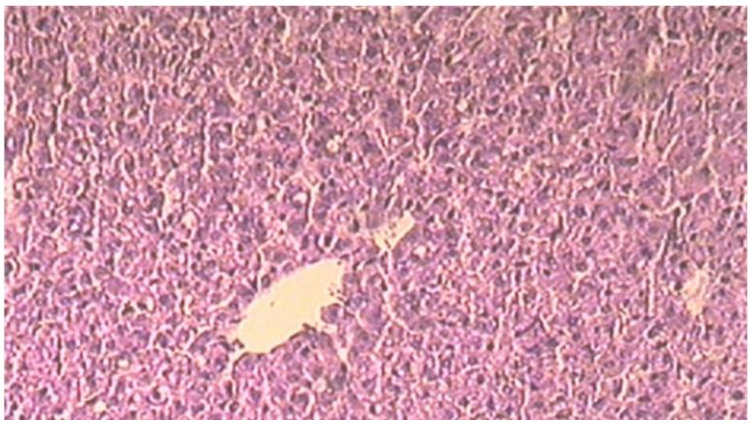

Figure (1) Cross section in liver of rat, (control) (H & E) (40x)

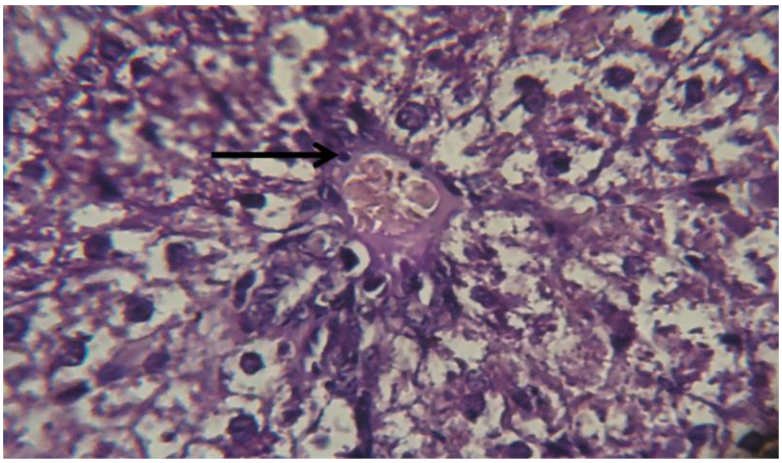

Figure (2): Cross section in liver of rat, treated with 25 mg/Kg-day of chitocal showing accumulation (Mononuclear cells) around central vein (H& E) (40x)

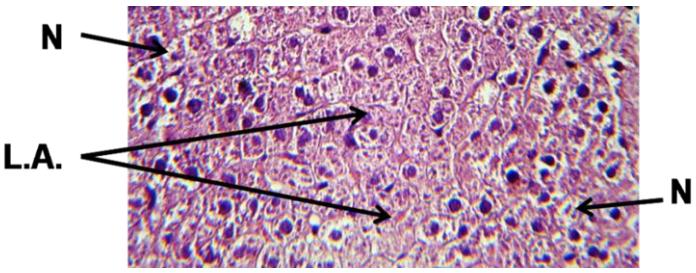

Figure (3) Cross section in liver of rat, treated with 25 mg/kg/day of chitoeal showing massive necrosis (N), Hydropic vacuolation (H.V.) Degeneration (D) and loss of architecture (L.A.) (H & E) (40x)

3-2- Effect of chitocal on the (PCV, Hb. and WBC)

This study showed significant decreased P<0.05 in some Parameters. of blood (PCV, Hb, and WBC) in the treated group of male rats with chitocal compare with the control group of male rats (Table-1). Administration of native chitocal apparently reduced the absorption of calcium and iron ions [9] malondialdehyde (MDA) level is the most important Factor indicating increased peroxidative level. While glutathione is substance with an important role in cell detoxifican and protection from hazardous compounds Glutathione is synthesized in the erythrocytes and is found in living cells. It has been reported that cellular glutathione has important function against chemical agents by protection the cell membrane integrity, A decrease in the amount of glutathione and increase in the amount of MDA may result in the destruction of membrane integrity [26,27]. In this study the decrease in the reduced P.C.V. and WBC indicated that chitocal damaged the integrity of the erythrocyte and WBC membrane. Chitocal induced the increased peroxidative liver that causes decrease in the reduced glutathione and increase in the amount of MDA. The reduced absorption of iron ions causes decreasing in the Level of Hb [9].

Table -1: Effect of chitocal (25 mg/kg B.W) on the some Parameters of blood and body weight

Group	B.W gm	PCV %	Hb Gm/dl	WBC mm^3
G_1	270.87 ± 7.29	43 ± 1.41	13.73 ± 0.31	12425 ± 301.18
G_2	*218.12 ± 3.94	*29.3 ± 0.98	*10.92 ± 0.92	*9000 ± 298.56

Significant difference p<0.05

3-3- Effect of chitocal on the (GPT, GOT, and ALP).

The present study indicates that the exposure of rats to chitocal results in significant elevation in the serum activity of aminotransferase erase enzymes.

The results of statistical analysis showed significant increase (p<0.05) in serum activity of GPT, GOT, and ALP of groups treated with chitocal when compared with control group (Table -2).The serum activities of enzymes such as GOT, GPT and ALP increase with the increase in cellular membrane permeability resulting in liver cells degeneration [26,27]. Rubbins (2003)[28] reported that biotransformation of some drug and chemical make them to be converted to reactive toxic metabolite which then act on the target cells. Usually these target cells were mostly liver cells since that modification of drug and chemical is accomplished by cytochrome (P450) in smooth endoplasmic reticulum of liver than other organs. These toxic substances may stimulate the damage of plasma membrane. Mitochondria were the major target in drug-induced liver injury [29,30,31]. The enzymes such as GPT, GOT and ALP were released into blood. These increase in the plasma activities of these enzymes was directly proportional to the degree of cellular damage [15]. The chitocal caused some alteration on liver function in the rat [32].

Print ISSN: 2073-8854 & Online ISSN: 2311-6544
Magazin of Al-Kufa University for Biology / VOL.6/ NO.1/ Year : 2014

Table -2: Effect of chitocal (25 mg/kg B.W) on liver enzymes in blood serum)

Group	GOT mg/dl	GPT mg/dl	ALP mg/dl
G1	20.63 ± 0.14	16.89 ± 0.35	11.51 ± 0.21
G2	*24.64 ± 0.36	*23.07 ± 0.35	*15.21 ± 0.38

Significant difference $p<0.05$

3-4- Effect of Citocal on the body weight (B. W)

This study showed that B.W. of chitocal-treated group. for 5 weeks were significant $p<0.05$ decreased when compared with the control group as in (Table-1). Chitocal with higher degrees of deacetylation (DD) have more lree amino groups and more positive charge in solution. Hypo-cholesterolemic effects of chitocal with different degrees of deacetylation the fat binding capacity of chitocal was increased with the increase of both DD and Mw (viscosity-average molecular weight) and occurs with lowered plasma triglyceride, total cholesterol and low-density-lipoprotein cholest-erol (LDL-C) level [33]. [3], chitocal with higher molecular weights limits the body-weight gain of adult rats [33,3].

The hepatic lipase (HL) and lipoprotein lipase (LPL) activity were also reduced by chitocal, which could regulate lipase activity. chitocal could not only prevent the hyperlipdmia induced by long-term administration of ahigh- fat diet but also reduce serum lipid level and liver-fat accumula-tion in hyperlipdemic rats [34].

The gymnema sylvestre (present in chitocal) may also decreases glucose and some nutrient absorption in the gastrointestinal tract [35,36]. This study about B.W. were showed the same results in the provious concentra-tion that occurs reduced B.W. because the effect of chitocal.

Reference

1-Muwwarelli, R.A.A. (1977). Enzymatic synthesis of chitin and chitosan. occurrence of chitin. In chitin, Muzzarrelli, R.A.A. (Ed), pergamon press, New York, P.5,

2- Liu, J.N., Xia, W.S., and Zhang, J.L. (2008). Effects of chitosans physico-chemical properties on binding capacities of lipid and bile salts in vitro *Chinese food science*. 29: (1). 45-49..

3- Liu, J.N., Zhang, J.L., and Xia, W.S. (2008). Hypocholesterolemic, effects of different chitosan samples in vitro and in vivo. *Food chemistry*. 107: 419-425.

4- Zhou, K., Xia, W., Zhang, C. and Yu, L. (2006). In vitro binding of bile acids and trigly derides by selected chitosan preparations and their physicochemical properties. *LWT-Food science and Technology*. 39. 1087-1092.

5- Kobayashi, T., Otsuka, S.I. and Yugari, Y. (1979). Effect of chitosan on serum and liver cholesterol levels in cholesterol- Fed rats. *Nutr. Rept.* Int., 19, 327-334.

6- Chung, G.H., Kim, B.S., Hur, J.W. and Chung S.Y.: (1996). Effect of dietary lobster shrimp chi to san, on lipid metabolism in diet induced hyperlipidemic rats (in corean). *J.Korea soc. Food nutr* 25, 384-391.

7- Ormrod, D.J., C.C. Holmes and T.E. Miller. (1998). Dietary chitosan inhibits hypercholesterollaemia and atherogenesis in the apolipoprotein E-deficient mouse model of atherosclerosis. *Atheros-clerosis*, 138: 329-334.

8- Gallaher, C.M., J. Munion, J. Hesslink, J. Wise and D.D. Gallaher. 2000, Cholesterol reduction by glucomannan and chitosan is mediated by changes in

Cholesterol absorption and bile acid and fat excretion in rats. *J. Nutr*., 130: 2753-2759.

9- Deuchi, K., Kananchi, O., shizukuish, M., and Kobagashis E., (1995). Continuous and massive Intake of chitosan effects mineral and fat-soluble vitamine status in rats fed on ahigh-fat diet- Bioscience *Biotechnology and Biochemistry*. 59, 1211-1216.

10- Chiang, M.T., H.T. Yao and H.C. Chen, 2000. Effect of dietary chitosans with different viscosity on plasma lipid and lipid peroxidation in rats fed on a diet enriched with cholesterol. *Biosci. Biotechnol. Biochem*., 64: 965-971.

11- Tai, T.S., W. H. Sheu, W.J. Lee, H.T. Yao and M.T. chiang (2000). Effect of chitosan on plasma lipoprotein concentration in type 2 diabetic subjects with hyper-cholesterolemia. *Diabetes care*, 23: 1703-1704.

12-Yao, H.T., S.Y. Huang and M.T. Chiang 2008. A comparative study on hypoglycemic and hypoch-olesterolemic effects of high and low molecular weight chitosan in streptozoto (in-induced diabetic rats. *Food chem. Toxicol*., 46: 1525-1534.

13- Souba, W.W. and D.W. Wilmore, (1983). Postoperative alteration of arteriovenous, exchang of amino acids across the gastro-intestinal tract. *Surgery*, 94: 342-350.

14- Sudhahar, V., S.A., Knmar, P.T. Sudharsan and P. Varalakshmi, (2007). Protective effect of lupeol and its esteron cardiac abnor-malities in experimental hyperch-olesterolemia. *Vascul. Pharmacol.*, 46: 412-418.

15- Osman, M., S.A. Fayed, G. I. Mahmoud and R.M. Romeilah (2010). Protective effects of chitosan, ascorbic acid and gymnema sylvestre against hyper-cholesterolemia in male rats: *Australian Journal of Basic and Applied sciences*, 4 (1): 89-98.

16- Carson, F.L. (1997). Histote-chnology: A-Self instructional *Text. Ascp press*.

17- Reitman. S. and Frankel, L. S. (1957). A colorimetric method for determination of serum giutamic oxaloacetic and glutamic pyruvic transaminase. *Amer. J. Clin. Path.* 28, 56-63.

18- Kind, P.R.N. and King, E.J. (1954). Estimation of plasma phosphatase by determination of hydrolysed phenol with amino-antipyrin. *J. Clin. Pathol*. 7: 322-326.

19-Adebayo A.H. (2010). Biochemical, hematological and histological studies of extract of *Ageratum conyzoides* L. is sprague Dawley rats. *Journal of medicinal plants* Research. 4 (21) 2264-2272.

20- Fisher, R.A., 1970. Statistical method for research workers Edinburgh ed. 14. *Oliver and Boyed*, p. 140.

21-Allan G. Blumn (1998). Elementary statistics step by step approach. *Bosten Press*.

22- Steven, A., Lowe, J. and Young, B. (2003). Wheater's Basic histopa-thology. *4th Ed. Churchill Living-ston. London-New York.*

23-Butterworth, B., Popp, J., Conolly, R., and Goldsworth, T., (1992). Chemically induced cell proli-feration in carcinogensis *IAPCSCI. Pub*. 279-209.

24-Plaa, G., and M., Charbonneau., (2001). Detection and evalutions of chemically induced liver injury. In principle and Methods of toxicology 4thed. Hayes. *A.W. (ed) Tylor and Francis, Philadelphia*, pp. 1145-1187.

25- Kim, S.J. Kang, S.Y. Park S.L. and Ko. Y.H. (1998). Effect of chitooligosaccharides on liver function in the mouse. *Korean J. Food SCI. Technol*. 30. (3): 693-696.

26- Kempaiah, R.K. and K. Srinivsan, 2005. Influence of dietary spices on the fluidity of erythrocytes in hypercho-lesterolemic rats. *Br. J. Nutr*., 93: 81-91.
27- Tauseef, M., Sharma K.K. and Fahim, M. (2007). Aspirinor-estores normal barore flex function in hypercholes-terolemic rats by its antioxidative action. *Eur. J. Pharmacol.*, 556: 136-143.
28- Rubbins, L., (2003). Basic pathology. 7th (ed) *W.B. Saundays Co. philadelphia, Pennsylvania*. PPXIV + 850.
29- Bernstein, J., Videla, L. and Israel, Y: (1973). Metabolic alteration produced in the liver by choronic ethanol adminis change related to energetic parameters to cell. *Biochem. J.*, 134, 515-521.
30- Kass, G.E. (2006). Mitochondrial involvement in drug induced hepatic injury. *Chemico Biological Interactions* 163: 145-159.
31- Boelsterli, U.A. and Lim, P.L. (2007). Mitochondrial abnorm-alities –a link to diosyncratic drug hepatotoxicity. *Toxicology and Applied pharmacol*. 220: 92-107.
32- Lehoux, J.G. and Grondin, F. (1993). Some effects of chitosan on liver function in rat. *Endocinology*: 132. 1078-1084.
33- Li, L., Zhang. J. L., Liu, J. N., and Xia, W. S. (2007). Effects of chitosan serum lipid and fat Liver. *Chinese journal of marine Drugs*, 26 (2), 7-9.
34- Xia, W., Liu, P., Zhang, J., and Chen, J. (2010) Biological activities of chitosan and chito-oligosaccharides. *Food Hyroco-lloids.* 10. 1016.
35- Shanmugasundaram, E.R.B., Gopinath, K.L. Shanmugas-undaram K.P. and Rajendran, V.M.(1990). Possible regeneration of the islets of Langerhans in streptozocin diabetic rats given gymnema sylvestre leaf extract. *J. Ethnopharmacol*. 30: 265-279.
36- Daisy, P., J. Eliza and K.A.M. Farook, 2009. Anovel dihydroxy gymnemic triacetate isolated from gymnema sylvestre possessing normoglycemia and hypolipidemic activity on STZ- induced diabetic Rats. *J. Ethnopharma Col*., 126: 334-344.

تأثير الشيتوكال في التركيب النسجي والوظيفي للكبد وبعض معايير الدم ووزن الجسم في ذكور الجرذان الابيض

عبد حسن براج

جامعة بغداد/ كلية العلوم/ قسم البايولوجي

المستخلص

أجريت هذه الدراسة للكشف عن تأثير الشيتوكال المعطى عن طريق الفم على الكبد وبعض معايير الدم ووزن الجسم في ذكور الجرذان الابيض.استخدم لهذا الغرض 16 جرذ أبيض من الذكور الناضجة وقسمت الى مجموعتين متساويتين مجموعة السيطرة (G_1) تم تجريعها بالمحلول الملحي الفسيولوجي الطبيعي (0.9% NaCl) والمجموعة الثانية (G_2) تم تجريعها لمدة 5 اسابيع بـ 25 ملغم/ كغم/ يوم بمادة الشيتوكال.

Print ISSN: 2073-8854 & Online ISSN: 2311-6544
Magazin of Al-Kufa University for Biology / VOL.6/ NO.1/ Year : 2014

تم اخذ نسيج الكبد وعينات الدم بعد تشريحها لغرض اجراء الدراسات. اظهر الدراسة ان المعاملة بالشيتوكال سببت تغيرات نسجية مرضية في الكبد حيث تظهر اضرار متنوعة مثل تجمع الخلايا وحيدة النواة (الخلايا الالتهابية) حول الوريد المركزي والتنخر الواسع في الخلايا الكبدية مع التحلل المائي وظهور الفجوات في السايتوبلازم للخلايا الكبدية وفقدان البنية التركيبية لنسيج الكبد. وكما سبب الشيتوكال انخفاض معنوي ($P<0.05$) في بعض معايير الدم مثل (PCV, WBC, Hb)، وارتفاع معنوي ($P<0.05$) في مستوى فعالية الانزيمات الكبدية ALP, GOT, GPT والتي تعتبر مؤشر لحصول الضرر في الكبد وكذلك لوحظ انخفاض معنوي $P<0.05$ في معدل اوزان الحيوانات التجريبية مقارنة مع مجموعة السيطرة