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A canine model of carbon tetrachloride (ccl_4)induced liver cirrhosis: I. Clinical ,hematological and biochemical evaluations

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ABSTRACT

The liver is a vital organ of vertebrates and plays a major role in metabolism in addition to other numerous functions in the living body, including regulation of glycogen storage, decomposition of red blood cells, plasma protein synthesis, hormone production, and detoxification. Damage of hepatic cells "Liver cirrhosis" is apotentially life-threatening condition. This could be accredited to a variety of pharmacological agents, viruses, alcohol abuse, autoimmune inflammation and exposure to metabolic metals such as iron, copper and other toxic substances. Carbon tetrachloride (CCl_4)is a known highly hepatotoxic agent.This study aimed to assess the degree of liver cirrhosis through clinical and biochemical and hematological analysis after the administration of CCl_4 in order to establish a canine model of liver cirrhosis for further research work.Eighteen skeletally mature mongrel dogs received weekly oral administration of CCl_4 (1ml/kg 98%) for 16 weeks. Dogs were examined for symptoms of liver cirrhosis and the degree of cirrhosis was recorded through clinical and biochemical and hematological tests. Dogs were of clinical manifestation of liver insufficiency with abnormal hematological and biochemical tests values so we can conclude that, the levels of serum markers of the liver (liver biochemical tests) and other hematological parameters can be taken as a staging indicator of liver cirrhosis.

Keywords: dogs, CCl_4, liver, cirrhosis, hematological and biochemical tests.

INTRODUCTION

Liver is the largest metabolic organ that performs several vital functions including immune defense, metabolism of carbohydrates, lipids, proteins, hormones, xenobiotics, as well as the secretion of plasma proteins and bile (Piryaei et al., 2010 and Ren, 2015).

The hepatocytes are the functional unit of the hepatic lobule that makes up to 80% of cellular mass of the liver(Cullen, 2006).Functional disorders of these cells are related to a variety of pharmacological agents, hepatitis viruses, alcohol abuse, autoimmune inflammation and exposure to metabolic metals as iron and copper leading to hepatitis and ending with cirrhosis and hepatocellular carcinoma (Piryaei et al., 2010).Liver cirrhosis is a potentially life-threatening condition as it is often an indolent diseasebecause it remains asymptomatic until the occurrence of cirrhosisor hepatocellular carcinoma(Chan et al., 2008).

There are large numbers of toxins that can induce hepatitis and liver cirrhosis. The most frequently employedhepatotoxins to induce experimental liver degeneration are D-galactosamine, ethanol, thioacetamide, acetaminophen and CCl_4(Palmes and Spiegel, 2004).CCl_4isa highly classical toxic agent (Junnila et al., 2000 and Palmes and Spiegel, 2004). It is activated in the liver to a highly reactive trichloromethyl radical which initiates free radical mediated lipid peroxidation of the cytoplasmic membrane phospholipids and causes functional and morphological changes in the cell membrane, which leading to an accumulation of lipid derived oxidants causing liver injury (Nagmoti et al., 2010 and Cordero-Pérez et al., 2013).

The objective of this study was to assess the degree of liver cirrhosis through clinical, hematologicaland biochemical evaluations after the administration of CCl_4 in order to establish a canine model of liver cirrhosis for further research work.

MATERIALS AND METHODS

Eighteen dogs,24 – 30 months old, weighing 15-20 kg of both sex (11 male and 7 female) were acclimated to the kennel environment for at least 2 weeks before enrollment in the study. Complete clinical, laboratory were conducted

on each dog to exclude the evidence of systemic and/or hepatic diseases. All study procedures were approved by the Institutional Animal Care and Use ethical Committee (IACUC) of Faculty of Veterinary Medicine-Cairo University (Cu F Vet/F/SUR/2013/16). During the study, dogs were fed on standard dry food twice daily and were allowed a free access to drinking water.

Weekly oral administration of 1 ml/kg of CCl_4 (98%) using orogastric tube was done for 16 weeks under light sedation (Lemke, 2007). Dogs were examined for liver cirrhosis by clinical hematologicaland biochemical evaluations.

Clinical evaluation:

Routine physical examination including; activity, general body condition (hair, skin and body weight),appetite and mucous membrane was carried out for each dog.

Hematological and biochemical evaluation:

Blood samples were collected at two weeks intervals along the study period (16 weeks) for the assessment of alanine aminotransferase (ALT), aspartate aminotransferase (AST), total protein (TP), albumin (ALB), total bilirubin (T.bili), direct bilirubin (D.bili), alkaline phosphatase (AP), hemoglobin (HB), platelates (PLT) and prothrombin time (PT) (Vaden at al., 2009).

Statistical analysis:

Data of biochemical and hematological evaluation was expressed as mean ± SD. Independent-samples T test was used to determine the statistically significant differences between the values at the base line (0 day) and 16 week (SPSS version 17.0 computer Software). Results were considered significant at $P<0.05$.

RESULTS

Clinical symptoms:

At the first 4 weeks, dogs had no obvious clinical symptoms.At the 6th week, weight loss, fatigue and dullness werenoticed in all animals.From the 8th week and updecreased activity thatmanifested by depression and dullness withsevere weight loss were observed in all animals.Furthermore, mucous membrane started to become pale in color (Fig.1) andprogressedto deep yellow discoloration at the end of the 16th week follow up period. This icteric color was also noticed on the mucous membranes of the eyeand gum (Figs 2 and 3).

Hematological andbiochemical and evaluation:

ALT, AST, T.bili, D.bili, AP significantly increased till 16th week. ALB, TP, HB, PLT, PT significantly decreased throughout the follow up period. Detailed obtained biochemical and hematological data are shown in table (1).

From our data, liver fibrosis could be graded through hematological and biochemical testsas shown in table (2) parallel with Laennec staging system (figs 4 and 5).

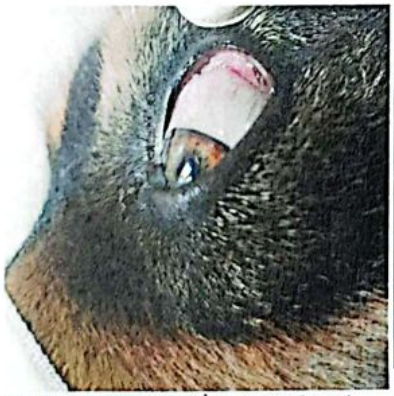

Fig (1): dog after 8th week of CCl_4 administration showed pale mucous membrane

Fig (2): dog after 16thweek ofCCl_4 administration showing severe icteric mucous membrane and sclera.

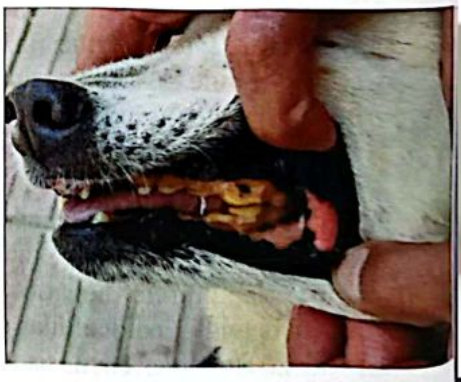

Fig (3): dog after 16thweek ofCC administration showing severe icter gum.

Table (1) Mean hematologicaland biochemical parameters of CCl_4administartionfrom day 0to 16 weeks in dogs.

Week	Alt (u/l)	Ast (u/l)	Ap (u/l)	T.bili (mg/dl)	D.bili (mg/dl)	Alb (g/dl)	Tp (mg/dl)	Hb (u/g)	Plt (plt/ul of blood)	Pt (seconds)
0	32.3*	29.89*	110.22*	0.69*	0.37*	4.53*	7.08*	11.8*	409.02*	12.71*
2	34.57	40.04	147.8	0.95	0.71	3.81	6.62	11.25	380.66	11.94
4	52.62	51.62	180.3	1.2	1.01	3.37	6.3	10.58	354.61	11.55
6	62.48	60.22	203.9	1.5	1.34	3.02	5.66	9.91	318.53	11.28
8	72.94	71.32	231.05	1.84	1.63	2.59	5.1	9.35	283.68	10.92
10	83.27	82.25	253.87	2.18	1.9	2.2	4.69	8.7	247.94	10.56
12	96.38	92.86	296.46	2.48	2.17	1.84	4.29	8.23	224.81	10.22
14	110.9	104.89	323.08	2.77	2.48	1.57	3.81	7.6	204.16	9.86
16	130.9*	117.83*	352.65*	3.12*	2.74*	1.36*	3.37*	6.89*	189.98*	9.44*

***Statistically significant at P < 0.05**

Table (2) grading of CCl_4 induced liver cirrhosis in dogs according to hematologicalandbiochemical tests parameters

weeks	*Percentage difference from the baseline (%)*										Grade (F)
	ALT	AST	AP	T.Bili	D.Bili	ALB	TP	HB	PLT	PT	
2	107	134	134	138	192	84	94	95	93	94	F1
4	163	173	164	174	273	74	89	90	87	91	
6	193	201	185	217	362	67	80	84	78	89	F2,3
8	226	239	210	267	441	57	72	79	69	86	
10	258	275	230	316	514	49	66	74	61	83	F4A
12	298	311	269	359	586	41	61	70	55	80	
14	343	351	293	401	670	35	54	64	50	78	F4B
16	405	394	320	464	740	30	48	58	46	74	

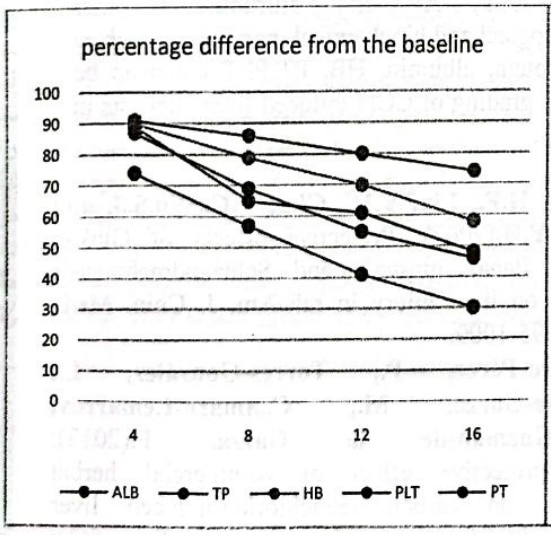

Fig (4) showing the decline percentage of ALB, TP, HB, PLT, PT.

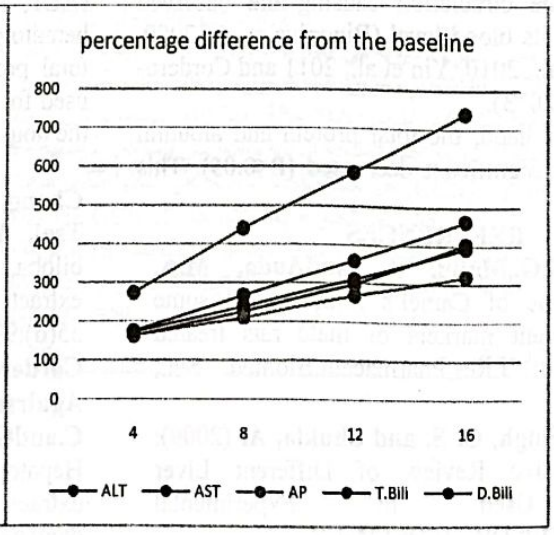

Fig (5) showing the increased percentage of ALT, AST, AP, Bilirubin.

DISCUSSION

Carbon tetrachloride is one of the most known classical hepatotoxicagent used in the experimental study of liver diseases(Bigoniya et al., 2009). Therefore, thus study aims to evaluate the degree of liver cirrhosis induced by CCl4 in dogs. This evaluation based on clinical, hematological and biochemical evaluations.

In the present study, the loss of body condition in intoxicated dogs are enforced by the toxic effects of CCl_4 on hepatocyte, that are responsible for carbohydrate, lipid and protein metabolism, causing mal-utilization of the consumed food. These results agreed with those obtained earlier (Venukumar and Latha, 2002 and Chang et al., 2007). Furthermore, Moreover, hepatocytes damage lead to impairment of metabolism of bilirubin in the liver with subsequent excessive breakdown of red blood cells in the blood, bilirubin increase in the blood (hyperbilirubinemia) leading to increase serum levels of total and direct bilirubin and anemia occur(Sourabie et al., 2012). This causes the development of the yellowish discoloration of the skin, gum and mucous memrane of the eye (icterus) similar findings agree with (Sourabie et al., 2012)

Biochemical were reported that the serum levels of hepatic markers (ALT, AST and AP) had significantly increased ($P<0.05$).This is due to the fact that a healthy hepatocyte exhibit these enzymes within the cytoplasmic area of the cell. When the cell membrane of the hepatocyte is damaged or injured, these enzymes gains free access to the circulation causing the observed elevation of its blood level (Bigoniya et al., 2009, Nagmoti et al., 2010, Yin et al., 2011 and Cordero-Pérez et al., 2013).

On the other hand, the total protein and albumin levels had a significant decreased ($P<0.05$). This could also be explained throughtheadversetoxic effect of CCl_4 on hepatocytes that leads to the impairment ofproteinsynthesis, reduced absorption of amino acids and loss protein in urine. All these factors lead to the observed emaciation conditions in all intoxicated dogs. Thesefindingsare in agreement with (Al-Fartosi et al. 2012).

As a result of hepatocellular damage, there is impairment in the secretion of coagulation factors which was indicated by the significant decrease in PT level and PLT count. These results agree with (Mammen, 1992).

Through the obtained results, grading the stage of liver cirrhosis would be possible with the help of biochemical and hematological tests. For instance, an increase of the cytoplasmic liver enzymestogether with decreased in TP, ALB, HB, PLT and PT by 1.7 folds than the baseline indicates liver fibrosis grade one. Similarly, a2.5 fold increaseof the cytoplasmic liver enzymeswith a 0.8 fold decreased in TP, ALB, HB, PLT and PT indicates, the liver fibrosisof grades 2-3. With a 3 fold increase of the cytoplasmic liver enzymes and 0.6 fold decreased in TP, ALB, HB, PLT and PT, liver cirrhosisgraded as 4A. Finally, when the cytoplasmic liver enzymes has increased 3.6 fold than the baselineandthe TP, ALB, HB, PLT and PT had decreased 0.5 fold than the baseline, the liver cirrhosis graded as4B. theseresultsare parallel to Laennec staging system.

CONCLUSION

In conclusion, the levels of the serum markers (ALT, AST, AP and bilirubin)and other hematological and biochemical parameters such as total protein, albumin, HB, PT,PLT countcan be used for grading of CCl4 induced liver cirrhosis in the dogs.

REFERENCES

Al-Fartosi, K.G.,Majid. A. andAuda, M.A. (2012): The role of Camel's milk against some oxidant-antioxidant markers of male rats treated with CCl_4. Int. J.Res.Pharmaceut.Biomed. Sci., 3(1):385–389.

Bigoniya, P., Singh, C. S. and Shukla, A. (2009): A Comprehensive Review of Different Liver Toxicants Used in Experimental Pharmacology.IJPSDR., 1(3): 124-135.

Chan, H.L.,Tse, C.H.; Mo, F.,Koh, J. and Wong, V.W.(2008): High Viral Load and Hepatitis B Virus SubgenotypeCe Are Associated With Increased Risk of Hepatocellular Carcinoma. J.Clin.Onco.,26: 177-182.

Chang, H.F., Lin, Y.H., Chu, C.C.,Wu,S.J. and Tsai, Y.H.(2007): Protective effects of Ginkgo biloba, Panax ginseng, and Schizandrachinensis extract on liver injury in rats.Am. J. Chin. Med., 35(6):995-1009.

Cordero-Pérez, P., Torres-González, L., Aguirre-Garza, M., C.,amara-Lemarroy, C.andGuzmán-de la Garza, F.(2013): Hepatoprotective effect of commercial herbal extracts on carbon tetrachloride-induced liver damage in Wistar rats. Pharmacognosy Res., 5(3): 150–156.

Cullen, J.M.(2006): Pathologic Basis of Veterinary Disease, chapter 8 (liver, biliary system and exocrine pancrease). **In McGavin, M. D and Zachary, J.F. 4thEd, Elsevier Health Sciences.**

Junnila, M., Rahko,T., Sukura, A. and Lindberg, L.A. (2000):Reduction of Carbon Tetrachloride-Induced Hepatotoxic Effects by Oral Administration of Betaine in Male Han-Wistar Rats: A Morphometric Histological Study. Vet.Pathol., 37: 231-238.

Lemke, K.A. (2007): Anticholinergics and sedatives. In: Lumb and Jones Veterinary Anesthesia and Analgesia, 4thEd. (Tranquilli W. J., J. C. Thurmon, K. A. Grimm, Eds.). Blackwell Publishing, Iowa, USA.203-239.

Mammen, E.F. (1992): Coagulation abnormalities in liver disease. Hematol.Oncol.Clin. North. Am., 6 (6):1247-1257.

Nagmoti, D.M, Yeshwante, S.B., Wankhede, S.S. and Juvekar, A. (2010): Hepatoprotective effect of averrhoabilimbilinn against carbon tetrachloride induced hepatic damage in rats. Pharmacol., 3: 1- 6.

Palmes, D. and Spiegel, H.U. (2004): Animal models of liver regeneration. Biomaterials., (25): 1601–1611.

Piryaei,A., RezazadehValojerdi, M. and Baharvand, H. (2010): Therapeutic Potential of Mouse Bone Marrow Mesenchymal Stem Cells in Carbon Tetrachloride (CCl_4)-Induced Liver Fibrosis. J. Iran. Anat. Sci., 7(28-29):99-112.

Ren, S., Irudayam, J.I., Contreras, D., Sareen, D. and Adame, D.T. (2015): Bioartificial Liver Device Based on Induced Pluripotent Stem Cell-Derived Hepatocytes. J. Stem Cell Res.Ther., 5(2):1-9.

Sourabie, T.S., Ouedrogo, N., Sawadogo, W.R., Yougbare, N. andNikiema, J.B.(2012): Evaluation of the anti-icterus effect of crude powdered leaf of Argemone Mexicana L. (Papaveraceae) against CCl_4-induced liver injury in rats. IJPSR., 3(10): 491-497.

Vaden, S.L., Knoll, J.S., Smith, F.W.K.and Tilley, L.P. (2009): Blackwell's five-minute veterinary consult. Laboratory tests and diagnostic procedures: canine and feline, 1st ed., 2121 State Avenue, Ames, Iowa 50014-8300, USA.25-36.

Venukumar, M.R. and Latha, M.S. (2002): Antioxidant activity ofcurculigoorchioides in carbon tetrachloride-induced hepatopathy in rats. Indian J.Clin.Biochem.,17(2):80-87.

Yin,G., Cao, L., Xu, P., Jeney, G. and Nakao, M. (2011): Hepatoprotective and antioxidant effects of Hibiscus sabdariffa extract against carbon tetrachloride-induced hepatocyte damage in Cyprinuscarpio. In Vitro Cell Dev. Biol. Anim., 47(1):10-1

الملخص بالعربى

استحداث نموذج لتليف الكبد فى الكلاب باستخدام رابع كلوريد الكربون

1:الفحص الاكلينيكي واختبارات الدم و الكيمياتية الحيوية

الكبد هو أحد الأجهزة الحيوية في الفقاريات ويلعب دورا رئيسيا في عملية التمثيل الغذائي، بالإضافة إلى العديد من الوظائف الأخرى في الجسم الحي، بما في ذلك تنظيم تخزين الجليكوجين، وتحلل خلايا الدم الحمراء والبلازما تخليق البروتين، وإنتاج الهرمون، وإزالة السموم. ولذلك يعتبر تلف خلايا الكبد "تليف الكبد" حالة ربما تهدد حياتهم والتي ترجع أسبابها إلى مجموعة متنوعة من المكونات الدوائية، والفيروسات، وتعاطي الكحول، وامراض المناعة الذاتية والتعرض للمعادن الأيضية مثل الحديد والنحاس وغيرها من المواد السامة. ومن المعروف أن رابع كلوريد الكربون (CCl_4) هو مادة شديدة السمية على خلايا الكبد. هدفت هذه الدراسة إلى فحص تليف الكبد من خلال الفحوص الأكلينيكية والتحاليل الدموية والكيمياء الحيوية بعد التعرض ل CCl_4 من أجل وضع نموذج لتليف الكبد في الكلاب لإجراء مزيد من البحوث المستقبلية. تلقت ثمانية عشر كلاب بالغة مادة ال CCl_4 لمدة 16 أسبوعا وتم فحصها لمتابعة أعراض تليف الكبد وسجلت درجة تليف الكبد من خلال الفحوص الأكلينيكية واختبارات الدم والكيمياتية الحيوية. وقد لوحظ تغيرات اكلينكية علي الحيوان وكذلك تغيرات في معدلات التحاليل الدموية والكيمياتية ولذلك قد قمنا باستنتاج ان الاعراض الاكلينيكية المصاحبة لتليف الكبد وكذلك التحاليل الدموية والكيمياتية تكون مؤشر لحدوث تليف الكبد