# Galectin-3 in Repairing Damaged Mice Liver Induced By $Ccl_4$: Role of Apoptosis and Oxidative Stress.

***Tahia H. Saleem[1], Mohamed A. Abd EL-Aziz[2], Mona A.H. El-Baz[1], Tarek Okda[2], Hekmat O Abdel-Aziz[3]***

Department of Medical Biochemistry, Faculty of Medicine, Assuit Univrsity[1], Department of Biochemistry, Faculty of pharmacy, Al-Azhar University (Assiut) [2] Department of Histology, Faculty of Medicine, Sohag university[3]

## ABSTRACT

***Background:*** *Carbon tetrachloride ($CCl_4$) causes hepatic injury. Galectin-3 is a member of the lectin family; several studies have suggested that Gal-3 could repair liver damage.* ***Objective:*** *To estimate Gal-3 expressions in different periods after $CCl_4$ administration and to explore the mechanism of repair of the injured liver by Gal-3 either through modulation of apoptosis or oxidative stress.* ***Materials and methods****: Twenty male mice (age 6 weeks; weight 25-30 g) were divided into 4 groups of 5 mice each in separate cages with free access to food and water. Group (I): Control group. Groups II, III and IV administered orally $CCl_4$ as a single dose 50% (W/W); $CCl_4$ in olive oil at 2 ml/kg of body weight and left for 48, 72 and 96 hours respectively. The period of repair of hepatocytes injured by $CCl_4$ and signaling proteins intrinsic to these periods were examined.* ***Results:*** *A 30 kDa polypeptide was detected by both RT-PCR and Western blot analysis using anti-galectin-3 antibody in livers from mice 48 to 96 hours after administration of a single dose of $CCl_4$ and was identified as galectin-3 in hepatocytes. Levels of Gal-3 were significantly higher in liver of mice at 48 to 72 hour after $CCl_4$ treatment compared to the control. Its level was reduced at 96 hours after $CCl_4$ administration. Bcl-2 levels increased significantly during the experimental period after administration of $CCl_4$, where presented in low amount in the control mice. Caspase-3 was detected in trace amount in control mice, increased after 48 and 72 hours from administration of $CCl_4$ and then decreased gradually at 96 hours. Both tissue homogenate levels of nitric oxide and lipid peroxidation showed marked increase at 48 hours as compared to controls. Their levels decreased gradually at 72 and 96 hours after $CCl_4$ administration. The tissue homogenate levels of antioxidants CAT, GSH and SOD activity of all groups were significantly decreased at 48 hours and then increased gradually at 72 and 96 hours after $CCl_4$ administration but did not reach to normal level.* ***Conclusion:*** *Gal-3 plays an important role in repairing the hepatocellular damage which occurred by $CCl_4$ through its role as anti-apoptotic agent and against free radical generation.*

***Abbreviations:*** *Carbon tetrachloride ($CCl_4$), Gal-3(galactin-3), ROS (reactive oxygen species), RT-PCR (reverse transcriptase-polymerase chain reaction).*

## INTRODUCTION

**Galectin-3** is a member of the galectin family, which consists of animal lectins[(1)]. It is approximately 30 kDa and contains a carbohydrate-recognition-binding domain of about 130 amino acids that enable the specific binding of β-galactosides[(2)]. Also, it is encoded by a single gene, LGALS3, located on chromosome 14, locus q21–q22[(3)]. During development, Gal-3 is detected in most types of cells including the hepatocytes in human and mouse embryos[(4)]. It is also present in rat neonate livers by 9 days after birth, but fells to a trace level in the adult liver[(5)].

Animal models of hepatic damage provide a means to study the cell and molecular mediators of damage in a serial manner during both progression and recovery. Several approaches to induction of liver damage have been described. One of these, $CCl_4$ intoxication in rats and mice is probably the most widely studied. In addition, the $CCl_4$ model is the best characterized with respect to histological, biochemical, cellular, and molecular changes associated with the development of the damage. $CCl_4$ can be given intraperitoneally or by oral administration; it induces hepatic damage mainly through hepatocyte apoptosis [(6)].

Galectin-3 was induced in the cytoplasm of periportal hepatocytes and in livers from rats at 48 to 72 hours after administration of a single dose of CCl4; suggesting that it plays a role in repair or survival of the injured hepatocytes [(7)].

**Apoptosis** is the process of programmed cell death that may occur in multicellular organisms. Apoptosis plays a very important role in regulating a variety of diseases that may result from an abnormal ratio of pro- and anti-apoptotic factors[(8)].

**B- cell leukemia/lymphoma 2 (Bcl-2)** is the founding member of the Bcl-2 family of apoptosis regulator proteins. Bcl-2 proteins interact with each other to promote and inhibit apoptosis. It was suggested that Gal-3 binds Bcl-2 through its carbohydrate recognition domain so the increase of Gal-3 increases its binding with Bcl-2 and so suppresses of apoptosis [(9)].

**Caspases** or cysteine-dependent aspartate-directed proteases are a family of cysteine proteases that play essential roles in apoptosis. Caspase-3 is the key member of effector caspases [(10)].

Damage due to oxidative stress and free radicals is one of the important factors for hepatic damage using $CCl_4$. This action could be through the increase in the level of nitric oxide, lipid peroxidation and decreased activities of antioxidant enzymes and generation of free radicals [(11)]. The antioxidant activity or inhibition of the generation of free radicals is important in providing protection against hepatic damage. It has been proposed that antioxidants, which maintain the concentration of reduced glutathione (GSH), super oxide dismutase (SOD) and catalase activity, may restore the cellular defense mechanism and block lipid peroxidation [(12)].

**Aim of the study** The aim of the present study was to estimate Gal-3 expression in different periods after

$CCl_4$ administration and to study its role in repair of the injured mice liver, then explore the mechanism of repair of the injured liver either through modulation of apoptosis or oxidative stress.

## MATERIALS & METHODS

***Animals and treatments:***

The experimental procedures were carried out according to the National Institute of Health Guidelines for Animal Care and approved by The Ethics Committee of the Al-Azhar University, Assuit. Twenty male mice (age 6 weeks; weight 25-30 g) were divided into 4 groups of 5 mice each, in separate cages with free access to food and water. Group (I): Control group. Groups II, III and IV administered orally $CCl_4$ as a single dose 50% (W/W); $CCl_4$ in olive oil at 2 ml/kg of body weight [13]. and left for 48,72 and 96 hours respectively.

The animals were sacrificed by cervical decapitation. Liver was separated from each mouse and washed several times with 0.9% sterile saline solution to remove any blood from the tissues and was transferred to filter paper to remove excess saline solution. Homogenization buffer was added to liver tissue (0.5 g from the liver with 4.5ml from 0.25M sucrose buffer). The tissue samples were homogenized by tissue homogenizer (IKA ULTRA-TURRAX® T25) at 40,000 rpm. The supernatant extracts were collected in Ependorf tubes and frozen at -40°C till assay of NO, TBARS, CAT, GSH and SOD. All steps of that operation were performed rapidly and in ice path.

**Drugs and Chemicals:**

$CCI_4$ and olive oil were purchased from Sigma chemical Company (St., Louis, MO, USA).

**Methods:**

***1- Detection of galactin-3, Bcl-2, and Caspase-3 by RT-PCR analysis:***

**a) RNA isolation:**

Total RNA fractions were prepared using RNA Kit (Omega Bio-Tek) to provide a rapid method for the isolation of total RNA. The concentration of RNA was determined by measuring the absorbance at 260 and 280 nm using double stream spectrophotometer (T80 UV/Vis. Spectrometer PG Instruments LTd, UK) and through the following equation:

$$Concentration\,of\ RNA(ug/ul) = \frac{[(62.9xA260 - 36A280)xDilution\ factor]}{1000}$$

**b) RT-PCR assay:**

RT/PCR premix kit (Bioron Cat No.:122020-96) which consists of premix tubes that contain all the components necessary for cDNA synthesis and amplification in one tube as Reverse transcriptase and DNA polymerase enzymes.

The various cDNAs were polymerase chain reaction (PCR) amplified with specific primers (Pupmed, gene bank, nih, RNA workbench 4) in 50 µl of PCR

reactions containing 10× PCR buffer (10 µl), 2µl deoxyribonucleoside- 5′-triphosphate, RT-PCR preMix (2 µl), distilled water (18 µl), 8µl primer (4µl for each primer), and 10µl RNA. The amplification conditions consisted of an initial denaturation at 94°C for 5 minutes, followed by 37 cycles of 94°C for 30 seconds, and 55°C for 30 seconds, with a final extension at 72°C for 1 minute. The PCR products were analyzed with 1.5% (w/v) agarose electrophoresis.

**Primer design:**

The primer for galectin-3 (Gal-3); sense primer; 5'-GGGTGT CCT CTC CAT TTG AC-3' and antisense primer 5'-CCG GCG TAT ATC ATG ATG GG-3' (Pupmed, gene bank, nih, RNA workbench 4).

The primer for Bcl-2; sense primer 5'-GAC GGA GAA GAA TGT CCT TA-3' and antisense primer 5'-CGA CCT TGT CGT ACT GAG CA-3' (Pupmed, gene bank, nih, RNA workbench 4).

The Primer for Caspase-3; sense primer 5'-GAC CAT GGA GAA CAA CAA AAC-3' and antisense primer 5'-GGC AGG CCT GAA TGA TGA AG-3'(Pupmed, gene bank, nih, RNA workbench 4).

***2- Detection of galectin-3 by* Western blot analysis*:***

Liver was homogenized in 10 mM Tris–HCl buffer (pH 7.5) containing 140 mM NaCl, then denatured at 95°C for 5 min in the presence of 2% SDS and 5% 2-mercaptoethanol and stored at -70°C until the time of experiment. 50µg. of total supernatant liver homogenate was used in each lane [(14)].

Anti Gal-3 antibody 1:200 dilution and anti-rat Ig secondary antibody was used in 1:1000 dilutions. Enhanced chemiluminescence (ECL) detection kit was used to detect the chemiluminescence were used in western blot.

**3-*Biochemical analysis:***

The tissue levels of NO and TBARS (oxidative stress markers) were determined by chemical methods according to the methods described by **Van Bezooijen et al., [(15)]**, and **Buege and Aust [(16)]** respectively. Also, the tissue levels of catalase (CAT), glutathione (GSH) and superoxide dismutase (SOD) activity (anti oxidants) were determined by chemical methods according to the methods of **Clairborne [(17)]**, **Beutler et al. [(18)]** and **Marklund [(19)].**

**Histological Examination:**

After scarifying animals, parts from the livers were rapidly removed, preserved in 10% buffered formalin, washed, dehydrated in ascending grades of 95% ethanol and cleared in xylene. The specimens were embedded in paraffin blocks and 5µm thickness tissue sections were cut by Leica microtome, mounted on slides and stained with hematoxylin and eosin. The slides were examined by light microscope (Olympus BX50) and photographed. All histological evaluations were made under blind conditions.

**Statistical analysis:**

Statistical analysis was achieved using Graph Pad In Stat. software Inc, Program, version 4.0 Philadelphia, San Diego CA, (2003). Data were presented as mean ± SD and the levels of significance were accepted with $p < 0.05$. Multiple comparisons were done using one way ANOVA followed by Tukey-Kramer test as

multiple comparison post ANOVA test.

## RESULTS

***1- RT-PCR Results:***

Figure (1) shows the expression of Gal-3 in all studied groups. The band besides of marker's band (DNA ladder) shows the low level of Gal-3 in the control. Over expression of Gal-3 is markedly increased after 48 and 72 hr from treatment with $CCl_4$ in comparison to controls. In other hand, the expression is markedly reduced after 96 hours .

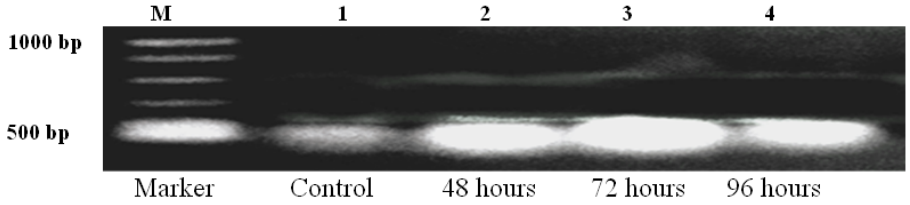


**Figure (1)**

Figure (2) shows the expression of Bcl-2 in all studied groups. The Bcl-2 increased gradually during the experimental periods (48, 72& 96 hours after administration of $CCl_4$) as compared to controls using DNA ladder markers.

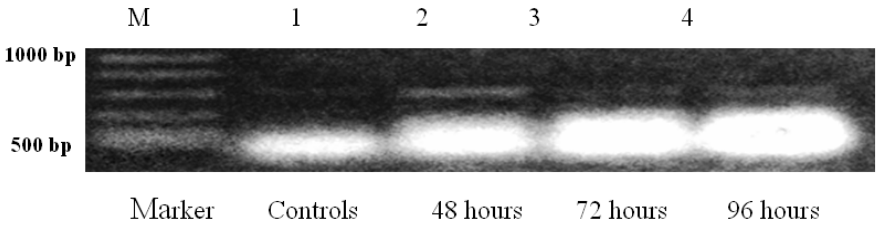


**Figure (2)**

Figure (3) shows the expression of caspase in all studied groups. The caspase increased after 48 hours after $CCl_4$ administration as compared to controls and then decreased gradually during the experimental stages.

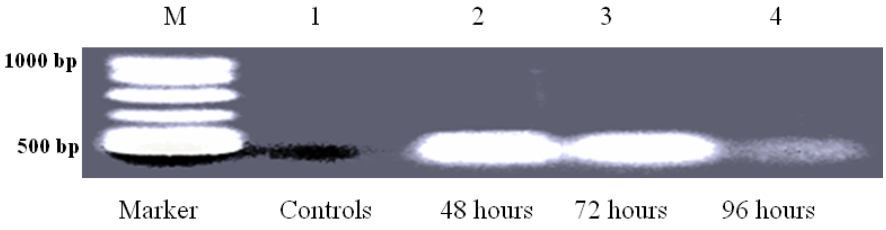


**Figure (3)**

**2- Western Blot results:** Figure (4) shows Galectin-3 protein expression determined by western blot in all experimental groups (control, mice treated with $CCl_4$ and left for 48, 72 and 96 hours) using Gal-3 antibody. The detection a 30 kDa protein appearing in liver of mice at 48 to 72 hours after $CCl_4$ administration, the expression of Gal-3 reached its maximum at 48 to 72 hours after treatment with $CCl_4$. Over expression of Gal-3 is markedly increase after 48 and 72 hours from treated with $CCl_4$. The molecular weight (demonstrated according to position in acrylamide gel, because it is not visible in western analysis On other hand, the expression markedly reduced after 96 hours. Immunoblot analysis using Gal-3 antibody as a probe demonstrated that liver extract prepared from normal mice contained trace amount of Gal-3. Thus, Gal-3 appears in a process of liver injury. Since immunoblot analysis using Gal-3 antibody indicated that Gal-3 is present in cytosolic liver from 48 to 72 hours.

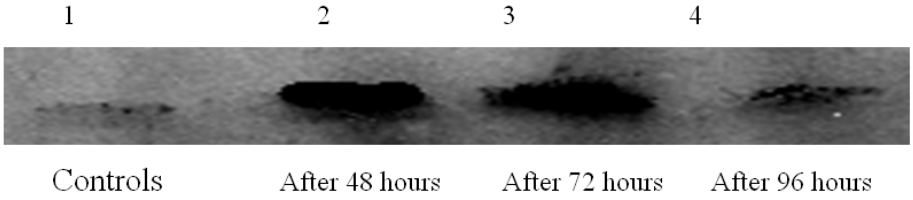


**Figure (4)**

***Biochemical changes:*** Biochemical changes induced by oral administration of $CCl_4$ (2 ml/kg) in adult male mice are presented in table (1). There was a significant increase in TBARS and NO and decrease in antioxidants (GSH and SOD activity) at 48, 72 and 96 hr than controls.

**Table (1):** Tissue levels of NO, TBARS, CAT, GSH and SOD activity in all studied groups.

| ***Biochemical*** | ***Group I: Controls (n=5)*** | ***Treated mice with $CCl_4$*** | | |
|---|---|---|---|---|
| | | **Group II: Mice left for 48 hr (n=5)** | **Group III: Mice left for 72 hr (n=5)** | **Group IV: Mice left for 96 hr (n=5)** |
| **NO (µM/g wet tissue)** | 34.15±4.47 | 78.48±5.66<br>P*< 0.001 | 59.65±5.5<br>P*< 0.05 | 51.20±6.4<br>P**< 0.05 |
| **TBARS (µM/g wet tissue)** | 1.192±0.25 | 4.04± 1.08<br>P*< 0.001 | 3.53±0.47<br>P*<0.001 | 2.45±0.23<br>P*< 0.05<br>P**< 0.01 |
| **CAT (U/mg protein)** | 0.0370±0.009 | 0.0137±0.007<br>P*<0.001 | 0.0145±0.003<br>P*<0.01 | 0.0236±0.008 |
| **GSH** (uM/mg protein) | 17.36±2.18 | 10.44±2.434<br>P*<0.001 | 13.00±1.118<br>P*<0.05<br>P**>0.05 | 16.16±1.483<br>P**<0.001 |
| **SOD activity** (U/mg protein) | 14.83±1.87 | 4.793± 0.867<br>P*<0.001 | 8.008 ±1.167<br>P*<0.001<br>P**<0.05 | 10.70 ±1.425<br>P*<0.01<br>P**<0.00<br>P***<0.051 |

NO, nitric oxide; TBARS, thiobarbituric acid reactive substaances; CAT, catalase; GSH, glutathione reductase; SOD, superoxide dismutase. Presented values are means ±SD. n, number; hr, hours. *mice of group II, III, IV versus controls (group I).
** mice of group III, IV versus group II. *** mice of group III versus IV.

***Histopathological changes***

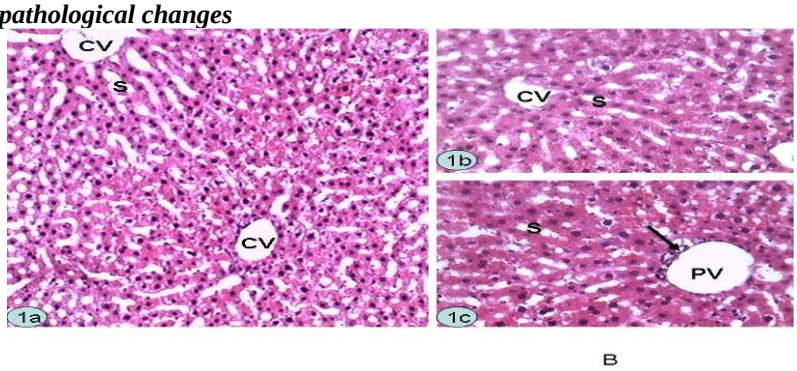


**Fig. 5:** Light photomicrograph of mice **liver from the control group:** demonstrating: **1a&1b:** Normal hepatocytes surrounding the central vein (CV). They are polygonal with pale vesicular nuclei and prominent nucleoli. They have an esinophilic granular cytoplasm. The hepatic sinusoids (s) are narrow and lined by endothelial cells and Von Kupffer cells. **1c** The portal vein tributaries (PV) and the branch of the bile duct (arrow) are enclosed in a fine connective tissue stroma within the portal tract. (H&E stain. Mic. Mag. 1a X 200 and 1b &1c X400)

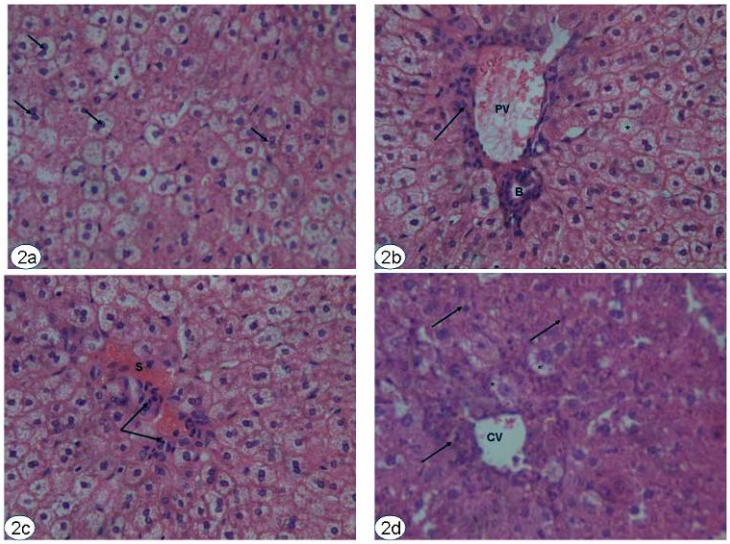


**Fig. 6:** Light photomicrograph of the liver of mice treated with **$CCl_4$ after 48 hours revealing: 2a:** Vacuolar changes (*) in the hepatocytes. Note many of the hepatocytes have two nuclei (arrows). **2b:** Dilated and congested portal vein (PV). The portal tract containing the portal vein (PV) and the bile duct (B) also showed inflammatory cell infiltration. **2c:** Focal area of congested blood sinusoids within the hepatic tissue (S) surrounded with inflammatory cells (arrows). **2d:** Degenerated cells around the central vein (**CV**) with pyknotic nuclei (arrows) and vacuolated cells (*). (H&E stain. Mic. Mag. X400)

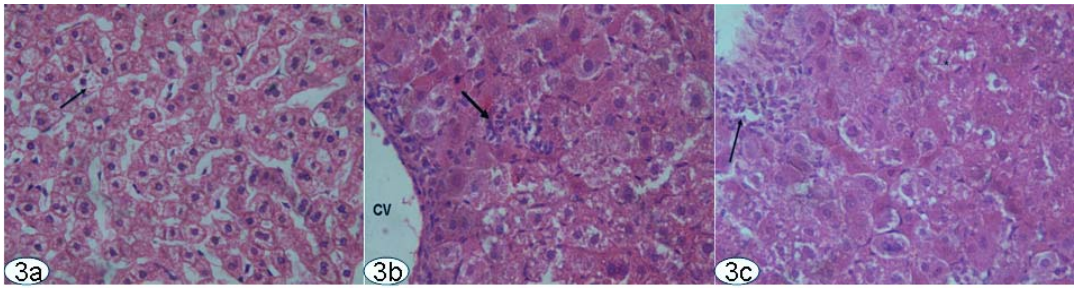


**Fig. 7:** Light photomicrograph of the liver of mice treated with **$CCl_4$ after 72 hours revealing: 3a:** An intact hepatic architecture with mild degree of vacuolation (arrow). **3b:** Most of the hepatocytes with normal cytoplasmic appearance around the central vein (CV). Occasional small aggregates of inflammatory cells also appeared (arrow). **3c:** Many hepatocytes with normal cytoplasmic appearance and few with vacuolated cytoplasm (*) with focal inflammatory cell at the periphery of the classic lobule. (H&E stain. Mic. Mag. X400)

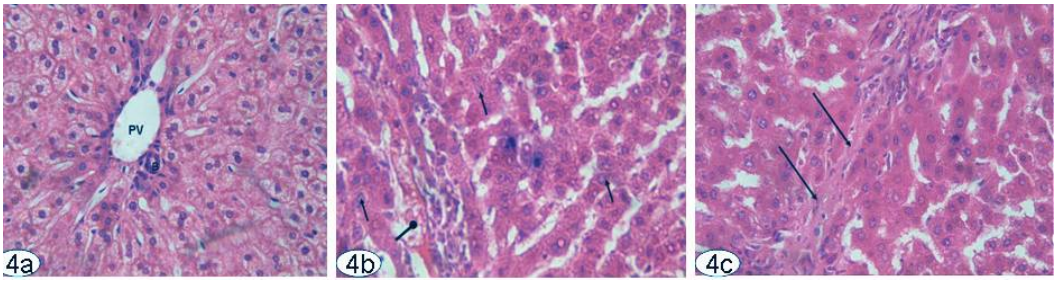


**Fig. 8:** Light photomicrograph of the liver of mice treated with **$CCl_4$ after 96 hours demonstrating: 4a:** An intact hepatic architecture with no inflammatory cells in the portal area (PV: portal vein, B: bile duct). **4b:** Hepatocytes with normal nuclear and cytoplasmic appearance (arrows). Few degenerated cells also appeared (oval arrow). **4c:** Prominent connective tissue stroma between the hepatic lobules (arrows).

## DISCUSSION

In the present study, the appropriate dose of $CCl_4$ causes pericentral hepatocyte death approximately 24 hr after its administration, the pericentral lesion thus formed can be restored to a normal architecture by repairing and regeneration of the cellular components during an approximately 2-week period post-treatment[6]. The reproducible nature of the chemically induced liver injury allows the study, at a tissue level, of the proteins involved in the regulation of hepatocyte death and regeneration. On the other hand, acetaminophen and some other therapeutics can similarly injure the liver in humans [20];

implying that drug induced liver injury is a serious problem in patients under particular conditions. Therefore, identification of proteins that protect and ameliorate the injury is useful for understanding the mechanisms of serious liver injury and developing suitable treatments, as well as the understanding of mechanisms for viability control in hepatocytes.

Liver damage after $CCl_4$ treatment is generally considered to result from two main effects; firstly, hepatocyte apoptosis[6]. Secondly, decrease in the activity of some hepatic antioxidant enzymatic mechanisms leading to an increase in some free radicals like lipid peroxidation and nitric oxide [21].

The result of the present study exhibited marked increase in the RNA expression and protein synthesis of Gal-3 at 48 and 72 hours after administration of $CCl_4$ than the controls. On the other hand, the expression was markedly decreased at 96 hours after treatment with $CCl_4$ as compared to controls. These results are comparable with previous studies which showed that Gal-3 was induced in the cytoplasm of periportal hepatocyte of adult rat liver with inflammation caused by $CCl_4$ treatment. The Authors study clarified that Gal-3 is induced as a consequence of augmentation of Gal-3 gene transcription [7, 22].

**B- cell leukemia/lymphoma 2** is an anti-apoptotic protein molecule involved in regulation of apoptosis. In the current study, the RNA expression of Bcl-2 increased gradually during the experimental stages, it was present in low amount in the controls and increased after treatment with $CCl_4$ in all stages of the experiment (from 48 to 96 hr) after $CCl_4$ treatment.

The previous findings of the increase in Bcl-2 could be compensatory to overcome the damage occurred in the liver by $CCl_4$[23].

The present results are in harmony with other results that explained the relation between Bcl-2 and Gal-3 that was due to the similarity between Bcl-2 and Gal-3 in structure. Both proteins are rich in proline, glycine and alanine amino acid residues in their N terminal and contain Asp-Trp-Gly-Arg in the C terminal [9].

Another explanations for the anti apoptotic role of Gal-3 regarding to Bcl-2 was reported; firstly, it was suggested that Gal-3 binds Bcl-2 through its carbohydrate recognition domain, so the increase of Gal-3 at 48 and 72 hours after $CCl_4$ treatment increases its binding with Bcl-2 so this explains the anti-apoptotic action of Gal-3[9]. Secondly, it was reported that Gal-3 is equally significant for its anti-apoptotic activity as it is for Bcl-2. Bcl-2 translocation to the mitochondrial membrane leads to anti-apoptotic activity resulting from blocking cytochrome release so this is another explanation for the anti-apoptotic action of Gal-3[24].

**Janicke *et al.*** [25] study suggests that caspase activation is essential for the occurrence of the apoptotic phenotype of cell death. A variety of caspase substrates are involved in the regulation of DNA structure, repair and replication. Carbon tetrachloride induces marked histopathological changes and increase in the levels of apoptotic proteins. $CCl_4$ treatment significantly increased the levels of

apoptotic proteins such as caspases-3[26].

In the present study, both RNA expression and tissue levels of caspase-3 were detected in trace amount in the control group, moreover markedly increased at 48 hr after $CCl_4$ treatment compared to the control group, then gradually decreased in both 72 and finally at 96 hr after $CCl_4$ treatment but not reached to the control level. These results are in harmony with similar results reported by **Moon et al.**[27] who showed that caspase-3 which is pro-apoptotic protein increased at 48 hr after $CCl_4$ treatment, which indicates the damage occurred in the liver by $CCl_4$ due to hepatocellular apoptosis, followed by decreased levels due to the partial repairing occurred in the liver cells as a result of the release of Gal-3 with its anti-apoptotic effect.

Carbon tetrachloride is a hepatotoxic agent and it is the well established animal model for free radical-induced liver injury, $CCl_4$ treatment significantly increased the levels of one of the free radicals inflammatory mediators which is iNos and decreased the levels of GSH SOD and CAT with subsequent increase in the apoptotic proteins [26]. In the present study, oxidative stress was assessed by measuring TBARS, a product of lipid peroxidation, NO as free radical and some antioxidants as GSH, SOD and CAT.

Data from the current study revealed that mice at 48 hr after treatment with $CCl_4$ showed significant increase in tissue levels of both TBARS and NO and significant decrease in GSH, SOD and CAT. Increased generation of ROS and decreased antioxidants in liver tissues has been reported in many models of $CCl_4$ induced hepatic injury in animals. **Boll et al.** [28] explained the mechanism of lipid peroxidation using $CCl_4$ which was through a reaction with oxygen to form $CCl_3$-OO* which initiates lipid peroxidation reactions.

On the other hand, the repair of hepatic cells as a result of the release of Gal-3 at 72 hr after CCl4 treatment and its regeneration of hepatic cells may explain the reduced levels of both TBARS and NO in comparison to 48 hr group, with a subsequent increase of the anti-oxidant parameters. At 96 hr group the liver supposed to be repaired so the lowering of the generation of the free radicals and the increase in the anti-oxidant parameters would be more pronounced.

**In conclusion:** Gal-3 plays an important role in repairing the hepatocellular damage which occurred by $CCl_4$ through different mechanisms as the following: Firstly, Gal-3 has a role as anti-apoptotic agent due to the similarity between Gal-3 and Bcl-2 in structure and functions. Secondly, through its role against free radical generation

## REFERENCES

1. **Rudolf, A., Boer, Lili, Y.U. and Dirk, J. van Veldhuisen (2010):** Galectin-3 in Cardiac Remodeling and Heart Failure. Current Heart Failure Reports, Journal 7(1): 1-8.
2. **Dumic, J., Dabelic, S. and Flögel, M. (2006):** Galectin-3: an

open-ended story. Biochim. Biophys. Acta 1760 (4): 616–635.

3. **Sharma, U.C., Pokharel, S., van Brakel, T.J. (2004):** Galectin-3 marks activated macrophages in failure-prone hypertrophied hearts and contributes to cardiac dysfunction. *Circulation* 110 (19): 3121–3128
4. **Van den Brule, F.A., Fernandez, P.L., Buicu, C., Liu, F.T., Jackers ,P. and Lambotte, R. (1997):** Differential expression of galectin-1 and galectin-3 during first trimester human embryogenesis. Dev. Dyn., 209: 399-405.
5. **Cerra, R.F., Gitt, M.A. and Barondes, S.H. (1985):** Three soluble rat beta galactoside-binding lectins. J. Biol. Chem., 260: 10474–10477.
6. **Constandinou, C., Henderson, N. and Iredale, J.P. (2005):** Modeling liver fibrosis in rodents**.** Methods Mol Med., 117:237-50.
7. **Yamazaki, K., Kawai, A., Kawaguchi, M., Hibino, Y., Li, F. and Sasahara, M. (2001):** Simultaneous induction of galectin-3 phosphorylated on tyrosine residue, p21(WAF1/Cip1/Sdi1), and the proliferating cell nuclear antigen at a distinctive period of repair of hepatocytes injured by $CCl_4$. Biochem. Biophys. Res. Commun., 280: 1077–1084.
8. **Glantz, L.A., Gilmore, J.H., Lieberman, J.A. and Jarskog, L.F. (2006):** "Apoptotic mechanisms and the synaptic pathology of schizophrenia". Schizophr. Res., 81 (1):47-63.
9. **Yang, R.Y., Hsu, D.K. and Liu, F.T. (1996):** Expression of galectin-3 modulates T-cell growth and apoptosis. Proc. Nat. Acad. Sci. U. S. A., 93: 6737–6742.
10. **Yuan J. and Horvitz H. R. (2004):** A First Insight into the Molecular Mechanisms of Apoptosis. Cell 116: 53–56.
11. **11-Poli, G. (1993):** Liver damage due to free radicals. Br. Med. Bull., 49(3):604-620. **Pupmed, gene bank, nih, RNA workbench 4.**
12. **Montilla, P., Cruz, A., Padilto, F.J., Tilnez, I., Gascon, F., Mufioz, M.C., Gdmez, M. and Perq, C. (2001):** Melatonin versus vitamin E as protective treatment against oxidative stress after extra-hepatic bile duct ligation in rats. J. Pineal Res., 31 (2): 38-**44.**
13. **Rao, P.S., Mangipudy, R.S. and Mehendale, H.M. (1997):** Tissue injury and repair as parallel and opposing responses to CCl4 hepatotoxicity: a novel dose response. Toxicology 118: 181-193.
14. **Kim, H., Lee, J., Hyun, J. W., Park, J. W., Joo, H. and Shin, T. (2007):** Expression and immunohistochemical localization of galectin-3 in various mouse tissues. Cell Biology International 31(7): 655-662.
15. **Van Bezooijen, R.L., Que, I., Ederveen, A.G., Kloosterboer, H.J., Papapoulos, S.E. and Lowik, C.W. (1998):** Plasma nitrate+nitrite level are regulated by ovarian steroids but do not

correlate with trabecular bone mineral density in rats. J. Endocrinology 159: 27-34.

16. **Buege, J.A. and Aust, S.D. (1978):** Microsomal lipid peroxidation. modified by adding the BHT solution to prevent further lipid peroxidation during boiling. Method Enzymology 52: 302-310.
17. **Clairborne, A. (1985):** Catalase activity. In: Greenwold R.A,(ed.). Handbook of Methods for Oxygen Radical Research CRC press, Boca Raton, Florida. 283-284.
18. **Beutler, E., Duron, O. and Kelly, B.M. (1963):** Improvement method of the determination of glutathione in tissue homogenate. J. Lab. Clin. Med. 61: 882-888.
19. **Marklund, S.L. (1985):** Superoxide dismutase isoenzymes in tissue and plasma from New Zealand black mice. Mutat. Res.,148: 129-134.
20. **Zhang, J., Huang, W., Chua, S.S., Wei, P. and Moore, D.D. (2002):** Modulation of acetaminophen-induced hepatotoxicity by the xenobiotic receptor CAR. Science 298: 422–424.
21. **Gonzalez-Reimers, E.**, **Lopez-Lirola, A**., **Olivera, R.M**., **21-Gonzalez-Reimers, E.**, **Lopez-Lirola, A**., **Olivera, R.M**., **Santolaria-Fernandez, F**., Galindo-Martín L, Abreu-González P, Sánchez-Sanchez JJ, Martínez-Riera A**.(2003):** Effects of protein deficiency on liver trace elements and antioxidant activity in carbon tetrachloride-induced liver cirrhosis. Biol Trace Elem. Res., 2003 93(1-3):127-40.
22. **Stein, G. H., Drullinger, L. F., Soulard, A. and Dulic, V. (1999):** Differential roles for cyclin-dependent kinase inhibitor p21 and p16 in the mechanisms of senescence and differentiation in human fibroblasts. Mol. Cell. Biol., 19: 2109–2117.
23. **Takeda, K., Watanabe, J., Inoue, K. and Kanamura, S. (2000):** Rifampicin suppresses hepatic CYP2E1 expression and minimizes DNA injury caused by carbon tetrachloride in perivenular hepatocytes of mice. Alcohol. Clin. Exp. Res., 24(4): 87-92.
24. **Burlacu, A. (2003):** Regulation of apoptosis by Bcl-2 family proteins. J. Cell. Mol. Med. 7(3):249–257.
25. **Janicke, R.U., Spregart, M.L., Wati, M.R. and Porter, A.G. (1998):** Caspase-3 is required for DNA fragmentation and morphological changes associated with apoptosis. J. Biol. Chem., 273: 9357 - 9360.
26. **Srilaxmi, P., Sareddy, G.R., Kishor, P.B., Setty, O.H. and Babu, P.P. (2010)**: Protective efficacy of natansnin, a dibenzoyl glycoside from Salvinia natans against $CCl_4$ induced oxidative stress and cellular degeneration in rat liver. B.M.C. Pharmacol., 12: 10-13.
27. **Moon, B.K., Lee, Y.J., Battle, P., Jessup, J.M., Raz, A. and Kim, H.R. (2001):** Galectin-3 protects human breast carcinoma cells against nitric oxide induced

apoptosis. Implication of galectin-3 function during metastasis. Am. J. Pathol. 159(3): 1055-1060.

**28. Boll, M., Weber, L.W., Becker, E. and Stampfl, A. (2001).** Mechanism of carbon tetrachloride-induced hepatotoxicity. Z. Naturforsch C. 56 (7-8):649-659.

## الجلاكتين-٣ فى اصلاح التلف الحادث فى كبد ا لفئران نتيجة اعطاء١١١١ الكربون تتراكلوريد: دور الانحدار الخلوى الذاتى و الضغط التأكسدى

***تحية هاشم سليم*، محمد أنور عبد العزيز**، منى عبد الحميد الباز*، طارق محمود عقده**، حكمت عثمان عبد العزيز******

أقسام الكيمياء الحيويه الطبيه-كلية الطب-جامعة أسيوط*، الكيمياء الحيويه -كلية الصيدله-جامعة سوهاج**، الهستولوجى--كلية الطب-جامعة سوهاج***

يعد الجلاكتين-٣ ( الذى يتكون من اللا كتين الحيوانى مرتبط مع الجلاكتوز) أحد أفراد الجليكوبروتينات المكتشفة حديثاً. توجد كميات ضئيلة من الجلاكتين-٣ فى خلايا الكبد الطبيعية ولكن وجد ان كمية الجلاكتين-٣ تزداد فى خلايا الكبد لدى فئران التجارب المصابة بتكسير خلايا الكبد إثر اعطائها مادة رابع كلوريد الكربون. تم فى هذه الدراسة تقدير نسبة الجلاكتين-٣ فى خلايا الكبد بواسطة إستخدام مضادات الاجسام للجلاكتين -٣ والتحليل باستخدام جهاز فصل البروتينات وايضاً بواسطة (أر.تى.بى.سى.أر). كما تم تعيين نسبة ال(بى سى ال-٢) و الكاسباس- ٣ بواسطة (أر.تى.بى.سى.أر) . وجد أن أعلى مستوى للجلاكتين-٣ يكون ما بين ٤٨-٧٢ ساعة من حقن رابع كلوريد الكربون للفئران. كما وجدت زيادة ذات دلالة احصائية فى مستويات (بى سى ال- ٢) اثناء جميع مراحل التجربة اثر حقن رابع كلوريد الكربون عند مقارنتهم بالمجموعة الضابطة.

بينما وجد ان الكاسباس-٣ قد زاد زيادة ملحوظة فى كبد فئران التجارب بعد ٤٨ و ٧٢ ساعة من حقن رابع كلوريد الكربون عند مقارنهم بالمجموعة الضابطة ثم نقص بشكل تدريجى حتى ٩٦ ساعة بعد الحقن .

و يلعب الجلاكتين-٣ دوراً مهماً فى اصلاح التلف الحادث فى خلايا الكبد نتيجة حقن مادة رابع كلوريد الكربون ومحاولة إعادة خلايا الكبد المدمرة إلى وضعها الطبيعى .تقوم مادة رابع كلوريد الكربون خلال ال٢٤ ساعة الاولى من الحقن بزيادة دلالات الضغط التاكسدى عن طريق زيادة مستوى أكسيد النيتريك و فوق أكسيد الدهون زيادة ملحوظة فى خلايا الكبد إذا ما قورنت بالمجموعة الضابطة. كما يقوم الجلاكتين-٣ بازالة التلف القابل للاصلاح فى خلايا الكبد بتقليل مستوى دلالات الضغط التاكسدى عن طريق الاقلال من مستوى أكسيد النيتريك و فوق أكسيد الدهون لكى يصل الى مستوى يقارب مستوى المجموعة الضابطة بعد ٩٦ ساعة من حقن رابع كلوريد الكربون