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Therapeutic Effect of Camel Milk Against Hepatotoxicity Induced by CCl_4 in Rats.

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ABSTRACT

This study was aimed to assess the therapeutic potential of camel milk on the carbon tetrachloride (CCl_4)-induced hepatic injury in rats. A total of 24 Rats were randomized and divided into 4 groups (6 rats for each). Group 1: control untreated, group 2 was orally treated with camel milk (5 ml/ rat/day) through gastric intubation with duration of 3 times weekly for 2 weeks and 5 times weekly for another 4 weeks.Group 3: CCl_4 intoxicated rats (intraperitoneally injected with CCl_4(1ml/kg b.w, 3 times weekly for 4 weeks). Group 4 was treated with $CCl_4$and camel milk with the same dose and treatment protocol of the group 2 &3 .Blood samples were collected at the end of experiment for determination of serum levels of liver enzymes, albumin and total proteins.Determination of inflammatory cytokines (TNF-α and IL-1β) in liver homogenate was done and detection of the hepatic m-RNA expression of CYT p450 2E1. Other liver specimens were routinely fixed and processed for histopathological evaluationin addition,immunohistochemical evaluation of α- SMA expressionin hepatic sections was performed. Results revealed thatCCl_4induced significant ($P<0.01$) increase in serum hepatic enzymesand significant ($P<0.01$) reduction of serum total proteins and albumin with elevated levels of TNF-α and IL-1 β compared with control animals. Genetic results showed that the administration of CCl_4 caused a significant down - regulation of the expression of CYP2E1 genes in liver tissue compared to control.On other hand the treatment with camel milk markedly improve serum hepatic functions and inhibit the down regulation of inflammatory cytokines and CYP2E1 genes in liver .Varioushistopathological alterations were detected in CCl_4- intoxicated group that was markedly ameliorated by camel milk administration. Conclusion: the present study proved the therapeutic potential of camel milk against CCl_4 - induced hepatic damage though improvement of hepatic function,decrease level of inflammatory cytokines and inhibit the down regulation of CYP2E1 genes in liver as well as markedly reduced hepatic histopathological alterations .

Keywords: CCl_4, Hepatotoxicity, Camel milk,Rats.

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INTRODUCTION

The liver is pivotal organ in body it has several biochemical, metabolic functions, and energy production(Asija et al., 2015). Liver disease is considered as one of most common diseases among humans at different ages and causes great mortalities among public as 20,000 deaths occur every year (Pramod et al., 2012) .Viral hepatitis, alcohol liver disease, non-alcoholic fatty liver disease, autoimmune liver disease, metabolic liver disease, drug induced liver injury, gallstones are the most encountered hepatic disorders. Hepatocellular carcinoma is one of the ten most common tumors (Hurkadale*et al*., 2012).

Carbon tetra chloride (CCl_4)was known to be hepatotoxin that induced liver damage(Gnanaprakash*etal*., 2010). Within the body, CCl_4 breaks down to highly toxic trichloromethyl and trichloromethyl peroxyl free radicals by cytochrome P450 enzyme (Khan *etal*., 2009) which cause damage to hepatocytes (Girish *et al.*, 2009).CCl_4-induced hepatotoxictyvia activationof the toxicant by the microsomal cytochromeP450-dependent monooxygenase system to thetrichloromethyl radical (CC13) and in the presence of oxygen will converted to a peroxy radical (CCI3-OO).These free radicals further induced lipid peroxodation and degenerative cellular changes damage(Boll *et al*.,2001).These processes are followed by release of inflammatory cytokines and growth factors and depletion of CYP2E1 activity the infiltration of inflammatory cells and release of various cytokines and growth factors (Simeonova*et al*., 2001,Fahmy*et al*., 2009, Al-Seeni*et al.*,2016).

Current available drug treatment is unable to meet the demand clinically due to lack of complete cure, numerous adverse effects, lower safety, etc. Therefore, interest concerned the use of alternative medicines for treatment of hepatic disease has been arisen. Camel milk (CM) is different from other ruminant milk; it has low levels of protein, cholesterol and sugar, but has high levels of vitamins, minerals, and insulin. (Yousef, 2004). It has no allergic features and can be used by lactose-intolerant persons as well (Cardoso *et al*., 2010). Additionally, CM exhibits a wide range of biological activities; antioxidative, antimicrobial, antihypertensive, antithrombotic, and immuno-modulatory effect (FitzGerald and Meisel, 2000 and Saltanat*et al*., 2009).

The present study aimed to evaluate the camel milk as a therapeutic agent against CCl_4- induced hepatic injury.

MATERIALS AND METHODS

Chemicals:

Carbontetrachloride (CCl_4) is of molecular weight 153:84.It was obtained fromLobaChemie,India.

Materials:

Camel milk: Early morning, hand milking CM samples were daily collected from western desert camel farm in sterile screw capped containers and were transported to the laboratory in cool boxes. CM was given to rats in a dose of 5 ml/ rats according to (El Miniawy*et al*., 2014), once daily, 3 times weekly for 2 weeks and 5 times weekly for 4 weeks more.

Experimental Animals and design

Twenty four adult male albino rats with average body weight 180gm.Animals were maintained in the animal holding room under controlled environmental conditions (12/12 h light/dark cycle and 24°C) and fed rodent diet (NRC, 1977)and tap water *adlibitum*.They were housed in a well-ventilated vivarium of the animal house of Nuclear Research Centre, Inshas, Egypt. The rats were fed standard diet and water ad libitum. The animals were divided into four groups of six animals each and labelled (Groups G1 to G4). Group 1 (G1) served as normal control group. Group 2(G2) served as camel milk group with oral intubation of rats at dose of5ml / kg once daily, 3 times weekly for 2 weeks and 5 times weekly for 4 weeks more. Group 3(G3) served as CCl_4 – intoxicated group,intraperitoneally injected (I.P) with CCl_4 (1 ml/kg 3 times weekly for four weeks according to Abdel-Moneim*et al*. (2015).Group 4 (G4) served as therapeutic camel milk group, the rats were I.P injected

with CCl_4 for 4 weeks, beginning from 3rd week rats were orally treated with CM along with CCl_4 then increasing the times of CM treatment to 5 times weekly for 4 weeks more.

Determination of liver enzymes activities and total bilirubin

Serum samples were used for determination of alanine and aspartate aminotransferase activities (ALT&AST) (Reitman and Frankel, 1957), alkaline phosphatase (ALP) (Belfied and Goldberg,1971) and total bilirubin (TP) (Walter and Gerade, 1970).

Determination of serum Total protein and albumin.

The level of total proteins and albumin were determined by a colorimetric method as described by Gornal*et al*. (1949) and Doumas*et al*.(1971) resepectivelyusing available commercial kits.

Determination of serum globulin (G)

The globulin value was obtained by subtracting the albumin value from the corresponding total proteins value for each sample.

Determination of serum globulin /albumin (A/G) ratio

The A/G ratio was calculated by dividing each sample's albumin value by its corresponding albumin value.

Determination of TNF-αand interleukin -1- beta(ELISA assay):

liver levels of tumour necrosis factor alpha (TNF-α) and interleukin -1- beta (IL-1-β) in rats were measured using enzyme-linked immunosorbent assay (ELISA) kit by the methode described by Taylor.(2001) and Grassi*et al*. (1991) respectively according to manufacturer's instructions.

Histopathological examination and lesion scoring

Liver specimens were collected from different treated groups and were fixed in 10% buffered formaline.Tissue specimens were routinely processed to obtain paraffin embedded histological section which were routinely stained by H&E stain in addition to special Masson`s Trichrome for collagen staining .(Bancroft and Gamble, 2008).Hepatic sections were microscopically examined and lesion scoring was performed as follow:-

Examination of 10 low power fields (4x for fibrosis and 10x for the other hepatic lesions) per liver section and five liver sections per group were examined. Fibrosis was graded according to Ishak*et al*. (1995) into six grades.grade 0 indicates no fibrosis, grade 1 indicates fibrosis of some portal areas with or without short septa expansion into hepatic lobule, grade 2 indicates fibrosis of most portal areas with or without short septa expansion into hepatic lobule, grade 3 indicates fibrosis of most portal areas with occasional portal to portal bridging, grade 4 indicated indicates fibrosis of most portal areas with marked portal to portal bridging and finally grade 5 that indicates fibrosis of most portal areas with marked portal to portal bridging and occasional nodule formation and finally grade six that indicates complete cirrhosis. the confluent necrosis was also graded into six grades according to Ishak*et al*. (1995) as follow :grade 0 for no obvious necrosis, grade 1 indicated focal confluent necrosis,grade 2 indicates Zone 3 necrosis in some areas, grade 3 indicates Zone 3 necrosis in most areas,grade 4 indicates Zone 3 necrosis with occasional bridging portal-central,grade 4 indicates Zone 5 necrosis with multiple bridging portal-central and grade 6 for Panacinar necrosis, while ballooning degeneration, steatosis and hepatocellular vacuolization were graded into four grades according to Shackelford *et al*. 2002).Grade 1 indicated that lesion was present but so minor, grade 2 indicate that lesion was identified but not prominent, grade 3 indicates that lesion was prominent feature and finally grade 4 indicated that lesion was overwhelming feature in the tissue.

Immunohistochemicalanalysis of α -SMA:

Liver sections were deparaffinization in xylene for 15 minutes, rehydration in graded ethanol, blockage of endogenous perioxidase was done adding few drops of H_2O_2 .antigen retrieval was carried out using heat treatment in microwave at 500 W for 10 min by adding 10 mM citrate buffer, pH 6.0 over the slide and put the slides in the microwave . Sections were incubated overnight at 4 °C in a humidified chamber with one of the following primary antibodies: mouse monoclonal antibody to α-SMA diluted 1:100(mouse anti-α -SMA, clone 1A4, DAKO). Anti-mouse IgG in rabbit (cat no. M7023; 1:500; Sigma-Aldrich) was used as the secondary antibody. The sections were washed with PBS. sections were incubated with Streptavidin peroxidase (Thermo Scientific). slides were incubated for 10 min with 3,3′ -diaminobenzidine tetrahydrochloride (DAB, Sigma). Finally, the slides were counterstained with haematoxylin then dehydrated and mounted. The cells that were stained brown staining in the cytoplasm/nucleus were considered to be positive.

For immune his to chemical examination, anti-α -SMA (Santa Cruz, CA, USA) was used as primary antibodies to detect its targeted protein using standard immune his to chemical method. Stained tissues were determined using a light microscope (Olympus BX 51, Olympus America, Melville, NY) and photographed with a digital camera (Olympus DP11) connected to the microscope.

Gene expression assay

Semi-quantitative RT-PCR

Isolation of Total RNA and Real-Time PCR (qPCR)

Total RNA was purified from 30 mg of liver tissue using Qiagen tissue extraction kit (Qiagen) according to the manufacturer's protocol. The purity (*A*260/*A*280 ratio) and the concentration of RNA were obtained using spectrophotometry (dual wave length Beckman, Spectrophotometer, USA). GAPDH was used as a housekeeping gene for normalizing mRNA levels of the target genes. The mRNA expression levels of CYP2E1 gene was assessed using qPCR standardized by co-amplification with the housekeeping gene GAPDH. Briefly, the total RNA was reverse transcribed into cDNA by reverse transcriptase kit (Fermentas, USA). cDNA was added to a Quantifast SYBR Green qPCR Master Mix (Qiagen) containing 3 µl of each primer (Table 1).

Table 1: Primers sequences

Gene	Primer Sequence	Reference
CYP2E1	F: 5'- TCCAGGTTTGCACCAGACTCT-3' R: 5'- TCCTCGCTCCTCCTGAGAAG-3'	**(Galal*et al*., 2014)**
GAPDH	F: 5'-ACCACAGTCCATGCCATCAC-3' R: 5'-TCCACCACCCTGTTGCTGTA-3'	**(Ogaly*et al*., 2015)**

The thermal profile included 40 cycles of denaturation at 95 °C for 15 s, annealing at 60 °C for 15 s and extension at 72 °C for 45 s. During the first cycle, the 95 °C step was extended to 1 min. The GAPDH gene was amplified in the same reaction to serve as the reference gene. Gene expression levels were calculated and determined following the method described byLivak and Schmittgen. (2001).

Statisticl analysis:

Statistical differences between the means were assessed by analysis of variance (ANOVA) according to Snedecor and Cochran (1982) followed by Duncan's multiple range test(Duncan, 1955)using(SPSS for Windows, version 19). Lesion scoring was done using T- test. Values of $P<0.05$ were considered statistically significant.

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RESULTS

Table (2) showedinsignificant difference in the mean values of serum total protein, albumin, AST, ALT, ALP, total bilirubin between normal control group (G 1) and CM- treated group (G3).

Table (2) Effect of camel milk treatment the activities of alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP) enzymes, total bilirubin and serum protein fractions of normal rats and ccl4 - intoxicated ones.

Parameters / Group	T. Protein (g/dl)	Albumin (g/dl)	Globulin	A/G ratio	T. Blilrubin (mg/dl)	AST (U/ml)	ALT (U/ml)	ALP (U/ml)
Normal control	8.38^{a}±0.15	4.46^{a}±0.04	5.27^{a}±0.19	0.85^{b}±0.04	0.89^{c}±0.02	164.17^{b}± 0.91	63.33^{b}±0.84	193.21^{b}±37.07
CCl_4	5.42^{b}±0.26	3.17^{b}±0.21	2.25^{c}±0.08	1.41^{a}±0.0.08	3.30^{a}±0.11	203.43^{a}±4.96	173.71^{a}±13.89	464.42^{a}±9.03
CM	8.56^{a}±0.17	4.39^{a}±0.18	4.17^{b}±0.08	1.06^{b}±0.06	0.79^{b}±0.02	172.33^{b}±7.40	70.17^{b}±2.01	173.57^{b}±2.54
CCl_4 +CM	9.04^{a}±0.42	4.23^{a}±0.11	4.81^{ab}±0.45	0.92^{b}±0.09	1.26^{c}±0.0	168.00^{b}±1.79	75.83^{b}±2.39	180.65^{b}±0.74

Data are expressed as Mean ± S.E

^{a,b,c,} Mean values with different letters in the same column are significantly different at (P< 0.01)

CCl_4 treatment of the rats resulted in a significant elevation ($P < 0.01$) in the mean values of liver functions (AST, ALT and ALP), A/G ratio andtotal bilirubin. Meanwhile , the mean values of total protein, albumin and globulin were significantly decreased ($P < 0.01$) compared to control rats. Concomitant oral administration of camel milk with CCl_4 showed significant decrease ($P < 0.01$) in the mean values of liver enzymes and total bilirubin and the mean values of total protein, albumin and globulin were significantly increased as compared with CCl_4-treated rats alone (G2).

Effects of camel milk on the levels of proinflammatory mediators (hepatic IL-1β and TNF-α Levels).

It was evident from table (3) that CCL_4 intoxicated rats showed elevated levels of proinflammatory mediators, including TNF-α and IL-1β in the liver tissue that were significantly increased when compared with the control group ($p<0.01$), suggesting induction of a severe inflammatory response. Nevertheless, camel milk (G4) markedly inhibited the levels of these proinflammatory mediators compared to CCl4- intoxicated group (G2).

Table 3: Effect of camel milk treatment on the values of hepatic IL-1β and TNF-αof normal rats and CCl_4 – intoxicated ones.

Parameters / Group	IL-1β	TNF-α
Normal control	32.55^{c}±1.15	31.43^{c}±1.68
CCl_4	127.41^{a}±4.53	164.64^{a}±9.42
CM	27.58^{c}±0.42	30.57^{c}±0.87
CCl_4 +CM	56.57^{b}±3.85	58.38^{b}±1.09

Data are expressed as Mean ± S.E
^{a,b,c,}Mean values with different letters in the same column are significantly different.

Gene Expression Analysis:

Real-time quantitative PCR analysis showed a significant decrease in CYP2E1 mRNA level in CCl_4-intoxicated group compared to that of control rats.However, CM treatment to CCl_4-intoxicated rats (G4) induced amarkedly attenuated CYP2E1down-regulation by increasing its mRNA levelsignificantly compared to the CCl_4 group (G2).No significant difference between CCl_4 group (G2) and normal control ones(Table 4).

Table 4: Effect of camel milk treatment on the values of CYP 2E1 of normal rats and CCl_4 – intoxicated ones.

Group parameter	Normal control	CCl_4	CM	CCl_4+CM
CYP 2 E1	**1.09^{a}±0.03**	**0.17^{b}±0.05**	**1.01^{a}±0.02**	**1.04^{a}±0.01**

Data are expressed as Mean ± S.E
^{a,b}Mean values with different letters in the same column are significantly different.

Effect of camel milk on CCl_4-induced liver histopathological alternations and lesion scoring:

The results of hepatic histopathological lesion scoring of different organs were shown in Table (5) when compared with the normal liver tissues of controls, liver tissue in the rats treated with CCl_4 (G2) revealed extensive liver injuries, characterized by cholangiofibrosis represented by portal fibrosis ,oval cell proliferation with formation of bile ductules (fig 1a) and macro and micro vesicular steatos is of hepatocytes associated with hepato cellular necrosis of periportal hepatocytes(Fig 1b). However, the histopathological hepatic lesions induced by I.P injection of CCl_4 were remarkably ameliorated by treatment camel milk.This finding was consistent with the levels of the enzymes markers. These effects were markedly rehabilitated by CM. The liver

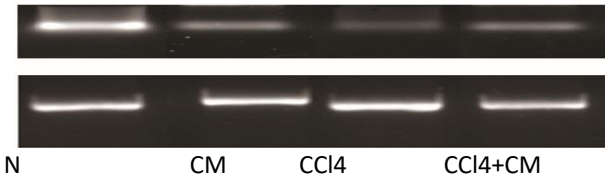
specimens obtained from the rats intoxicated with CCl_4 and post- treated with CMresulted in reduction in the severity of hepatocellular lesions induced by CCl_4, lesions were restricted to c) minimal portal fibrosis with maintaining of hepatic lobular structure and vacuolization of hepatocellular cytoplasm.(fig 1c) and Minimal portal mononuclear cell infiltration and vacuolization of periportal hepatocytes (fig 1d).Concerning hepatic lesion in camel milk – treated rats was restricted to showing diffuse vacuolization of hepatocytes (fig 1e).Rat of untreated control group showing normal histological hepatic structure (fig 1f).

Table 5: Histological injury score of liver intoxicated with CCl_4 and treated with camel milk.

Score injury / group	Hepatic fibrosis	Confluent necrosis	Ballooning degeneration	Macro to microvesicularsteatosis
CCl_4	2.82^{a}±0.21	3.30^{a} ±0.81	1.65^{a} ±0.14	2.13^{a} ±0.32
CCl_4+CM	1.42^{b}±0.10	1.92^{b} ±0.16	0.00^{b} ±0.00	0.00^{b} ±0.00

Data are expressed as Mean ± S.E.

^{a,b}Mean values with different letters in the same column are significantly different.



His to chemical and immune his to chemical findings

Masson's trichrome staining of the liver was performed to assess collagen fiber distribution.The histochemical staining of hepatic section by Masson`s Trichrome revealed marked variation in fibrosis among $CCl_4$ treated groups. In $CCl_4$ group there was bluish staind collagenous tissue disrupting the hepatic parenchyma note the massive bridging fibrosis with pseudolobules formation (Masson`s Trichrome,X100) (fig 2a). Liver of rat from CCl_4+CM treated group showing bridiging fibrosis grade 4 note the collagenous tissue proliferation extending from portal to portal and portal to central (Masson`s Trichrome,X100). (fig 2b). On other hand Liver of CCl_4 treated rat showing positive stained cells are spindle to stellate with large amount of cytoplasm and long extending cytoplasmic processes (Immunohistochemistry for alpha-SMA, X400). (fig 2c). Liver of rat from CCl_4+CM treated group showing many brownish staining immune positive cells in the area of portal fibrosis (Immunohistochemistry for alpha-SMA,X400).(fig 2d).

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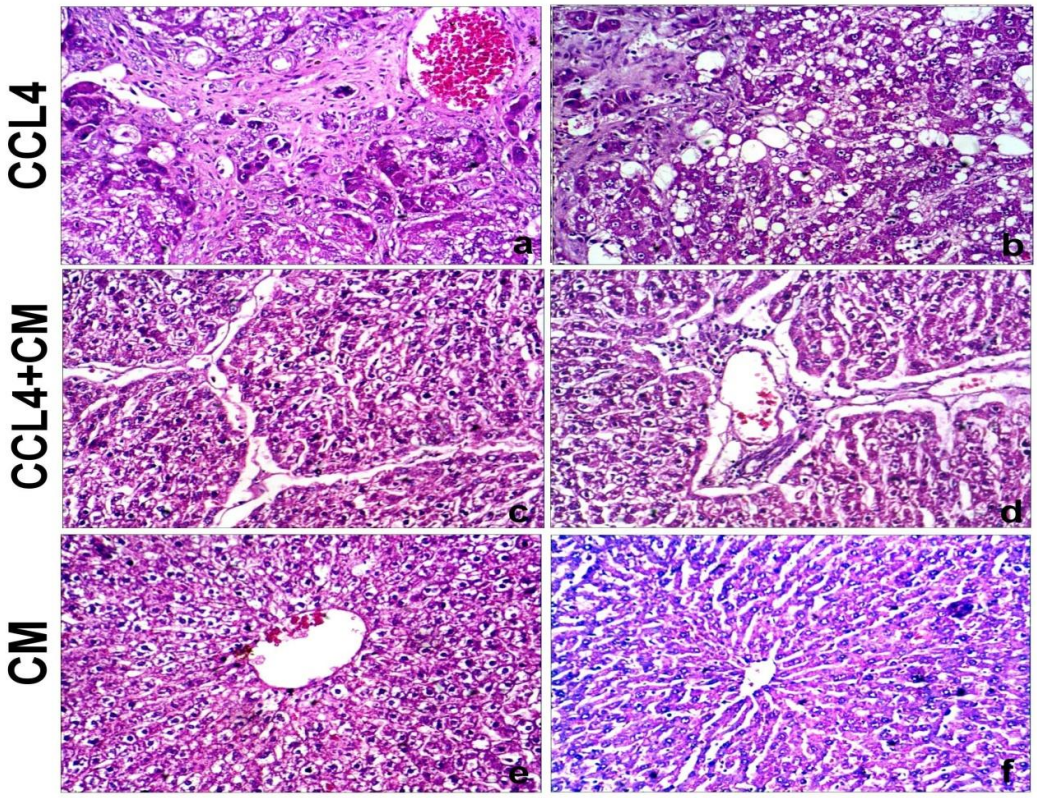

Fig 1: liver of rat from: CCl_4- intoxicated group showing:a) cholangiofibrosis represented by portal fibrosis,oval cell proliferation with formation of bile ductules and b) macro and microvesicularsteatosis of hepatocytes associated with hepatocellular necrosis of periportal hepatocytes.Liver of rat from CCl_4 - intoxicated group plus CM showing:c) minimal portal fibrosis with maintaining of hepatic lobular structure and vacuolization of hepatocellular cytoplasm. d) Minimal portal mononuclear cell infiltration and vacuolization of periportal hepatocytes.e)rat from CM treated group showing diffuse vacuolization of hepatocytes.f)rat of untreated control group showing normal histological hepatic structure(H&E,X200).

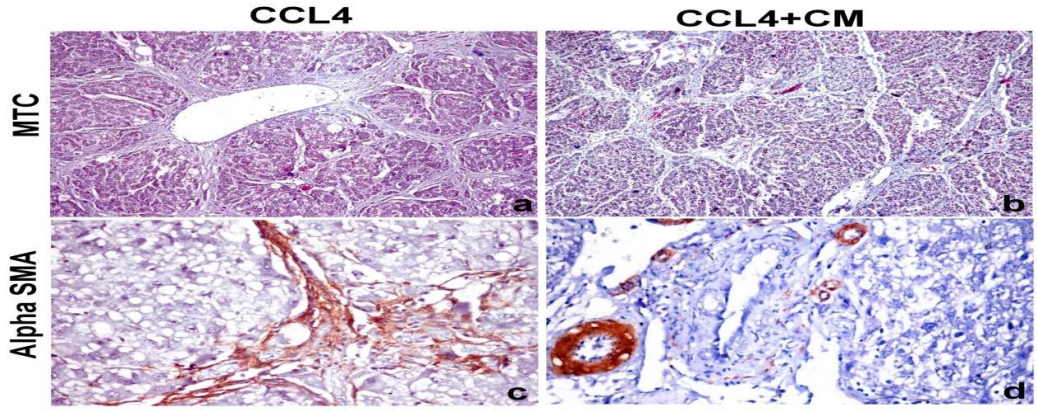

Fig 2:a)Liver of CCl_4 treated rat showing bluish staind collagenous tissue disrupting the hepatic parenchyma note the massive bridging fibrosis with pseudolobules formation (Masson`s Trichrome,X100) .b) liver of rat from $CCl_4$+CM treated group showing bridiging fibrosis grade 4 note the collagenous tissue proliferation extending from portal to portal and portal to central (Masson`s Trichrome,X100).c)Liver of CCl_4 treated rat showing positive stained cells are spindle to stellate with large amount of cytoplasm and long extending cytoplasmic processes (Immunohistochemistry for alpha-SMA, X400).d) liver of rat from CCl_4+CM treated group showing many brownish staining immune positive cells in the area of portal fibrosis (Immunohistochemistry for alpha-SMA,,X400).

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DISCUSSION

The present study was aimed to assess the therapeutic effect of camel milkin carbon tetrachloride treated rats. It is bio transformed by the cytochrome P 450 system. By this process, it produces the trichloromethyl free radical, which in turn covalently binds to cell membranes and organelles and elicits lipid peroxidation. It also disturbs Ca^{2+} homeostasis and finally results in cell death (Recknagel*et al*., 1989).Increased levels of ALT, AST, ALP, TP, Alb and globulin are conventional indicators of liver injury(Achiliya*et al*., 2004).The activities of serum marker enzymes, like ALT, AST and ALP when estimated can make the assessment of liver function when liver cell plasma membrane is damaged, a variety of enzyme which are normally located in the cytosol are released into the blood stream. Due to the structural damage of liver, the enzyme levels are increased in serum because of their location in cell cytoplasm. After damaging or injury they are released into blood circulation and raises the level of enzymes in serum (Sultana and Nazam, 2012).During hepatic damage, cellular enzymes like AST, ALT and ALP present in liver cells leak into the serum, resulting in increased concentrations(Patel and Jawaid,2014).

The estimation of serum marker enzymes like ALT, AST and ALP in the serum is a useful quantitative marker of the extent and type of hepatocellular damage (Mitra*etal*., 1998). In CM injested group, the tendency of these enzymes to return to near normally is a clear manifestation of antihepatotoxic effects of the extract. The reduction in the levels of enzymes like ALT and AST towards the normal value is an indication of regeneration process. Bilirubin, the endogenous product derived from the degradation of haemoglobin, builds up in the blood and extracellular fluid as its excretion is impaired. Usually, ALT and AST or in combination with total bilirubin are estimated for assessment of hepatocellular injury in rodents and non-rodents (Singh *etal*., 2011).The reduction in ALP levels with concurrent depletion of raised bilirubin levels is suggestive of the stability of the biliary function during injury with carbon tetrachloride. Histopathological liver sections also revealed that the normal liver architecture was disturbed by hepatotoxin, CCl_4 whereas, in the liver sections of the rat treated with the CM showed the normal cellular architecture was retained.Several studies revealed that CCl4 treatment increased levels of AST, ALT, ALP and bilirubin. (Ahsan *et al*., 2009, Soma *et al*., 2014 and Yengkhom*et al*., 2017)and Similarly, in present study there was significant rise of ALT, AST, ALP and bilirubin levels in the CCl_4 treated groups signifying the induced liver injury.

The levels of albumin and total proteins were reduced due to the CCl_4 - induced hepatotoxicity. The reduction is attributed to the initial damage produced and localized in the ER (endoplasmic reticulum) which results in the loss of P450 leading to its functional failure with a reduction in protein synthesis The albumin and protein levels were also raised suggesting the stabilization of ER (endoplasmic reticulum) leading to synthesis of protein.While, the biological value of bilirubin has been employed to assess the excretory role of the liver (Tietz, 1995), the metabolic alterations in the serum concentrations of albumin and total protein are used to monitor its secretory capability (Oloyede and Sunmonu, 2009). In the present study, the significantly increased serum level of bilirubin in the untreated hepatotoxic rats could be associated with CCl_4-mediated defect in the carrier-mediated saturable system at the sinusoidal surface of the hepatocytes that consequently obstruct bilirubin uptake and secretion into bile (Sabiu*et al.,* 2014). Similarly, the CCl_4-mediated significant reduction in the levels of albumin and total protein may be suggestive of diminished synthetic function of the liver (Sabiu*et al.,* 2015).CCl_4 - intoxication leads to hypomethylation of cellular components in the case of RNA the outcome is thought to be inhibition of protein synthesis. Hypoproteinemia and hypoalbuminemia in rats intoxicated with CCl4 for 6 weeks have been reported by Al-Yahya *et al*.(2013).The amelioration of rising serum enzymes in CCl_4 toxicity by CM may be due to the prevention of the leakage of intracellular enzymes by its membrane stabilizing activity. This is in agreement with the commonly accepted view that serum transaminases levels return to normal with the healing of hepatic parenchyma and the regeneration of hepatoocytes(BessemsandVermeulen, 2001 and Darwish et al., 2012). Several studies(Al-Fartosi et al., 2011, Dallak, 2009 and Al-Hashem, 2009)have provided an abundant support for evidencing the protective effects of camel milk on liver damage.The mechanism by which CM lowered liver enzymes may be referred to their ability to maintain liver cell integrity (Ibrahim *et al.*, 2017).

The administration of CM resulted in an increase in total proteinand stimulation of protein synthesis has been advanced as a contributory hepatoprotective mechanism which accelerates the regeneration process and the production of liver cells (Rip et al., 1985; Tadeusz et al., 2001).

Furthermore, the histopathological findings of liver samples are in agreement with the results of biochemical studies. CCl_4 caused damage to the hepatic architecture and produced histological changes such as inflammatory cell infiltration, necrosis of hepatocytes and sinusoidal dilatation. These results are in accordance with those obtained by Hsouna *ET AL*., *(2011)* and Kale *ET AL*., *(2012)* and Laouar et al.,(2017) which indicate that CCl_4 cause histopathological liver changes in rats. Liversections of CCl_4treated rats were characterized by significant intracellular lipidaccumulation, ballooning of hepatocytes, infiltration with inflammatory cells and hepatocyte necrosis.(Desai et al., 2012).Moreover, histopathological evaluation of livers revealed that camel milk reduced inflammation, necrosis and the number of liver lesions induced by CCl_4. The above results suggest that Camel milk inhibits CCl_4-induced oxidative hepatic damage by protecting cells from the effects of CCl_4 and by reducing insidious progressive inflammation-induced liver injury.

Since CYP2E1 is the major isozyme involved in the metabolism of CCl_4, the expression of CYP2E1 was investigated. ROS formed during the biotransformation process of CCl_4 are more reactive and toxic than the parental compound. Biotransformation of CCl_4 occurs in the endoplasmicreticulum and the isoenzyme implicated in this process is CYP2E1.As expected, CCl_4 induced a significant reduction of CYP2E1mRNA level (Figure). This specific CYP2E1 dysregulation in CCl_4 hepaticinjury was previously reported by Sakret al.(2011*)* and may result from a direct attack of reactive CCl4metabolites leading to CYP2E1 transcript degradation. Moreover, other mechanisms such as an inhibitionof CYP2E1 transcription subsequent to inflammatory responses could also have a role in CYP2E1 mRNA decrease(Riddick et al., 2004). An interesting observation in our study was the total blockade of CCl_4-inducedCYP2E1 downregulation by camel milk treatment . These data suggest that the hepatoprotective effect of camel milk possibly due to prevention of CCl_4-induced CYP2E1 downregulation.Chen et al., (2017) found that expression of CYP2E1 protein was decreased after CCl_4 treatment. Also, Mohamed, (2017) showed that cytochrome 2E1 was down regulated when 1ml /kg ccl4 30% in olive oil were given every 72h for 10 days.

HSCs with α-smooth muscle actin (α-SMA) expression play a key role in pathophysiological mechanism of hepatic fibrosis. Thus, α-SMA is a reliable unique marker of fibrosis and could be useful in monitoring the efficacy of the antifibrotic therapy(Carpinoet al., 2005, Domitrović*et al*., 2009 and Parikh et al., 2014). Hepatic stellate cells are regarded as the most relevant cell for the development of liver fibrosis, and their activation is the key step in the process of liver fibrogenesis(Tsukamoto, 2005).α-SMA, the marker of activated HSCs following liver injury, were used to evaluate the degree of HSC activation by immune his to chemical staining.CCl_4 treatment significantly increased the accumulation of α-SMA in this study confirming that CCl_4 stimulated the activation of HSCs in the rat model and agreed with(Rockey*et al*., 2013). Camel milk significantly improved the liver histology and resolved the fibrotic changes induced by CCl_4 and decreased its progression with a marked reduction of in α-SMA immune reactivity in hepatocytes.

Shim *et al*. (2010) found that inflammatoryytokines, such as TNF-α and IL-1β were markedly induced in CCl_4-treated mice. TNF-α is a pleiotropic proinflammatory cytokine mainly produced by activated macrophages and monocytes and is involved in many different biological and pathologic processes including inflammation, autoimmune diseases and cancer. In the current study, we found that the levels of proinflammatory mediators, including TNF-β and IL-1β evidently increased in the liver tissue of rats treated with CCl_4, 3times weekly for 4 weeks, but administration of camel's milk markedly decreased their level. Tan *et al*.(2016)recorded significant elevation ofpro-inflammatory cytokines IL-6, TNF-α, and IL-1β with hepatic fibrosis in mice treated with CCl4. In the same line, Ahn*et al*.(2016) and El-Boshy*et al.*(2017)observed elevated pro-inflammatory cytokines, including TNF-α and IL-1β,mRNA expression with hepatic damage in rats treated with CCl4.These results suggest that CM inhibit the hepatic local inflammatory response due to the fact that lacto ferrin, an anti-inflammatory protein component of CM, has been reported to inhibit the production of pro inflammatory cytokines e.g., TNF-a in mononuclear cells in vitro and in vivo, in response to lipopolysaccharide activation. Mechanistically, the inhibition of the sepro-inflammatory cytokines production could result from inhibition of NF-j Bactivation following internalization of Lactoferrin into monocytes (Haversen*et al*., 2002).Arab*etal*. (2014) found that Feeding with CM decreased the levels of TNF-a along with IL-10 in the colons of animals with TNBS colitis. These results imply that CM partly exerted its beneficial effects on colon inflammation by lowering the colonic content of proinflammatory cytokines such as TNF-a. In fact, CM has been reported to suppress inflammation and elevated levels of TNF-a in ethanol-induced hepatic injury (Darwish *et al*., 2012).Camel milk treatment caused significant decrease in IL-1b, Besides, camel milk treatment caused significant decrease in the TNF-a level (Alhaider et al., 2013).

CM prevents oxidative injury and cell damage by several mechanisms, including scavenging free radicals and inhibiting lipid peroxidation(Althnaian, 2012).The protective effect of camel milk against APAP-induced toxicity, oxidative stress, and tissue damage in this study could be referred to its composition of high levels of vitamins C, A, B2 and E and very rich in magnesium and other trace elements (Barbagallo et al.,1999).These vitamins are antioxidants that are found to be beneficial in preventing the tissues injury caused by toxic agent. Magnesium protects the cell against oxy-radical damage and assists in the absorption and metabolism of vitamins B, C and E (Majerus et al., 1971) which are antioxidants important in cell protection.

Also, CM is rich in zinc (Zn)(Althnaian, 2012). Zinc is a trace element fundamental for living organisms. Many enzymes require Zn for their activity. It also plays a vital role in the DNA replication, transcription, and protein synthesis, affecting cell division and differentiation.(Frederickso, 1989).It has been documented that Zn has a link with many of body enzymes and can prevent cell injury through activation of the antioxidant system (Ozturk et al., 2003 and Ozdemir and Inanc, 2005).Based on the present results, the ability of camel milk to reverse of the severe alterations in the liver injury markers caused by CCl4 is a clear indication of the improvement of the functional status of hepatocytes with preservation of cellular architecture.

CONCLUSIONS

In light of all findings, the present study suggest that CM can evidently served as anti hepatotoxic agent, which might be related with ameliorating liver functions, improving histopathological alternations, reducing levels of proinflammatory mediators, inhibiting α-SMA production and collagen production and up-regulating CYP2E1 expression. CMmight act as a promising complementary treatment to combat hepatotoxicity.

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