

## **Histopathological and biochemical studies in male Wistar albino rats injected with diethylnitrosamine and treated with Camel's milk and *Curcuma longa***

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### **Abstract**

The purpose of this study is to evaluate the hepatoprotective effect of Camel's milk and the antioxidant effect of *Curcuma longa* on Diethylnitrosamine (DENA) induced hepatotoxicity in male Wistar albino rats. Thirty rats were divided into six groups and fed on standard diet and tap water was provided *ad libitum*. The first group received only tap water beside standard diet and considered as control group. The second group was injected with a single intraperitoneal (i/p) dose of 1ml DENA (60 mg/kg b. w.). The third group was injected (i/p) with 1 ml (60 mg/kg b.w.) of DENA and received 1ml of camel's milk / each rat by stomach tube. The fourth group was injected (i/p) with 1ml (60mg/kg b.w.)of DENA and received 2 ml of camel's milk /each rat. The fifth group was injected (i/p) with 1ml (60mg/kg b.w.) of DENA and 1ml of *Curcuma longa* extract /each rat and the sixth group was injected (i/p) with 1ml (60 mg/kg b. w.) of DENA and 2ml of *Curcuma longa* extract / each rat. This experiment lasted for 120 day and the injection was done daily. All animals fed on standard diet and tap water during the experimental period (120 days). Blood and liver samples were collected at the end of experiment for biochemical analysis and histopathological investigation. The present findings revealed that DENA increased the level of malondialdehyde (MDA) while it decreased the level of antioxidants glutathione (GSH), superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px). On the other hand,. All enzymes aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma glutamyl transpeptidase (GGT), alkaline phosphatase (ALP) and lactate dehydrogenase (LDH) showed significant increase with DENA. Moreover, Total proteins levels, albumin,  $\alpha_1$ ,  $\alpha_2$   $\beta_1$ ,  $\beta_2$ ,  $\gamma_1$  and  $\gamma_2$  showed significant decrease while albumin/globulin (A/G) ratio was insignificantly affected. Pathological examination of the liver showed that i/p administration of DENA caused significant histological damage represented by degeneration of hepatocytes, fibroma adenoma with severe congestion and hemorrhage. The results also revealed that both camel's milk and *Curcuma longa* extract have good roles in improving the treated groups biochemically and histopathologically. The protective effect of both camel's milk and

Curcuma longa may be due to their antioxidant activity. It could be concluded that drinking camel's milk and Curcuma longa extract may be beneficial for patients who suffer from liver diseases.

### Introduction

The liver is responsible for metabolism and detoxification of the most of components that enter the body (**Nunez 2006**). Nitrosamines are considered one of the most important classes of carcinogens, therefore posing a significant threat for human health (**Aiub et al., 2003**), and have been reported to occur in various foodstuffs (cheese, cured or cooked meat products, bacon, some beverages (**Levallois et al., 2000**). Moreover, nitrosamines may be formed in the human as a result of the reaction of between the nitrite ion and secondary and tertiary amines at a low gastric pH (**Ohsawa et al., 2003**). Nitrites are formed by reduction of nitrates, which are often used to preserve various types of food, especially meat products. Nitrites used in the meat industry help to develop flavor, stabilize the red colour in meat products and have antioxidant effects (**Hord et al., 2009**). Diethylnitrosamine (DNA) is known for its hepatotoxic, carcinogenic and mutagenic potential to cause tumors in the gastrointestinal tract, liver, skin and other organs. At low doses (10 mg/kg body weight) causes hepatic fibrosis (**Kim et al., 2005**), Despite the fact that DNA compounds have been shown to be carcinogenic in animals (**Aiub et al., 2003**), little evidence has been found in the case of humans .It is also known to cause oxidative stress and act as a mutagen after its activation by microsomal enzymes; (**Aiub et al., 2006**). Curcumin has been used in indigenous herbal medicine for the treatment of inflammatory and liver disorders (**Joe et al., 2004**). Curcumin has potent antioxidant, anti-inflammatory, antimutagenic and anticarcinogenic properties (**Araujo and Leon, 2001; Chainani-Wu, 2003; Surh et al., 2001**). The protective effects of Curcumin against chemically-induced hepatotoxicity are well documented, and have been attributed to its intrinsic antioxidant properties (**Nanji et al., 2003; Rukkumani et al.,2004**).

The unique characters of camel's milk make it used extensively in the field of medicine as anti-microbial, antidiabetic and hepatoprotective agent. It has been found that camel milk has antidiabetic, anti-hepatitis and bactericidal (**Kula 2016**). The lack of studies on the protective effect of camel's milk against hepatotoxic compounds was the main reason beyond the conduction of the current experiment which aimed to investigate the protective effects of camel's milk and Curcumin against diethylnitrosamine (DNA) induced hepatotoxicity .

## Materials and methods

### Animals and treatment

A total of 30 Wistar albino male rats (300-350g) were obtained from Laboratory Animal House of College of pharmacy King Saud University. Animals were acclimatized under controlled conditions in the laboratory for one week before starting the experiment. All animals were housed in standard cages (5rats/cage), feeding with standard laboratory diet and tap water *ad libitum*. The experimental animals were housed in air-conditioned rooms at 21-23°C and 60-65% of relative humidity and kept on a 12 h light/ 12 h dark cycle.

### Experimental groups and protocol

The rats were divided according to their weights into six groups 5 rats in each group. The experimental design is described as follow:

Group I: Rats fed only with basal diet and tap water and consider as control group.

Group II: Rats were injected with a single intraperitoneal (i/p) dose of 1ml DENA(60 mg/kg b. wt.).

Group III: Rats were injected (i/p) with 1ml of (60 mg/kg b.wt. DENA and received 1ml of camel's milk through stomach tube as protective agent.

Group IV: Rats were injected (i/p) with 1ml of (60 mg/kg b.wt. DENA and received 2ml of camel's milk through stomach tube as protective agent.

Group V: Rats were injected (i/p) with 1ml of DENA 60mg/kg b.wt and 1ml of Curcuma longa extract.

Group VI: Rats were injected (i/p) with 1ml of DENA 60 mg/kg b.wt and 2ml of Curcuma longa extract.

All animals fed on standard diet and tap water during the experimental period (120 days). Blood and liver samples were collected at the end of experiment for biochemical analysis and histopathological investigations.

### Camel's milk

Camel's milk was collected daily from Soliman AL-Omary Farm, Buridah-Al-Qassim region. Milk was collected from camels by hand milking. The samples were collected in sterile screw bottles and kept in cool boxes until transported to the laboratory. The rats were given this fresh milk (1ml or 2ml/each rat.) without any further treatment.

### Curcuma longa

The dried Curcuma longa L., Zingiberaceae, rhizomes were obtained from Riyadh and Curcumin was extracted according to the method described by (Viviane et

al., 2012) with some modifications. DENA dose as method described by (Tien-chun et al., 2013)

#### **Blood and tissues collection:**

At the end of experiment, the overnight fasted animals were sacrificed under light ether anesthesia. Blood samples were collected and 5 ml of blood samples were collected in tubes, serum was collected and frozen at -30°C until the time of analysis. Liver tissues were cut in small pieces and immersed in neutral buffered formalin 10% for histopathology.

#### **Chemicals and kits**

The activities of aspartate transaminase (AST) and alanine transaminase (ALT) in serum and liver tissue were estimated according to the method of (Reitman and Frankel, 1957). The activities of alkaline phosphatase (ALP) and lactate dehydrogenase (LDH) were estimated according to the methods detailed by (King, 1965a and b), respectively. Gamma glutamyl transpeptidase (GGT) was assayed in serum according to (Rosalki and Rau, 1972). Catalase activity, lipid peroxidation (TBARS) and reduced glutathione (GSH) in tissue were determined according to (Aebi, 1974); (Ohkawa et al., 1979 and Ellman, 1959), respectively. The activity of tissue superoxide dismutase (SOD) was measured by Marklund and Marklund method. Estimation of serum total protein and electrophoretic pattern were carried out after (Sonnen Wirth and Jaret 1980; Davis 1964), respectively and calculated according SynGene S. No. 17292\*14518 sme\*mpcs.

#### **Histopathological examinations**

Liver tissues were cut in small pieces and immersed in neutral buffered formalin for 24h. The fixed tissues were processed routinely, embedded in paraffin, sectioned, deparaffinized and rehydrated using the standard techniques (Bancroft and Gamble 2002). The extent of DENA-induced necrosis was evaluated by assessing the morphological changes in the liver sections stained with Hematoxylin and eosin (H and E), using standard techniques.

#### **Statistical Analysis**

The data for various biochemical parameters were analyzed using ANOVA and the group means were compared by Duncan's multiple range test. Values were considered statistically significant when  $p < 0.05$ . Zarj (1984)

## Results and Discussion

The liver plays an astonishing array of vital functions in the maintenance, performance and regulating homeostasis of the body. It is involved with almost all the biochemical pathways to growth, fight against disease, nutrient supply, energy provision and reproduction. And it functions as a centre of metabolism of nutrients such as carbohydrates, proteins and lipids and excretion of waste metabolites. The bile secreted by the liver has, among other things, plays an important role in digestion. Therefore, maintenance of a healthy liver is essential for the overall well being of an individual (**Arulselvan and Subramanian, 2007**).

Liver cell injury caused by various toxicants such as certain chemotherapeutic agents, carbon tetrachlorid, nitrosamine, chronic alcohol consumption and microbes are common. Enhanced lipid peroxidation during metabolism may result in development of hepatitis leading to cirrhosis (**Agarwal, 2001**).

The nature has bestowed some substances with the property to prevent, treat and cure hepatic disturbances with interception of fewer side effects. Hepatoprotective agents are a class of therapeutic agents that includes synthetic as well as natural product which offer protection to liver from damage or help in regeneration of hepatic cells.

Two of the most common substances acts as antioxidant and hepatoprotective agents are curcuma longa and camel's milk. Over the years many studies have been conducted investigating the role of antioxidants in experimentally reduced liver damage. We have used the antioxidant Curcumin (CUR) which is derived from the plant *Curcuma longa* (turmeric) and is commonly used in food preparation and is reported to display biological activities such as antioxidant, anticarcinogenic (**Anand et al., 2008**) anti-inflammatory (**FU et al., 2008**) and immunomodulatory activities (**Nanji et al., 2003**). Curcumin has been studied in different models of acute and chronic liver injuries, some of which are alcohol-related or carbon tetrachloride induced liver injuries associated with high levels of oxidative stress and inflammation. However, only limited knowledge is available on the possible effects of Curcumin on the development and progression of non-alcoholic steatohepatitis) and is similar to alcoholic steatohepatitis in term of histopathology and characterized by the presence of signs of hepatocellular damage and inflammation, as well as ballooning degeneration and hepatocytes death, formation of Mallory-Denk bodies, and infiltration with inflammatory cells (**Yeh et al., 2007**) these changes are associated with different degrees of fibrosis or with the presence of cirrhosis. Although some aspects of the pathogenesis of non-alcoholic steatohepatitis (NASH) have not been elucidated in detail, others are now well established such as the accumulation of excess lipids particularly in the form of free

fatty acids, causes toxic damage to the hepatocytes, which in turn initiates inflammation and tissue repair in the form of fibrosis (**Choi et al.,2007 and Marra et al.,2008**).

The impaired liver functions may be due to the oxidative tissue damage caused by the massive production of reactive oxygen species (ROS) and disturbance in the protective physiological moieties (as antioxidant defense mechanism systems) causing lipid peroxidation, a process leading to damage to the macromolecules in vital biomembranes (**Mohamed et al.,2010 and Ismailetal.,2011**). Table (1) exhibits the effect of (DENA) diethylnitrosamine on the levels of (MDA) malondialdehyde, (GSH) glutathione, (SOD) superoxide dismutase, (CAT) catalase and (GSH-Px)glutathione peroxidase and also exhibits the effect of treatment with camel's milk and curcuma longa extraction. The MDA(which act as biomarker of oxidative stress)level was significantly increased in group (II) compared to normal control group(I).Activities of GSH, SOD,CAT,and GSH-Px were significantly reduced in group(II) compared to normal control group(I).Administration of different doses of Camel's milk groups (III and IV)and *Curcuma longa* extraction groups (Vand VI) significantly reduce the level of MDA significantly increased levels of GSH, SOD, CATand GSH-Px compared to DEN group(II).Our results were in agreement with results obtained by (**Dewa et al.2009 and Farombi et al., 2009**).

Nitrosamines compounds induce oxidative stress and retro gradation of the antioxidant defense mechanism due to overproduction of reactive oxygen species (ROS) and lipid peroxidation which result in a wide range of disorders in a variety of rodent organs, especially liver (**Thirunavukkarasu and Sakthisekaran, 2003**). This defense system operates through enzymatic and non-enzymatic components. GSH-Px and SOD are key enzymes in the body to eliminate free radicals. SOD can change the highly toxic superoxide anions ( $O_2^-$ ) to  $O_2$  and  $H_2O_2$ , then  $H_2O_2$  and  $O_2^-$  react while the iron chelating compounds exist, and produce  $OH^-$  which has strong activity; meanwhile, GSH-Px can further catalyzed the reduction of GSH and  $H_2O_2$ , oxidize  $H_2O_2$  to  $H_2O$  and prevent the production of highly toxic  $OH^-$  [40] Superoxide dismutase (SOD) and catalase (CAT) can counteract the deleterious action of reactive oxygen metabolites and protect from cellular and molecular damage (**Kaushik and Kaur, 2003**). They also can act as anticarcinogens, and inhibitors at initiation and promotion/transformation stage in carcinogenesis. Reduced glutathione (GSH)and glutathione peroxidase have been assumed as significant markers of chemoprevention owing to their antioxidant and detoxification properties (**Saydam et al.,1997**) As oxidative stress plays a central role in diethylnitrosamine induced hepatotoxicity, the use of antioxidants would offer better protection to counteract liver damage(**Vitaglione et al.,2004**). One strong mechanistic link between chronic inflammation and cancer is through the increased production of

free radicals at the site of inflammation and the resulting molecular changes, which include lipid peroxidation and oxidative DNA damage (**Hussain et al., 2003**). Indeed (MDA) considered as lipid peroxidation marker is commonly elevated in liver of patients with chronic hepatitis C virus infection and correlate well with the degree of viral infection and inflammation, known risk factors for Hepatocellular carcinoma (**de Maria et al., 1996**). Administration of camel's milk and curcuma longa extraction significantly enhanced liver GSH, SOD, CAT & GSH-Px. Several studies have provided a considerable support for evidencing the protective effects of camel milk on liver damage (**Hamad et al., 2011; Khan and Alzohairy, 2011; Al-Fartosi et al., 2012**). Also, these studies declared that the protective effect of camel milk against DEN-induced oxidative stress in the rat is due to its antioxidant properties. Camel milk was found to contain high concentrations of vitamins A, B2, C and E and is very rich in magnesium and other trace elements, these vitamins act as antioxidants and have been found to be useful in preventing toxicant-induced tissue injury (**Yousef 2004**). Also Curcumin represents a class of antioxidants reported to be a potent inhibitor of ROS formation (**Venkatesan et al., 2000; Biswas et al., 2005**). **Reddy and Lokesh (1994b)** indicated that Curcumin is a potent scavenger of a variety of ROS including superoxide anion radicals ( $O_2^-$ ) and hydroxyl radicals ( $\cdot OH$ ). This high antioxidant activity of Curcumin can account for the decrease in lipid peroxidation after Curcumin treatment of DEN-exposed rats, observed in this study.

Serum AST, ALT and ALP are biomarkers in the diagnosis of hepatic damage because they are released into the circulation after cellular damage (**Naik et al., 2007**). In this study, we demonstrated that DENA-induced hepatocellular damage is confirmed by a marked elevation in the levels of serum AST, ALT, ALP, LDH and GGT. High concentration of serum transaminases is taken as an index of hepatic injury and it is observed during parenchymal hepatocellular damage induced by drugs and chemicals (**Vasudevan and Sreekumari, 2001**). Previous studies have shown elevation in the levels of serum AST and ALT on DEN treatment (**Wang et al., 2010**) and our present observations are in accordance with these reports. The elevated activities of these enzymes are indicative of cellular leakage and loss of the functional integrity of liver cell membranes (**Rajesh and Latha, 2004**). The elevation in the status of the serum transaminases observed in this study might be due to the release of these enzymes from the cytoplasm into the blood circulation rapidly after the rupture of the plasma membrane and cellular damage caused by the free radicals released during the metabolism of DENA. ALP, a non-specific membrane bound enzyme is also considered as a reliable marker of liver damage, which even in a mild elevated state is reported to produce parenchymal liver damage. Increase in the status of ALP, observed in this

study, might be suggestive of parenchymal cell damage induced on DENA exposure. LDH is a cytoplasmic enzyme and its activity in serum is found elevated during malignancy by 70% of normal (**Tolman and Rej, 1999**). GGT is the most sensitive indicator of hepatobiliary disease (**Vasudevan and Sreekumari, 2001**). Gross elevation in the status of GGT is an indication of liver metastasis (**Szczeklik et al., 1961**). The elevated levels of LDH and GGT found in this study are an indication of parenchymal cell damage and induction of hepatic necrosis and pre-malignant hepatocellular damage induced by DENA administration. Post-treatment significantly prevented these DENA-induced alterations in the status of the marker enzymes in both serum and liver tissue and restored their activities towards normalcy. Thus, from this investigation, it is suggested that antioxidant might have effectively attenuated DENA-induced hepatotoxicity by inhibiting liver damage, by maintaining the hepatocellular membrane integrity and by suppressing the leakage of cellular enzymes. On the other hand, treatment with camel milk was found to suppress ( $p < 0.05$ ) the increase of serum AST and ALT activities induced by DENA treatment in rats. This finding implies that camel's milk challenge to protect liver tissue from DEN injury. The reversal of increased serum enzymes in DENA-induced liver damage by camel milk 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 levels of transaminases return to normal with the healing of hepatic parenchyma and the regeneration of hepatocytes (**Thabrew and Joice 1987**). Several studies have provided a considerable support for evidencing the protective effects of camel's milk on liver damage (**Hamad et al., 2011; Khan and Alzohairy, 2011; Al-Fartosi et al. 2012**). Our result also revealed the reduce of such enzymes with Curcuma longa extract this result was supported by result obtained by (**Hismigogullar 2014**). Serum total protein is a biochemical test for measuring the total amount of protein in blood plasma or serum. Serum proteins have many functions, including the transport of other substances, immune defense, blood clotting, and inflammation defense. Serum protein levels are useful for evaluating nutritional status, infection, and various other disorders. Within the human body, albumin is an important component of life (**Aiad et al., 2004; Honarmand et al., 2014**). Our results revealed a significant decrease in total protein, albumin & other protein fractions and A/G ratio in (gpII) DENA group compared to (groupI) control group. The levels of previous items were increase significantly after Camel's milk and Curcuma extraction treatments. Our results coincide with result obtained by (**Bendong et al. 2012**). Indicating poor liver functions or impaired synthesis, either primary as in liver cells damage or secondary to diminished protein intake and reduced absorption of amino acids caused by a malabsorption syndromes or malnutrition,

or loss protein in urine, due to nephritic syndrome and chronic glomerulonephritis (**Al-Fartosi et al., 2012**). On the other hand, a significant ( $p < 0.05$ ) increase in concentration of serum albumin was observed in rats received camel milk. The increase of albumin concentration after treatment with camel milk may be attributed to the decrease in lipid peroxidation processes and increase in the activities of plasma protein thiols as a result of treatment with camel milk in both animal and human (**Al-Hashem et al., 2009; Al-Fartosi et al., 2012**). The decrease in decreased albumin levels may be associated with liver disease (di Stefano et al., 2002). An albumin deficiency can lead to medical issues. Albumin/Globulin (A/G) ratio indicates the calculated ratio of levels of these two serum proteins. A low A/G is found in certain liver diseases, kidney disease, myeloma, and inflammation, as well as other disorders (**Al-Shinnawy, 2009**).

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The biochemical findings are further supported by the histopathological observations of the liver tissue.

The histopathological findings confirmed the establishment of hepatic fibroma and adenoma upon DENA (group II). **George et al. (2004)** reported centrilobular congestion, marked dilatation of central vein and sinusoids rats treated DMNA, and our present observations are in agreement with these reports. Our histopathological finding revealed degeneration of hepatocytes with congestion, hemorrhages with marked dilation after injection with (DEN). Treatment showed improvement in the architecture of the liver tissue when compared to DENA (group II), indicating the hepatoprotective ability and antioxidant role of Camel's milk and *Curcuma longa* extraction against DENA induced hepatocellular damage.

**Table1: Effect of diethylnitrosamine (DENa) on male Wistar albino rats MDA and antioxidants before and after treatment with Camel's milk and Curcuma longa extract**

Groups	MDA nmol/l	GSH nmol/dl	SOD $\mu$ /ml	CAT $\mu$ /ml	GSH-PX $\mu$ /ml
GP (I)Control	1.73 $\pm$ 0.158 <sup>c</sup>	3.73 $\pm$ 0.23 <sup>ab</sup>	62.93 $\pm$ 6.53 <sup>b</sup>	15.78 $\pm$ 0.83 <sup>a</sup>	6.31 $\pm$ 0.89 <sup>bc</sup>
GP(II)DEN	6.57 $\pm$ 0.22 <sup>a</sup>	1.48 $\pm$ 0.22 <sup>c</sup>	28.67 $\pm$ 1.94 <sup>c</sup>	7.82 $\pm$ 0.91 <sup>c</sup>	4.66 $\pm$ 0.23 <sup>c</sup>
GP(III)DEN+1ml camel,s milk	3.95 $\pm$ 0.477 <sup>b</sup>	4.94 $\pm$ 0.59 <sup>a</sup>	65.38 $\pm$ 4.61 <sup>ab</sup>	11.46 $\pm$ 0.73 <sup>b</sup>	10.87 $\pm$ 1.37 <sup>a</sup>
GP(IV)DEN+2ml camel,s milk	2.55 $\pm$ 0.61 <sup>c</sup>	4.17 $\pm$ 0.46 <sup>a</sup>	57.84 $\pm$ 7.16 <sup>b</sup>	17.38 $\pm$ 0.65 <sup>a</sup>	8.48 $\pm$ 1.21 <sup>ab</sup>
GP(V)DEN+1ml curcuma longa	2.17 $\pm$ 0.189 <sup>c</sup>	4.68 $\pm$ 0.74 <sup>a</sup>	80.61 $\pm$ 5.97 <sup>a</sup>	15.36 $\pm$ 1.20 <sup>a</sup>	8.54 $\pm$ 0.68 <sup>ab</sup>
GP(VI)DEN+2ml curcuma longa	1.94 $\pm$ 0.13 <sup>c</sup>	2.56 $\pm$ 0.36 <sup>bc</sup>	57.63 $\pm$ 3.66 <sup>b</sup>	14.87 $\pm$ 0.6 <sup>a</sup>	8.39 $\pm$ 0.41 <sup>ab</sup>

N.B. the different litters of columns denote significant variations between means (at  $P \leq 0.05$ ).

**Table2: Effect of diethylnitrosamine (DENa) on male Wistar albino rats AST, ALT, GGT, ALP and LDH before and after treatment with Camel's milk and Curcuma longa extract**

groups	AST U/L	ALT U/L	GGT U/L	ALP U/L	LDH U/L
GP (I)Control	262.0 $\pm$ 13.93 <sup>c</sup>	124.0 $\pm$ 14.35 <sup>b</sup>	190.0 $\pm$ 21.91 <sup>c</sup>	128.0 $\pm$ 7.34 <sup>cd</sup>	225.40 $\pm$ 14.47 <sup>bc</sup>
GP(II)DEN	474.0 $\pm$ 18.87 <sup>a</sup>	226.0 $\pm$ 15.03 <sup>a</sup>	578.0 $\pm$ 52.67 <sup>a</sup>	190.0 $\pm$ 7.07 <sup>a</sup>	419.00 $\pm$ 8.63 <sup>a</sup>
GP(III)DEN+1ml camel,s milk	344.0 $\pm$ 5.09 <sup>b</sup>	138.0 $\pm$ 6.63 <sup>b</sup>	336.0 $\pm$ 26.94 <sup>b</sup>	158.0 $\pm$ 5.83 <sup>b</sup>	233.2 $\pm$ 20.13 <sup>bc</sup>
GP(IV)DEN+2ml camel,s milk	316.0 $\pm$ 21.35 <sup>b</sup>	104.0 $\pm$ 9.7 <sup>b</sup>	200.0 $\pm$ 17.61 <sup>c</sup>	144.0 $\pm$ 6.78 <sup>bc</sup>	224.00 $\pm$ 23.94 <sup>bc</sup>
GP(V)DEN+1ml curcuma longa	264.0 $\pm$ 14.35 <sup>c</sup>	108.0 $\pm$ 10.68 <sup>b</sup>	230.0 $\pm$ 28.28 <sup>c</sup>	116.0 $\pm$ 5.09 <sup>d</sup>	268.80 $\pm$ 22.62 <sup>b</sup>
GP(VI)DEN+2ml curcuma longa	340.0 $\pm$ 19.24 <sup>b</sup>	143.60 $\pm$ 18.01 <sup>b</sup>	190.0 $\pm$ 20.25 <sup>c</sup>	122.0 $\pm$ 3.74 <sup>d</sup>	200.80 $\pm$ 22.18 <sup>c</sup>

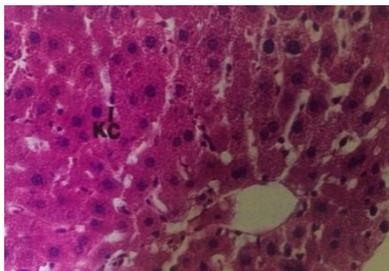
N.B. the different litters of columns denote significant variations between means (at  $P \leq 0.05$ ).

**Table3-Effect of diethylnitrosamine (DEN) on male Wistar albino rats Total protein, protein fractions and A/G ratio before and after treatment with Camel's milk and Curcuma longa extract**

Groups	Total protein g/dl	Albumin g/dl	$\alpha$ 1 %	$\alpha$ 2 %	$\beta$ 1 %	$\beta$ 2 %	$\gamma$ 1 %	$\gamma$ 2 %	A/G ratio
GP (I)Control	7.25±0.14 <sup>a</sup>	1.78±0.06 <sup>a</sup>	0.59±0.01 <sup>a</sup>	0.48±0.02 <sup>a</sup>	0.56±0.11 <sup>a</sup>	0.68±0.11 <sup>bc</sup>	2.49±0.06 <sup>a</sup>	0.67±0.06 <sup>a</sup>	0.33±0.28 <sup>b</sup>
GP(II)DEN	5.01±0.13 <sup>d</sup>	1.28±0.10 <sup>c</sup>	0.29±0.05 <sup>c</sup>	0.39±0.04 <sup>a</sup>	0.52±0.05 <sup>a</sup>	0.58±0.06 <sup>c</sup>	1.46±0.13 <sup>d</sup>	0.49±0.02 <sup>a</sup>	0.32±0.22 <sup>ab</sup>
GP(III)DEN+1ml camel,s milk	6.75±0.01 <sup>b</sup>	1.72±0.09 <sup>ab</sup>	0.45±0.04 <sup>b</sup>	0.52±0.07 <sup>a</sup>	0.48±0.06 <sup>a</sup>	0.76±0.10 <sup>abc</sup>	2.19±0.01 <sup>ab</sup>	0.49±0.14 <sup>a</sup>	0.34±0.24 <sup>ab</sup>
GP(IV)DEN+2ml camel,s milk	6.83±0.01 <sup>ab</sup>	1.78±0.07 <sup>a</sup>	0.43±0.0 <sup>b</sup>	0.49±0.09 <sup>a</sup>	0.51±0.03 <sup>a</sup>	0.95±0.01 <sup>a</sup>	2.06±0.17 <sup>bc</sup>	0.59±0.04 <sup>a</sup>	0.35±0.27 <sup>ab</sup>
GP(V)DEN+1ml curcuma longa	5.46±0.6 <sup>d</sup>	1.51±0.08 <sup>bc</sup>	0.29±0.03 <sup>c</sup>	0.36±0.02 <sup>a</sup>	0.57±0.09 <sup>a</sup>	0.74±0.04 <sup>abc</sup>	1.51±0.13 <sup>d</sup>	0.48±0.03 <sup>a</sup>	0.38±0.34 <sup>ab</sup>
GP(VI)DEN+2ml curcuma longa	6.22±0.17 <sup>c</sup>	1.77±0.07 <sup>a</sup>	0.43±0.06 <sup>b</sup>	0.46±0.04 <sup>a</sup>	0.52±0.06 <sup>a</sup>	0.86±0.04 <sup>ab</sup>	1.73±0.07 <sup>cd</sup>	0.49±0.05 <sup>a</sup>	0.39±0.36 <sup>a</sup>

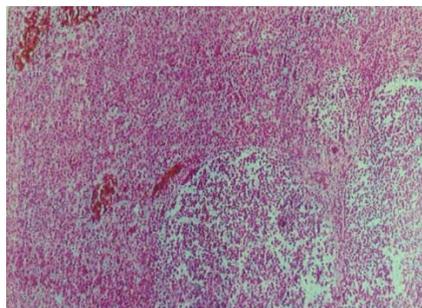
N.B. the different litters of columns denote significant variations between means (at  $P \leq 0.05$ ).

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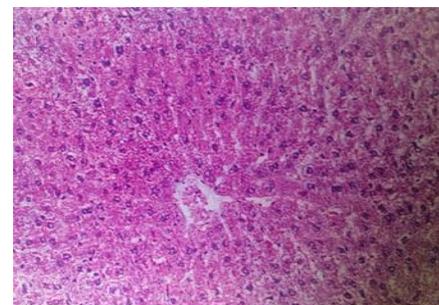
**Figure 1**

Fig( 1)Liver section in group (I) showing normal hepatocytes Hepatic Sinusoids and von Kupffer cells (KC) H&E X400



**Figure 2**

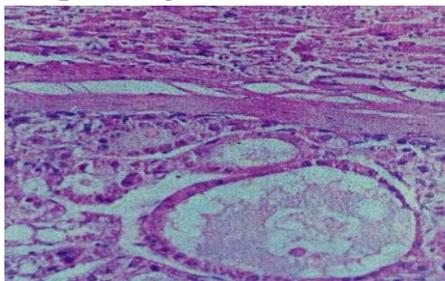
Fig(2 )Liver section in group (II) showing fibrosis with congestion of the hepatic parenchyma H&E X125



**figure 3**

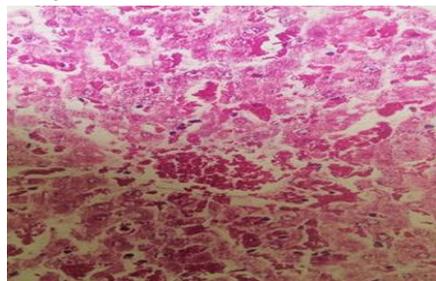
Fig( 3)Liver section of group (IV) showing normal hepatic architecture H&E X250

### Histopathological studies on liver of albino rats injected with (DEN) and treated with Curcuma longa extract



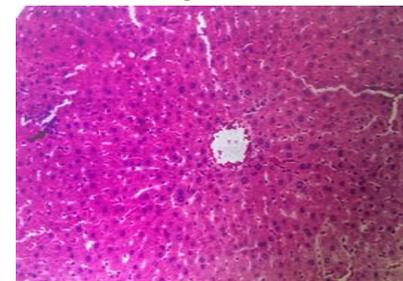
**Figure 4**

Fig( 4)Liver section in group (II) showing adenoma H &E X250



**Figure 5**

Fig ( 5 ) Liver section of group (II) showing marked dilation Of hepatic sinusoids and vascular congestion H&E X400



**Figure6**

Fig(6) Liver section of group (VI) showing normal hepatic architecture with enlarged Nuclei H&250

## Conclusion

In conclusion, our present study shows that both Camel's milk and *Curcuma longa* extract offers hepatoprotection against DENA-induced hepatotoxicity in rats by restoring the altered levels of the serum and liver marker enzymes, by preventing the elevation in the status of lipid peroxidation, and bringing back the levels of antioxidants towards normalcy and this is attributed to its free radical scavenging and antioxidant properties.

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