International Journal of Veterinary Science and Medicine (2014) 2, 136–141

HOSTED BY

ELSEVIER

Cairo University

International Journal of Veterinary Science and Medicine

www.vet.cu.edu.eg
www.sciencedirect.com

Full Length Article

Camel milk inhibits murine hepatic carcinogenesis, initiated by diethylnitrosamine and promoted by phenobarbitone

Hala M.F. El Miniawy [a], Kawkab A. Ahmed [a], Mohamed A. Tony [b], Sameeh A. Mansour [c], Marwa M. Salah Khattab [a,*]

[a] *Department of Pathology, Faculty of Veterinary Medicine, Cairo University, El Giza Square, Giza, Egypt*
[b] *Department of Animal Nutrition and Clinical Nutrition, Faculty of Veterinary Medicine, Cairo University, El Giza Square, Giza, Egypt*
[c] *Department of Toxicology, National Research Center, El Dokki, Giza, Egypt*

Received 2 September 2014; revised 27 October 2014; accepted 28 October 2014
Available online 27 November 2014

KEYWORDS

Diethylnitrosamine;
Camel milk;
Alpha fetoprotein;
Immunohistochemistry;
Hepatocellular carcinoma

Abstract This study was carried out in order to investigate the possible inhibitory effect of camel milk (CM) on induced hepatocarcinogenesis in rats. Twenty-eight male rats were assigned into 4 groups (7 rats per group). Group I served as control negative. Group II treated with camel milk. Group III was injected I/P with diethylnitrosamine (DENA) (200 mg/kg) as a single dose and after one week received 500 ppm phenobarbitone in drinking water. Group IV injected with DENA as group III and treated with camel milk. Estimation of AST, ALT, albumin, total protein and alpha fetoprotein (AFP) in the serum of euthanized rats was performed. Histopathological examination and immunohistochemical staining of AFP and placental glutathione s transferase of the liver were carried out. Biochemical result at 38th week revealed an increase in serum AFP and a decrease in serum albumin on group III although no significance was detected. Histopathologically, the size of altered hepatic foci was smaller in the milk treated group (group IV). The number of mitotic figures observed in group IV was lower than group III. Hepatocellular carcinoma developed only in group III but not group IV. Immunohistochemical staining of AFP demonstrated an intense positive staining in group III and a weak positive staining in group IV. Similarly, the area percent of pre-neoplastic P-GST positive foci in liver was higher in group III than group IV. In conclusion, camel milk halted the progression of hepatocellular carcinoma.

1. Introduction

Hepatocellular carcinoma (HCC) which is considered as primary cancer of liver remains the fifth common cancer and

* Corresponding author. Tel.: +20 100 127 64 69.
E-mail addresses: Marwakhattab@cu.edu.eg, maimar_topaz@yahoo.com (M.M. Salah Khattab).
Peer review under responsibility of Faculty of Veterinary Medicine, Cairo University.

http://dx.doi.org/10.1016/j.ijvsm.2014.10.004

the third leading cause of cancer mortality in the world [1]. The occurrence of HCC has been reported in several regions of the world such as Asia, sub-Saharan Africa, as well as parts of Europe and the North American continent [2]. Over the past decade, there was a progressive increase in the incidence of HCC among chronic liver disease patients in Egypt [3]. Many risk factors such as inflammation have been incriminated in the occurrence of HCC [4].

Diethylnitrosamine (DEN) is a potent hepatocarcinogen that, at low doses, acts only as an initiating agent in the rat liver [5] but when its combined with a promoting agent such as phenobarbital, development and progression of experimental liver cancer takes place as reported in the past 2 decades [6]. Many side effects have been associated with chemotherapy for cancer and the therapeutic outcome remains very poor [7].

Currently, camel milk has been used as a traditional medicine in Egypt in order to treat cancer and HCV infected patients. Camel milk contains numerous proteins in which caseins account for 80% (w/w) of the total milk protein content [8] and whey contains numerous proteins such as immunoglobulins, α-lactalbumin, lactoperoxidase, lysozyme and lactoferrin, among other proteins with biological functions [9]. In addition it contains the whey acidic protein [10] which is not found in ruminant or primate milk [11]. A wide range of biological activities have been exhibited by camel milk including antimicrobial, antioxidative, antithrombotic, antihypertensive, and immuno-modulatory effect [12,13].

Several studies have investigated the effect of camel milk and its constituents on tumor cell lines [14] and provided hopeful results. Therefore it was important to investigate the possible anti tumor potential of camel milk in vivo as well.

2. Materials and methods

2.1. Animals

Twenty-eight male Wister rats, weighing 100–120 g, were purchased from the animal house of the National Research Center (El Dokki, El Giza, Egypt). This experimental work was approved by Ethics of Animal Use in Research Committee (EAURC), Faculty of Veterinary Medicine, Cairo University, Egypt. The animals were housed in metal wire mesh cages (4–5 rats per cage) and were left for two weeks before beginning the experiment for acclimatization. The housing conditions including temperature 25 ± 2 °C, relative humidity 50–60%, and 12 h photoperiods were set. The rats were supplied with a pelleted diet and water ad libitum.

2.2. Chemicals

Diethyl nitrosamine (DENA) was purchased from Sigma Chemical Co. (St. Louis, MO, USA). Phenobarbitone was kindly supplied by the Egyptian International Pharmaceutical Industry Co. (EIPICO).

2.3. Camel milk

Camel milk of *Camelus dromedarius* was purchased from Ibn Sina camel farm (Ras sedr, South of Sinai, Egypt) and was transferred frozen to our laboratory.

2.4. Experimental design

28 male rats were divided into four groups (7 rats each).

Group I: served as a negative control group.

Group II: was treated with camel milk 5 ml by oral intubation after 28 weeks of experimental commence.

Group III: was injected intraperitoneally with a single dose (200 mg/kg body weight) of diethylnitrosamine dissolved in saline to initiate hepatocarcinogenesis. After one week, phenobarbitone was added to drinking water at a concentration of 0.05% (500 ppm) for 27 weeks.

Group IV: was treated as group III followed by treatment with camel milk after 28 weeks.

Three rats from each group were then euthanized after 34 weeks of DENA injection (6 weeks of camel milk treatment). The rest of the rats were euthanized after 38 weeks (9 weeks of CM treatment).

2.5. Biochemical analysis

Serum AST, ALT, albumin and total protein were analyzed spectrophotometrically using commercially available kits (spectrum, Egypt). Estimation of serum AFP was also carried out using rat alpha-fetoprotein ELISA kit (WKEA Med supplies corp., China).

2.6. Histopathology

Liver samples were fixed for 48 h in 10% neutral buffered formalin and processed by paraffin embedding technique. Sections of 5–6 μm thick were prepared and stained with H&E stain for microscopic examination [15]. Liver specimens from three different lobes were examined for each rat and lesion score for altered hepatocellular foci was performed. Diagnosis of liver cell foci and neoplasms was performed according to the histological criteria of the Institute of Laboratory Animal Resources [16] on the hematoxylin and eosin-stained sections. The mean area of foci was measured in group III and IV using image analyzer Leica Quin 500 (Pathology department, NRC).

2.7. Immunohistochemical detection of Alfa Fetoprotein

Liver paraffin tissue sections were stained by immunoperoxidase technique using anti alpha fetoprotein antibody (Pierce Biotech., USA) and the avidin biotin peroxidase complex method (UltraVision Detection System Anti-Mouse, HRP/DAB, Lab Vision Corp., USA). 3,3-diaminobenzdine (DAB) was used for color development. All staining procedure was carried out according to manufacturer protocol. The optical density of immunohistochemically stained AFP was measured using image analyzer Leica Quin 500, Pathology department, NRC.

2.8. Immunohistochemical staining of placental glutathione s transferase

Paraffin embedded liver tissue sections were immunohistochemically stained by using Anti-GST-P polyclonal antibody prepared in rabbit (MBL Co., LTD, USA) and the avidin biotin peroxidase complex method according to kit manufacturer protocol (Dako, LSAB + system-HRP, North America, Inc.).