Effect of Carcinogenic Substance (7,12 Dimethylbenz [a] anthracene (DMBA)) on Tissue, Hematology Character and Enzyme Activity in Rat

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Abstract

This study aimed to investigate the effects of 7,12 dimethylbenz [a] anthracene (DMBA) on some blood parameters and hepatic histopathology in rats and antioxidant enzyme. twenty female Wistar albino rats, weighing 180–200 g, were randomly divided into two group. DMBA group (positive group) who received 20 mg DMBA/kg body weight/ (single Douse). Control group (negative group) don't received any thing. The animals in the groups were sacrificed at the end of the 90 days: The histological structure of the liver tissues in the control group was normal. the liver exhibited hydropic degeneration and coagulation necrosis in hepatocytes, severe dilation in the sinusoids, congestion in the central and portal regions , DMBA groups, was degenerative and necrotic changes were detected. MDA levels increased in the DMBA group , compared to the control group, Antioxidant activity CAT, SOD and GPX, the results showed increased enzyme activity in positive control compared with negative groups , ,the hematological parameter was increase in WBC, PLT, GRA, and LYM and decrease in RBC value .Keywords: 7,12-dimethylbenz[a]anthracene (DMBA); Blood parameters; Antioxidant enzyme ; MDA ; Rat.

Introduction

Cancer, also termed as malignant neoplasm, is a type of diseases where in a group of cells show abnormal proliferation, invasion and sometimes metastasis [1] . Cancer begins when cells in a part of the body start to grow out of control [2] . There are more than 100 types of cancer have been identified [3] . The substances that promote cancers are called carcinogens, and agents that have the ability to change DNA in ways that are inherited by daughter cells are called mutagens. Most cancers are related to environmental, lifestyle, or behavioral exposures [4] The term "environmental", as used by cancer researchers, refers to everything outside the body that interacts with humans [5] . Other chemicals, like benzene, ketones, vinyl chloride, ethylene bromide, and dichloro-diphenyl-trichloroethane (DDT), are known carcinogens [6] . The accumulation of multiple factors, carcinogens and altered genes, transform a normal cell into a cancerous one. Carcinogens that have the ability to promote cancer have various sources [7] .The factors responsible for cancer development are classified as exogenous and endogenous [8] . The first group includes nutritional habits (food preservation and preparation), socio-economic status, lifestyle, physical agents (ionising and non-ionising radiation), chemical compounds (natural and synthetic) and biological agents (Helicobacter pylori, Epstein Barr virus, human T lymphotropic viruses I and II, human papilloma virus and the hepatitis B virus [9].

Meterial and Method

Experiential animals and Tumor induction in rats:

Twenty female of Wistar albino rats and aged between (7-8) weeks were weighing between 150 – 250 g used in this study. All they were kept in ventilated cages, with temperature of 25±2C°. A 12:12 h light:dark cycle is also regulated for these animals. Balanced rodent food pellet and water is provided. The rats were randomly assigned to 2 groups of 10 rats each.

The groups were as follow:

Group 1: Received a single dose of DMBA (positive control) 20 mg DMBA/kg body weight/ (single dose)

Group 2: Did not received any treatment (negative control).

DMBA administration: Mammary tumors were induced by 7, 12-

Dimethylbenz (a) anthracene (DMBA) [10] . A single dose of DMBA dissolved in corn oil was given by oral gavage to two groups using the syringe and needles. DMBA was purchased from Sigma Aldrich and dissolved in corn oil. The concentration of the solution was 20 mg DMBA per 1 ml corn oil for each rat [10] .

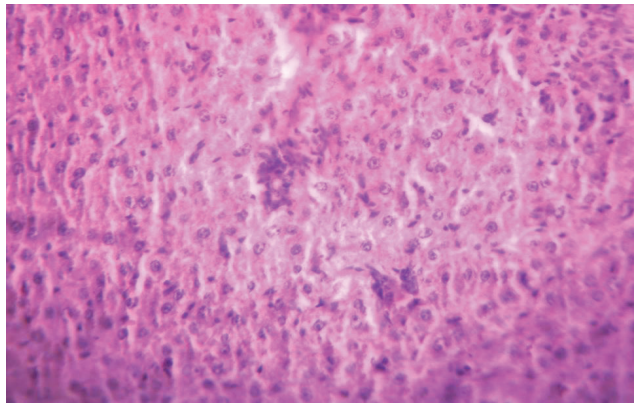
Preparation of tissue sample

The liver was exteriorized and excised. All specimens were immediately fixed in 10% formaldehyde solution. After fixation they were processed in usual manner, and embedded in paraffin for subsequent histopathological examination for liver. A scoring system (of no abnormalities, mild, moderate, severe) was used to classify the liver changes according to the severity of the damage and extent of histological changes. The histological sections were evaluated by a pathologist without prior knowledge of the treatment given to the animals [11] .

Blood parameters were determined in whole blood by the using rat mode of veterinary practice with a blood cell counter (Abocus Junior Vet-5, Austria). Measurements of biochemical parameters were made with a Modular PP autoanalyzer (Mindray BS800, China).

Rsults

Histopathological findings: The control group showed normal histological structure in the liver tissue (Figure 1A). In the DMBA group, the livers exhibited dilatation of the sinusoids, cholangiohepatitis in the portal region, and congestion in the sinusoidal and portal regions (Figure 1B). Hematological parameters also change in negative group compared of positive group Table 1 , in positive group : white blood cell (WBC) , LYM, MON ,GRA and PLT while other parameter such as RBC was decrease. The state of free radicals and antioxidants is given in Table 2. As seen in Table 2, the levels of MDA increased in liver groups compared to the control group, also SOD, GSH-Px, CAT, and GSH values decreased.



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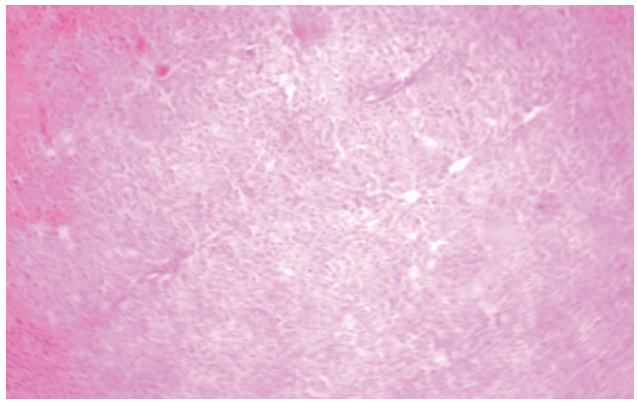

B

Figure1; A: DMBA group showing colangiohepatitis in portal region, mild dilation in sinusoids, hyperemia, and hydropic degeneration in hepatocytes;

B: Control group showing liver tissue with a normal histological structure

Table 1. Some hematological parameters in the groups (values are mean n=4)

No.	Parameter	Negative control	Positive control
1	WBC (103 /mm3)	8.2	15.1
2	RBC(103 /mm3)	8.8	1.4
3	LYM %	67%	78.5 %
4	MON%	1	0.9
5	GRA %	18.8 %	94 %
6	MCV(fl)	56.6	58.7
7	MCH(Pg)	16	21
8	MCHC(g/dl)	32.3	32.8
9	PLT(105 /mm3)	338	737

Table 2. Some biochemical parameters in the groups (values are mean n=4)

Treatments	Conc. of GPx(IU/mg)	Conc. Of SOD (IU/mg)	Conc. of CAT (IU/mg)	Conc. of MDA (nmol/ mg)
Negative group	127	458	122	134
Positive group	312.3	567	187	267

Dusccision

7, 12-dimethylbenz (α) anthracene (DMBA) is a well known carcinogen and immunosuppressor used in rodent models to study cancer [12]. DMBA is reported to induce mutations by making DNA adducts [13, 14]. Although, it is a well known skin carcinogen, yet many researchers have reported the deleterious effect of DMBA in liver [15, 16]. Liver is the primary site of metabolism and is often prone to damage by xenobiotics. Evidently, liver cancer is the second most common cause of cancer deaths worldwide [17]. several haematological and haematochemical parameters were changed when treated with DMBA and found that DMBA induced hepatocellular carcinoma. Experimental studies showed that DMBA-induced skin, oral, mammary and ovarian tumors [18]. The carcinogenic and mutagenic effect of DMBA requires its metabolic activation by mixed function oxidases. The hydroxylation of DMBA at 7-methyl group is a crucial step towards its carcinogenesis [19]. Further metabolism of DMBA leads to formation of a wide range of metabolites with varying toxicity. Among these, trans-3,4-dihydrodiol-1,2-epoxide is the carcinogenic product of DMBA [20, 21]. The metabolic products of DMBA, when present inside body, hampers ROS-antioxidant balance by overproduction of free radicals and the body in turn reacts by modulating activities of antioxidant enzymes to curb the damaging effects of an increased ROS [22]. Hematological and biochemical parameters may be affected by a variety of factors such as race, age, gender, pregnancy, lactation, muscular activity, region, season, environmental heat, maintenance, and nutrition. In the present study, the effects on blood parameters and hepatic, histopathology of fluoride . Oxidative products derived from mutagen metabolism , such as DMBA , might impair vital cellular function by damaging proteins and lipid membranes . Consequently , these changes induced by the chemical carcinogen 7,12-dimethylbenz[a]anthracene, have been reported to be leukemia, and the development of anemia [23]. Reactive oxygen species (ROS) are important as pathological agents for many diseases. Increased oxygen radical production and lipid peroxidation are associated with the pathogenesis of many diseases and the toxic effects of a wide range of compounds increase in total leukocyte count (WBC), eosinophil, neutrophil and monocyte values for rats exposed to DMBA was also reported by [24] . Hematological and biochemical parameters may be affected by a variety of factors such as race, age, gender, pregnancy, lactation, muscular activity, region, season, environmental heat, maintenance, and nutrition. a significant increase in the GPX, CAT, SOD level in rat treated with DMBA only was reported by [25]. On other hand [26] find MDA content increase significantly with rat treated with DMBA only.

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Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the College of Nursing, University of Warith Al-anbiya'a, Iraq and all experiments were carried out in accordance with approved guidelines.

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