.

Effect of *Cerastes cerastes* LAAO on Some Hematological Parameters in Hepatocellular Carcinoma-Induced in Rats

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ABSTRACT
Background: One of the most prevalent cancers globally is hepatocellular carcinoma, which is also the most prevalent kind of primary liver cancer in Egypt. Diethyl nitrosamine (DEN) is a well-known, highly carcinogenic substance for the liver. It is well known that DENA damages DNA repair enzymes and is typically utilized to cause liver cancer in rats used as experimental animals. **Objective:** To assess the hematological parameters in hepatocellular carcinoma induced in rats treated with paclitaxel and l-amino acid oxidase (LAAO) (PAC). **Materials and methods:** 25 adult, mature, healthy male albino rats (*Rattus rattus*) with an average weight of $(100 \pm 10 \text{ g})$ were used in this investigation. The rats were divided into 5 groups, each with 5 rats. At the conclusion of the experiment, hematological parameters were examined for all groups. **Results:** When treated with LAAO and PAC, the results demonstrated that all hematological parameters significantly changed from their respective levels in the control group. As compared to the DENA(HCC) group, the results revealed improvements for several criteria. **Conclusion:** WBCs, RBCs, Hb, and PLTs were all enhanced by the administration of LAAO and PAC. etc.

INTRODUCTION

The World Health Organization (WHO) estimates that there will be 10.3 million cancer deaths and 19.3 million new cases globally in 2020. The second most prevalent cause of cancer mortality (830180 fatalities, 8.3%) and the seventh most common cancer globally, respectively, is liver cancer (905677 cases, or 4.7% of the total). By 2040, liver cancer incidence and fatalities are expected to increase by more than 55% (Rumgay *et al*., 2022). According to estimates, Egypt's cancer burden would increase to 134632 new cases and 89042 deaths in 2020, with 278165 cases predicted to be prevalent (5-year) cases. Moreover, the most prevalent cancer in Egypt and the leading cause of cancer mortality (26523 fatalities, 29.8%) is liver cancer (27895 cases, 20.7% of the total).

Hepatitis B and C virus infections are the main primary liver cancer risk factors that can be changed (HCV). The burden of liver cancer is starting to increase as a result of the World Health Organization's (WHO) global hepatitis strategy's contribution to the success of HBV and HCV eradication efforts. Nevertheless, future changes in the incidence of liver cancer may be influenced by the rising frequency of other risk factors, such as type 2 diabetes and obesity. According to a recent research on the subject, lowering alcohol use might have prevented 17% of all liver cancer cases expected to be detected in 2020. Smoking tobacco is a significant contributor to liver cancer (Rumgay *et al.,* 2021). Chemotherapy, radiation, gene therapy, and hormone therapy are examples of current conventional therapies that have been discovered to have adverse effects on both malignant and healthy cells. As a result, using medications that are derived organically is becoming a more alluring option than using conventional pharmaceuticals. Natural biological resources are now among the promising options for treating cancer (Awad *et al.,* 2020; Magdy *et al.,* 2020). New pharmacological anticancer uses have been sparked by the bioactive compounds found in venoms recovered from a variety of venomous species, including snakes, scorpions, spiders, bees, and frogs (Gomes *et al.,* 2010; Abdel-Aziz *et al.,* 2017). Including cytotoxins, cardiotoxins, and neurotoxins, snake venom is a complex combination of bioactive peptides, proteins, enzymes, and toxins that have cytotoxic properties (Ebrahim *et al.,* 2016). According to reports, the venom of snakes has a cytotoxic impact on malignant cells (Ramos and Selistre-de-Araujo 2006; Vyas *et al.,* 2013). *Cerastes Cerastes* is one of the deadliest snakes in the world, and it resides in Egypt. Specific enzymes found in venom include phospholipase A2 (PLA2) and L-amino acid oxidase (LAAO). (LAAO) is a harmful protein that has potent anticancer therapeutic effects on a variety of cancer cell types (Guo *et al.,* 2015). By causing oxidative stress in cancer cells, LAAO triggers programmed cell death (Apoptosis) and can deliver hydrogen peroxide to cancer cells by binding to their surfaces at certain phospholipid compositions. (Abdelkafi-Koubaa *et al.,* 2016; Tan *et al.*, 2018; Ullah 2020).

Due to a lack of information about the anti-cancerous effect of LAAO produced by the venom of *cerastes cerastes* snake on human liver cancer (in vivo). Therefore, In the current study, was evaluated for its anti-cancerous activity against the proliferation of in vivo model of liver cancer compared to a conventional anticancer drug (Paclitaxel) to provide new anticancer agent in the future after further studies.

MATERIALS AND METHODS
1-Materials

Egyptian *Cerastes cerastes* (Cc) venom was bought from the Medical Research Center, The Holding Corporation for Biological Products-Vaccines and Drug companies (VACSERA), Egypt's laboratory animal division. The venom was combined, centrifuged at a low speed of 10,000 rpm for 10 minutes, and the supernatant was collected and kept at -80 °C until needed. L-amino acid oxidase (CC-LAAO) was isolated using size-exclusion and anion-exchange chromatography from the venom of *Cerastes cerastes* (El Hakim *et al.*, 2015). We bought diethylnitrosamine from Sigma-Aldrich (St. Louis, MO, USA). Purchases of Taxol (Paclitaxel) were made from (Hikma Co, Egypt).

2-Experimental Animals and *in vivo* Study:

Adult male albino rats weighing 100–110 grammes were purchased from the animal farm of the Egyptian Holding Company for Biological Products and Vaccines (VACSERA), Giza, Egypt, and transported to the animals were housed, college of science, Al-Azhar university. There, they were housed in plastic cages with five rats each and kept under standard conditions (23–2°C, humidity, and a 12-hour light/dark cycle throughout the experiment). Prior to the trial, the animals were given a week of free access to food and water for acclimation. According to the Al-

Azhar University Faculty of Science's ethics committee, all animals were treated humanely and in accordance with institutional standards for the care and use of experimental animals. This was done in Cairo, Egypt.

3-Induction of Hepatocellular Carcinoma:

Rats received a single intraperitoneal injection of DENA (200 mg/kg body weight), followed by weekly subcutaneous injections of carbon tetrachloride (CCl4) (200 mg/kg body weight) for three weeks (Sundaresan and Subramanian 2003).

4-Experimental Design:

Rats with and without HCC were placed into five groups of five rats each after HCC induction as follows:

Healthy animals made comprised Group (1) and acted as the control.

Healthy mice in group (2) received an intraperitoneal administration of LAAO dissolved in PBS (2.5 g/ml/48 hours) for one week (Abdelkafi-Koubaa *et al.,* 2021).

Animals with HCC that had been artificially created were involved in Groups (3) as a positive control.

Group (4) HCC patients received intraperitoneal administration of LAAO dissolved in PBS (2.5 µg/ml/48 hours) for a period of one week.

Animals in group (5) HCC received an intraperitoneal administration of Taxol (paclitaxel) at a dose of 1/10 LD50 (3.25 mg/kg/48 hr) for one week (park *et al.,* 2009).

5-Blood Sampling:

Animals were starved the night before diethyl ether anesthesia and at the conclusion of the experimental periods. The retro-orbital venous plexus was used to collect blood samples from all animals, and one part of the blood was transferred into tubes containing a 20-µl EDTA solution as an anticoagulant in order to determine the results of hematological tests such as the number of erythrocytes, hemoglobin concentration, the total number of leucocytes, and platelets, among other things.

6-Hematological Parameters:

A CBC analyzer (Sino Thinker Sk9000, U.S.) was used to estimate the blood's haematological investigations, such as erythrocyte count, total leukocyte count, platelet count, and haemoglobin concentration, and the Sysmex KX- 21N automated counter cell, Hematology Analyzer was used to confirm the results.

7-Statistical Analysis:

Using the Statistical Package for Social Sciences (SPSS/PC) computer application, all data were statistically evaluated using one-way analysis of variance (ANOVA), followed by the post hoc (LSD) test at p 0.05. (version 26).

RESULTS

Hematological Parameters:

White Blood Cell (WBCs) Count:

The WBC count increased significantly (p<0.05) in the HCC-animal groups compared to the control group, however, there was an insignificant change in the LAAO group. Moreover, as compared to rats treated with DENA alone, the WBC count on treated LAAO or PAC with DENA (HCC) also showed a substantial reduction (p<0.05) (Fig.1).
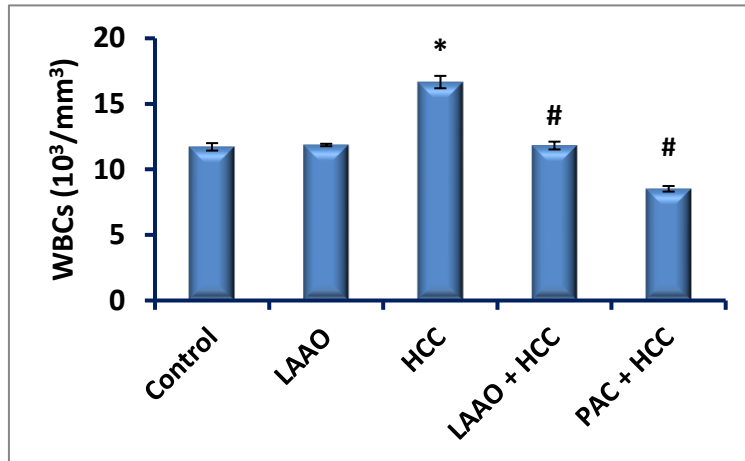
Fig. 1: White Blood Cell count (Mean values ± S.E) in the treated HCC-animal groups compared to control one. # is significantly different from HCC group. * is significantly different from the control group.

Red Blood Corpuscles (RBCs) Count:

The findings showed that, compared to their comparable value in the control group, the rats given DENA (the HCC group) exhibited a considerable drop in RBCs ($p < 0.05$), whereas healthy rats given LAAO only showed an insignificant difference.

Also, as compared to the group treated with DENA (HCC) alone, the RBCs count in the treated LAAO or PAC with DENA groups showed a significant increase ($p < 0.05$) (Fig. 2).
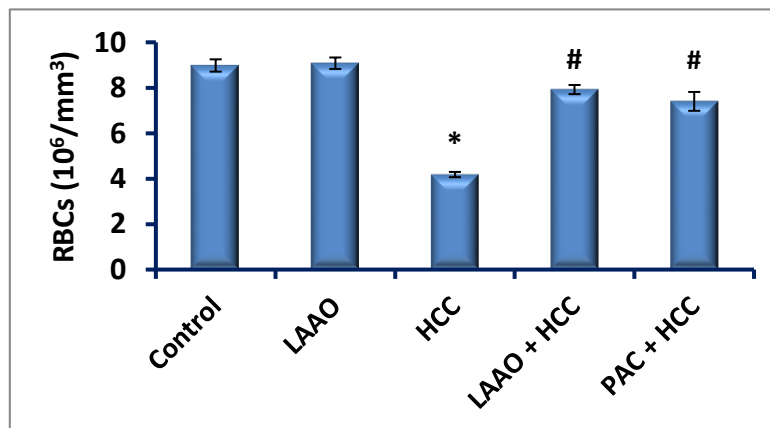

Fig. 2: Red Blood Cells count (Mean values ± S.E) in the treated HCC-animal groups compared to control one. # is significantly different from HCC group. * is significantly different from the control group.

Hemoglobin (Hb) Concentration (gm/dl):

When compared to the equivalent values in the control group, the rats intoxicated with DENA caused a significant drop ($p < 0.05$) in hemoglobin (Hb) concentration, while the rats in the LAAO group exhibited insignificant change.

In addition, as compared to the group treated with DENA (HCC) alone, the Hb concentration in the treated LAAO or PAC with DENA groups showed a significant increase ($p < 0.05$) (Fig. 2).
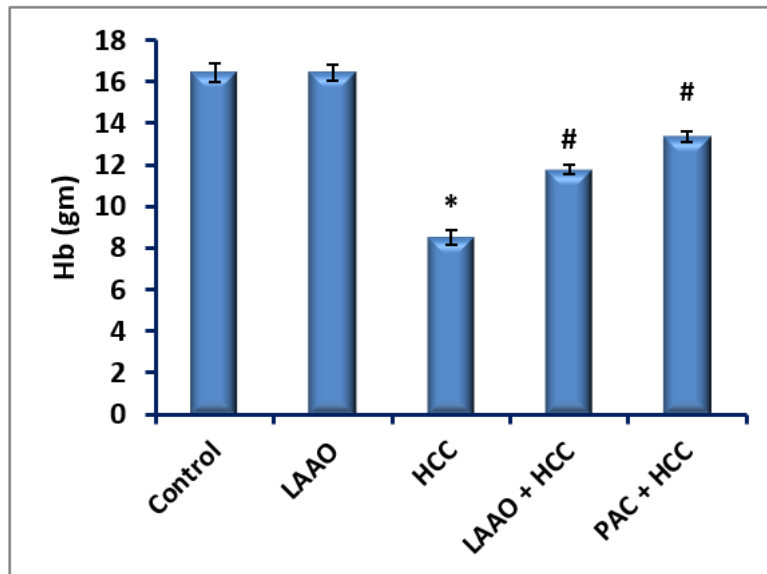
Fig. 3: Hemoglobin concentration (Mean values ± S.E) in the treated HCC-animal groups compared to control one. # is significantly different from HCC group. * is significantly different from the control group.

Platelets (PLTs) Count:

The findings showed that, when compared to their comparable value in the control group, the rats given DENA (HCC group) showed a substantial drop (p<0.05) in platelets count. Moreover, as compared to similar results in the control group, the healthy rats treated with LAAO only had negligible alterations in PLTs.

In addition, as compared to the group treated with DENA alone, the groups treated with LAAO or PAC also showed a substantial increase in PLTs (p<0.05) (Fig. 4).
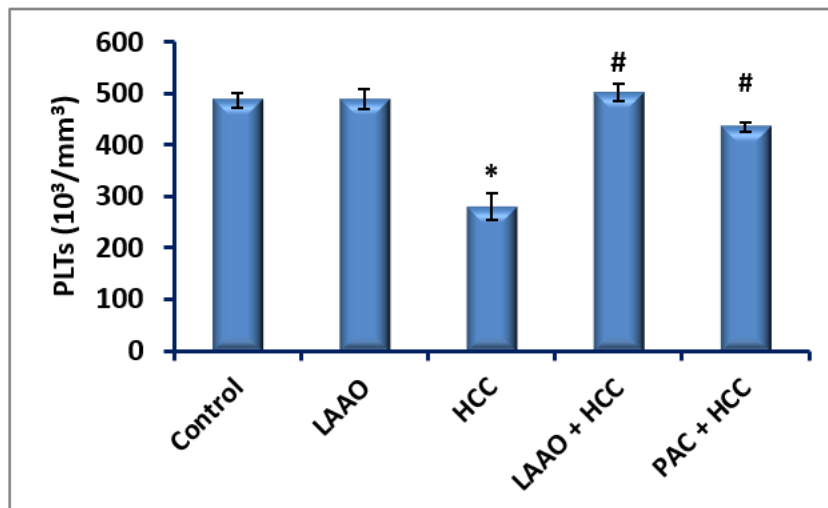

Fig. 4: platelets count (Mean values ± S.E) in the treated HCC-animal groups compared to control one. # is significantly different from HCC group. * is significantly different from the control group.

MCV and MCHC:

The outcomes demonstrated that MCV and MCHC in HCC rats treated with LAAO and PAC or LAAO administered groups showed negligible changes when compared to their corresponding values in control group, while the blood indices in the DENA (HCC) administered group showed a

significant decrease (p<0.05) when compared to their corresponding values in control group.

In addition to MCV and MCHC values, treatments with LAAO or PAC followed by DENA showed a substantial improvement (p<0.05) compared to the group receiving only DEN (Fig. 5).

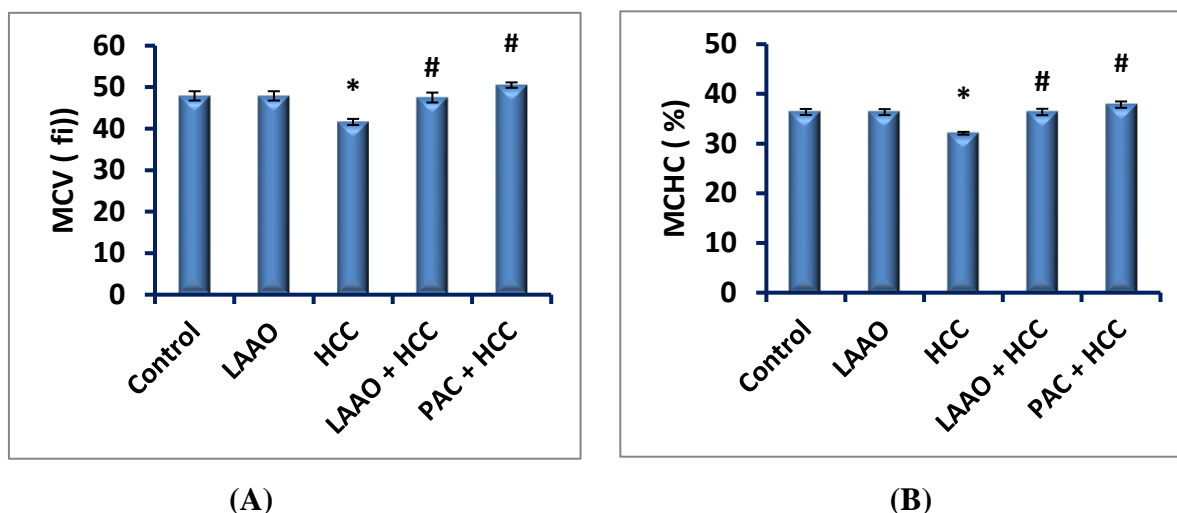**(A)**   **(B)**

Fig. 5: Hematological parameters of the treatment groups of HCC-affected animals in comparison to the control group: A) MCV and (B) MCHC. # is significantly different from HCC group. * is significantly different from the control group.

DISCUSSION

According to our findings, the determination of an agent's potential for carcinogenesis specifies the exposure parameters (dosage, time, and duration) under which the agent may cause cancer. Animals are used as substitute models for humans because of their resemblance in physiology and biochemistry. A lack of normal growth regulation, as shown in the current study, is one of several variables that play a significant role in the development of cancer (Hanahan and Weinberg 2011).

The hematopoietic system is incredibly susceptible to medicines' and other hazardous chemicals' potentially harmful impacts on human health. Hematological factors and indicators of the body's inflammatory response have also been linked to prognosis in a number of cancers. In light of the fact that any serious illness or anomaly directly affects blood parameters, it is essential to monitor changes in hematological parameters in liver cancer patients on a frequent basis during therapy (Ali, 2014; Shrivastava, *et al.,* 2016; Mokh *et al.,* 2019).

All of our findings indicated that the LAAO had no impact on the animals and had negligible effects when compared to the control group. According to Abdelkafi-Koubaa this indicates that when experimental animals are injected with 2.5g/mL, there are no negative side effects (Abdelkafi-Koubaa et al.,2021), unlike paclitaxel, which has many serious side effects (Abou-Donia *et al.,* 2015).

In the current study, the data showed that, when compared to their corresponding levels in the control group, the mean values of RBCs, Hb, Hct, and PLATS counts and concentration were significantly decreased while WBCs were significantly increased. This condition called pancytopenia was found in agreement with (Carr, 2016; Selvamani and Thomas, 2017) who stated that Leucopenia and thrombocytopenia are present in the majority of patients and are frequently present in the patients with splenomegaly and with a history of bleeding tendencies. As inferred from the study, decreased RBCs and hemoglobin are the most common anemia in primary HCC patients, and its type is frequently normochromic normocytic anemia (Solomon *et al.,* 2017). Additionally, these findings supported earlier findings that

suggested hematological abnormalities in chronic liver diseases may be caused by direct injury to the bone marrow, that blood components are sensitive to oxidative stress, and that a high percentage of polyunsaturated fatty acids in blood components' plasma membranes increases the production of lipid peroxidation products (Carr, 2016).

This is the first report demonstrating the effect of Cc-LAAO on Hematological parameters in rat model cancer, which may lead to the development of cancer treatment. In the present study, the data revealed that the mean values of RBCs, Hb, Hct, and PLATs counts and concentration were significantly increased while WBCs significantly decreased in the group treated with LAAO or PAC with DEN when compared to the group treated with DEN only (p< 0.05). This means that LAAO treatment could decrease or mitigate the toxic effects of anemia and leukocytosis against DEN-induced toxicity.

Conflict of Interest: The authors have no relevant financial or non-financial interests to disclose.

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