

## **Histological study of the effect of UV rays and active biomass EM-X in the livers of laboratory rats**

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### **Abstract**

The study includes showing the histological changes in the livers of male laboratory white rats resulting from exposure to ultraviolet rays and dosed with active biomass solution EM. (15) laboratory rats were used in this study, with an average weight of  $(250 \pm 5)$  grams divided into three groups, if the group was exposed The first group A for (30) minutes was exposed to ultraviolet rays on a daily basis, and the second group B was exposed to (30) minutes to ultraviolet rays and was orally dosed with an effective biomass solution EM on a daily basis for three weeks, while the control group (C) was not exposed to radiation and was not dosed with the block solution. Active biomass EM. The source of the radiation was a UVC Ray device that emits radiation with a wavelength of 275-nm 265 and active biomass EM (produced by the Amero company Japanese).The animals were dissected at the end of the experiment, and tissue samples were taken from their livers, which were prepared for the histological study to identify the resulting changes.

## Introduction

Ultraviolet rays are electromagnetic waves that are shorter in length than the waves of visible rays, and longer than X-rays, and they are called ultraviolet because they are the shortest among the colors of the spectrum. There are three types of ultraviolet rays called the first type UVA, which is the most common during most periods of the year and has a high penetration ability to epithelial and connective tissues. The second type UVB is less penetrating than the first type, so it causes superficial burns on the skin. The third type is UVC, which is the most Dangerous to various living organisms.

Among the types of ultraviolet radiation, it is considered carcinogenic because it is genetically mutagenic and a non-specific harmful factor that has all the characteristics of initiating tumors and cellular abnormalities.. [2] The most important natural factor for protection from ultraviolet rays is the ozone layer (O<sub>3</sub>), which is one of the layers of the atmosphere, which works to prevent the arrival of harmful ultraviolet rays to living organisms, but due to the increasing air pollution in recent decades, this layer has eroded, which led to the arrival of A high percentage of ultraviolet rays to the surface of the earth and the appearance of distorting, mutagenic and carcinogenic effects on living organisms [3].

The liver is the largest gland in the human body, and it performs many important functions. The liver occupies the upper right part of the abdomen as it is located directly below the diaphragm and partially covers the stomach. The liver is surrounded by a capsule consisting of loose connective tissue characterized by its thinness, and from which very thin septa barriers extend, dividing the hepatic tissue into lobules microscopic in size [4] One lobule consists of bands or branching sheets of hepatocytes, which are polygonal, large in size, light in color, with a homogeneous pale cytoplasm receptive to acidic colours. The liver, including the sinusoids, is arranged in a shape reticular around the branch of the central vein.

The sinusoids are also stellar-shaped phagocytic cells known as Kupffers cell, and the liver lobule is the structural and basic unit of the liver through which it performs its functions [5]. Effective Biomass Effective Microorganism, which is a natural preparation widely spread in East Asia, especially in Japan [6] It is produced by fermenting papaya plant, seaweed extract and unpolished rice, and it contains a compatible group of beneficial microorganisms, including photosynthetic bacteria, lactic acid bacteria, and yeasts Yeast as shown in Table [8,7].

The microorganisms used in the production of active biomass EM are classified under the standard (safety first class) and each food item classified under the safety first standard is one of the varieties that does not cause any kind of disease to adult human life, and this classification has been approved by the American Society for public health after undergoing hazardous testing, and also approved by the Scientific Advisory Committee for the Measurement of Microorganisms in the United States of America, all acid bacteria and yeasts used in active biomass products (EM) are included in the US Food and Drug Administration (FDA) classification list, an agency of the US Public Health Administration, and are recognized on the basis of their safety and freedom from risks to human health and the environment. [9,6].

### **Materials and methods:**

- Preparing the animals: 15 animals of white male *Rattus rattus* were used in this study, their ages ranged between 12-10 weeks, obtained from the animal house in the pharmacology department of the Samarra Pharmaceutical and Medical Appliances Industry Company. The animals were divided into three groups and placed in specialized cages.

As in Figure (1), appropriate laboratory conditions were created for it, including ventilation and temperature ranging between 26-24 °C, and a 12:12-hour illumination system, except for the exposure period. The cages were kept clean and sterilized with 70% ethanol alcohol disinfectant once a week.

Experiment groups :

- The first group (A) was exposed to ultraviolet rays for (30) minutes on a daily basis for three weeks [10].
- The second group (B) was exposed to ultraviolet rays for (30) minutes on a daily basis and was orally dosed with an effective biomass solution EM (produced by the Japanese company Amero) at a dose of 3 ml / kg of body weight with drinking water [11] for a period of three weeks.
- The control group (C) was not exposed to ultraviolet rays and was not dosed with EM active biomass solution.



Figure (1) Cages and experimental anima

#### Preparation of samples for study under light microscopy

After the termination of the treatment period of the animals, various organs of the animals such as the stomach, small intestine, large intestine and liver were taken, and these samples were fixed in 10% formalin saline solution for four hours, then the water was withdrawn from it by passing it with ascending concentrations of ethyl alcohol (50, 70, 95, (100)% Then the models were sanded using xylene, and then saturated with paraffin wax with a melting point of (58-56) °C by means of the oven and at 60°C for three hours, then the models were poured with special L-shape molds using clean paraffin wax, then they were cut by the cutter The rotor is (4-5) micrometers thick, and the sections were surfaced at a temperature of (45-48) °C in a special water bath to brush the sections, then they were loaded onto glass slides, and dyed with (hematoxylin and Eosin) .It was prepared for examination and imaging with a light microscope using a digital camera [12] .

#### Results

Histological changes in the livers of laboratory rats.

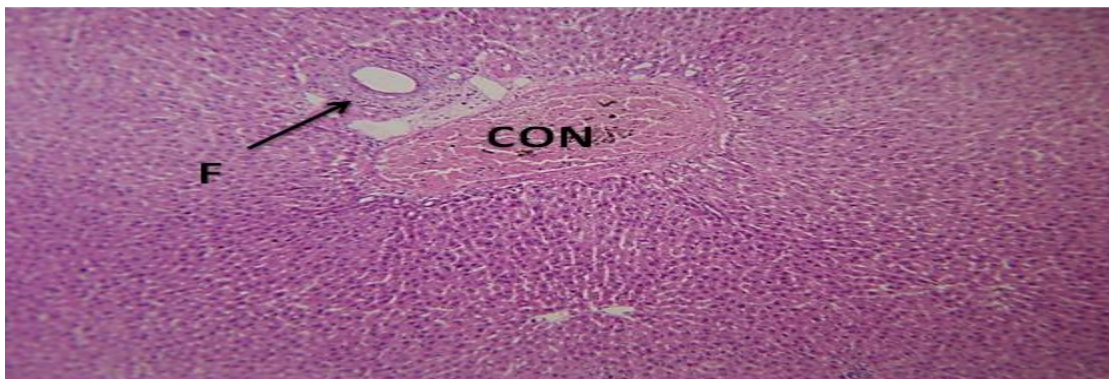


Figure (2) (A) group Just UV : Liver cross section show congestion (CON) of blood vessels with present fibrocytes (F) H&E X40.

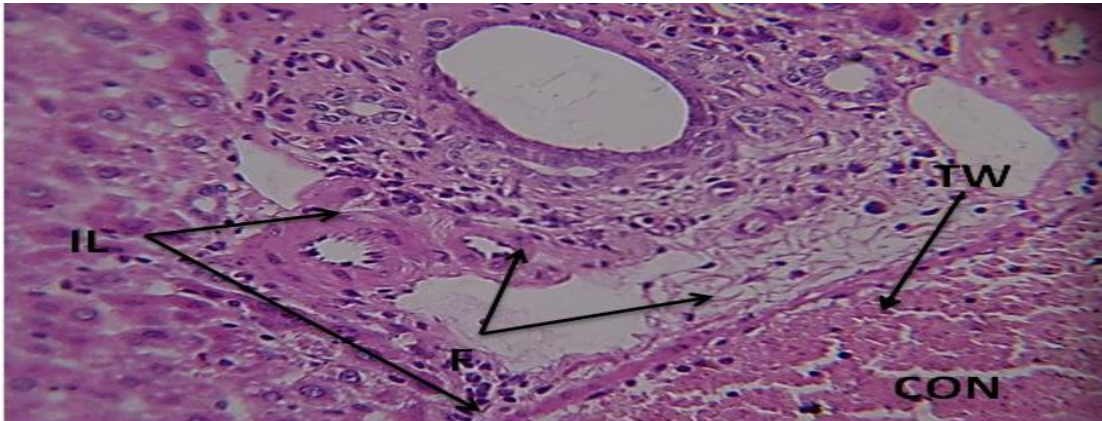


Figure (3) (A) group Just UV : Liver cross section show thickening wall (TW) and congestion (CON) of blood vessels and infiltration of lymphocytes (IL) with present fibrocytes (F) H&E X100.

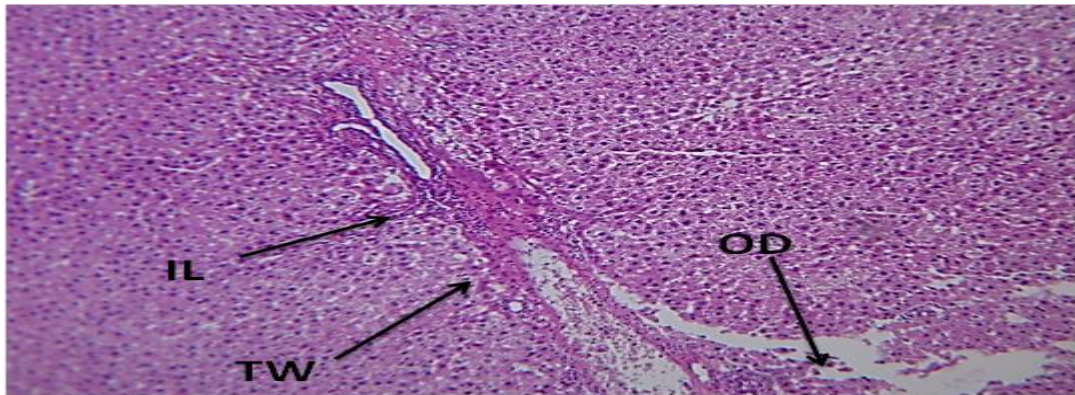
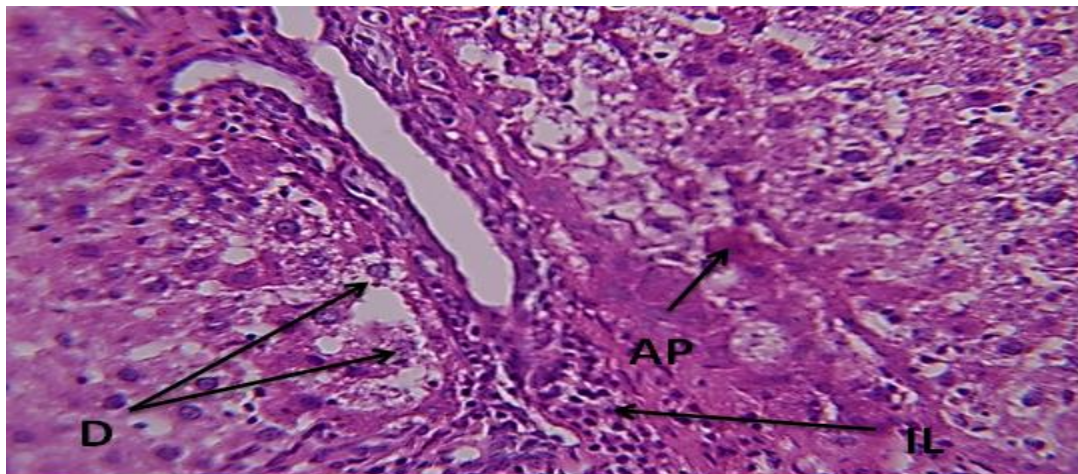


Figure (4) (A) group Just UV : Liver cross section show thickening wall (TW) of blood vessels and infiltration of lymphocytes (IL) with oedema (OD) H&E X40.



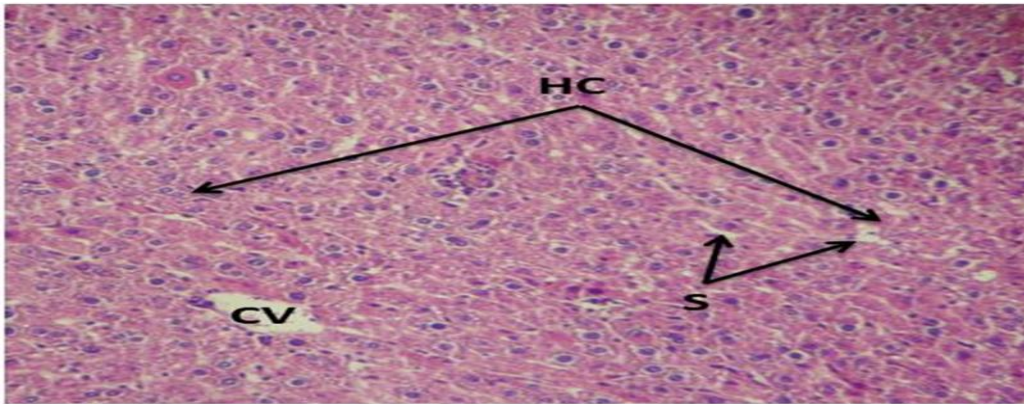


Figure (6) (B) group UV and EM: Liver cross section show central vein (CV), hepatocytes (HC) and sinusoids (S) with normal sizes, H&E X40.

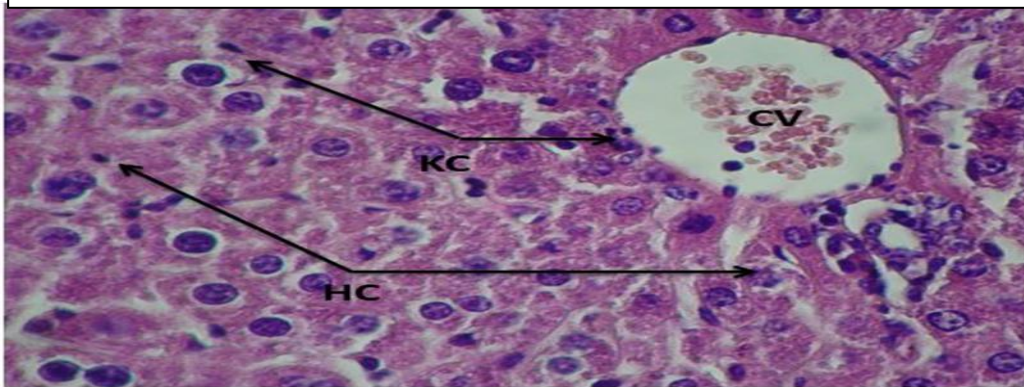


Figure (7) (B) group UV and EM: Liver cross section show central vein (CV), Kupffers cell (KC)and hepatocytes (HC) H&E X100.

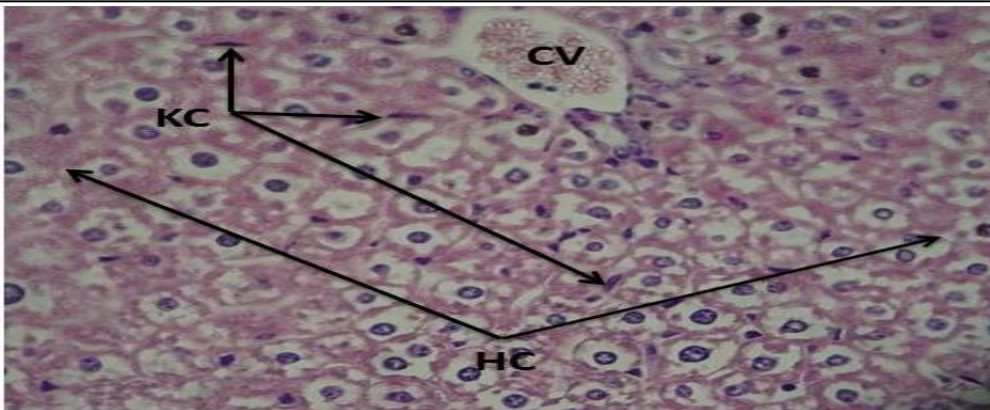


Figure (8) (B) group UV and EM: Liver cross section show central vein (CV), Kupffers cell (KC)and hepatocytes (HC) H&E X40.

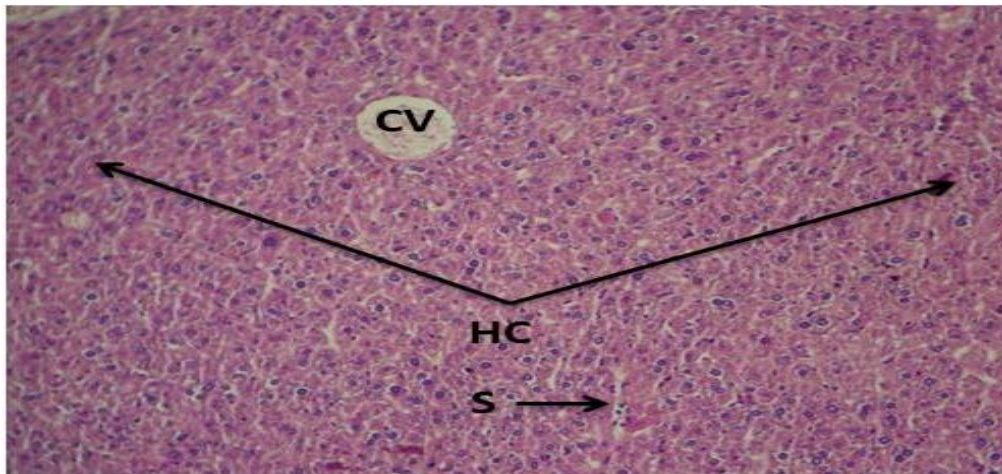


Figure (9) (C) control group: Liver cross section show central vein (CV), hepatocytes (HC), and sinusoids (S)H&E X 40.

## Discussion

The liver is the largest internal organ in the body and is the chemical factory of the body because it performs a wide range of chemical and vital functions, and it is the place in which all substances are metabolized, as it consists of tissues with great metabolic activity, possessing many mechanisms and metabolic systems [13] and one of these functions is The conversion of vitamin D<sub>2</sub> and vitamin D<sub>3</sub> to D<sub>3</sub> 25-hydroxyvitamin D<sub>3</sub> 25-hydroxy vitamin [D<sub>3</sub>-25(OH)] by the action of 25-hydroxylase enzyme [14],The most important source for obtaining vitamin D<sub>3</sub> is its formation through the skin, as it consists of 90% of vitamin D<sub>3</sub> and this is in the presence of ultraviolet rays [16,15].

As the cross-section of the liver shows congestion of blood vessels in addition to the accumulation of fibroblasts (Figure 2), and the cross-section of the liver in Figure 3 shows thickening in the wall of blood vessels, in addition to an infiltration of lymphocytes in the presence of inflammation in the area with the presence of fibroblasts, as the evidence indicates that Previous studies indicate that vitamin D is a modifiable factor according to the amount of chronic and continuous exposure to UV rays [18,17].Excessive vitamin D inhibits hepatic stellate cell (HSC) proliferation, which causes excessive collagen buildup—a hallmark of hepatic fibrosis and is the most potent pathogenic pathway for cirrhosis [19, 20] A cross-section of the

liver Figure (4) shows a thickening of the vessel wall Hematopoietic and lymphocyte infiltration with edema, as the histological results showed in the cross-section of the liver (Figure (5)), to the appearance of degenerative liver cells and the occurrence of inflammation, due to the occurrence of oxidative stress leads to the generation of free radicals that attack and combine with unsaturated fats, which causes lipid peroxidation Peroxidation in the structure of cellular membranes [21] that causes activation of programmed death pathways, the extrinsic pathway resulting from cell membrane damage, and activation of programmed apoptosis by a mitochondrial mediated pathway resulting from mitochondrial membrane damage.[22, 23].

The results of the histological study of the livers of male laboratory rats in group (B) exposed to ultraviolet rays and orally dosed with EM active mass solution showed a normal histological pattern similar to that of the control group, which is explained based on the action of EM biomass components, which included antioxidants such as flavonoids, including (kaempferol, panaxin, quercetin, lycopene, ascorbic acid, alpha-tocopherol, ubiquinone), studies have indicated the therapeutic ability of EM solution through its anti-inflammatory role, and by inhibiting the production of inflammatory mediators including cytokines and interleukins (IL-5, IL-6), IL-10) [24], and it also plays an antioxidant role, as it works to protect hepatocytes from the oxidative stress caused by free radicals resulting from exposure to ultraviolet rays, and this was explained by [25], It may be attributed to the action of flavonoids, saponins and lycopene, which are found in the components of EM, in the inhibitory effect of ultraviolet rays, which makes this substance a very effective antioxidant through its ability to increase the activity of enzymatic antioxidants inside the organism, including increasing the activity of Superoxide Dismutase (SOD).

Increasing the level of glutathione in the liver tissues and scavenging free radicals, which contributes to reducing lipid peroxidation and protecting membranes and cellular organelles from oxidative damage, especially DNA, which makes the tissue appear almost normal, and its stimulating action has led to compensation for cells damaged by the effect of radiation. Ultraviolet [24,25,26].

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