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Effect of Olive leaves extract on radiation exposed hepatic tissue: A histopathological study of albino rats

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Many extrinsic and intrinsic etiological factors are responsible for cellular injury. Radiation is a harmful etiological factor for cellular injury by producing free radicals. During cellular metabolism free radicals are produced and neutralised by anti-free radicals. Radiation causes imbalance between it thus leading to cellular injuries and changes accordingly. Olive leaves may play protective role to cells against injury. Histomorphological study establishes the role of it as a protective nature. The aim is to study histo-morphological changes in radiation induced injury of liver cells treated with olive leaves and to its protective role in albino male rats.Thirty male albino rats with an average weight 100-110 grams. The rats were divided equally into three groups that included control group without any treatment for one month. Group 2 irradiated by exposing to 6 Gy (fractional dose 2Gy each 3 days). Group 3 were irradiated as group 2 and treated orally with olive leaves extracts with a dose of 1ml/100gm of body weight every day for one month. The morphological and histological structures of the liver were compared in different groups of the rats.Significant changes of cellular injury were observed in group 2 as compared with control group. Group3 showed obvious decrease in pathological changes compared with group 2.These results indicate protective role of olive leaves against radiation induced injury in liver.

Keywords: Radiation, Liver, Olive, Histochemsitry, Gamma radiation

INTRODUCTION

Many etiological factors are responsible for cellular injury by action of oxidative stress. Radiation is an important cause of oxidative stress induced DNA damage and death (Tolman et al. 2004; Steel, 1996). The ionizing radiations react with the molecules of the cellular structures causing damage thus affecting normal functions (Hall, 2000) There is imbalance between pro-oxidants and anti-oxidant status in the cell (Samarth and Raghavan, 2003; Bhosle et al. 2005). The degree of protection against γ-radiation injuries depends on many complex factors such as dosage, time and distance (Vincent, 2008). Natural antioxidants play a resent source of protection against gamma radiation. Antioxidants may protect cells from injury caused by unstable molecules known as free radicals which is involved in several disorders (Gerber et al. 2002; Emam and Fadladdeen, 2016). The

effect of free radicals may be neutralized or reduced by appropriate anti-free radicals. A number of dietary antioxidants have been reported to decrease free radical attack on biomolecules like lycopene, hesperidin a flavanoglycone, olive leaves (El-Habit et al. 2000; El-Hady et al. 2018). Olive leaf is leaf from olive tree (Olea europaea). Its extract was used to treat many health problems such as pain, fever, infections by ancient Egyptians and Mediterranean (Omar, 2010 Taha et al. 2020). Unprocessed olive leaves contain many active chemical components such as oleuropeoside and oleuropein. Many studies proved that these components have anti-inflammatory, anti-atherogenic, anti-bacterial, antiviral and anticancer effect (Visioli and Galli, 2001; Carluccio et al. 2003; Hamdi and Castellon, 2005; Menendez et al. 2007).

This study is aimed to observe radiation induced histomorphological changes compared with olive leaves extract treated albino rats.

MATERIALS AND METHODS

Preparation of Olive Leaf Extracts

5.5 grams of the olive leaf powder were soaked in 100 ml boiled dist. water and covered for ten minutes, then cooled to room temperature and filtered. The extract was given orally with a dose of 1ml/100 gm of body weight (using the stomach tube) every day. This dose is equivalent to the therapeutic human dose (500mg) (Wainstein et al. 2012).

Animals

In this experimental study, 30 adult, healthy, male Wistar albino rats (300-350 grams, 3 months old) were used. They were gained from an animal house of the College of Medicine, Al Azhar University, Egypt. They were kept under controlled standard animal housing situations and moisture with access to food and water ad libitum at the Animal Care Facility. The pellet diet composed of 23% protein, 5% lipids, 4% crude fiber, and 55% nitrogen-free extract. The rats were kept under control for approximately 2 weeks before the start of the experimentation to allow acclimatization.

Gamma–irradiation procedure

Irradiation was performed at the National Centre for Radiation Research and Technology (NCRRT), Nasr City, Cairo, Egypt. The source of radiation was a Gamma Cell-40 (Cesium 137), irradiation unit manufactured by Atomic Energy of Canada Limited that ensure a homogeneous distribution of irradiation. The dose rate was 0.61 Gy/minute during the experimental periods. Animals were whole body exposed to 6 Gy delivered as a fractionated dose (2 Gy each 3 days).

Experimental design

Three experimental groups, ten rats for each, were used as follows:

The animals were randomly selected and divided into three equal groups. Group 1: These rats served as negative control (without any treatment for one month). Group 2: The rats were irradiated via exposing them to 6 Gy delivered as a fractionated doses of gamma radiation (2 Gy each 3 days delivered in 3.5 min) for one month. (Abu-Amara and Meselhy, 2016)

Group 3: These rats had a combination of irradiation plus oral intake of olive leaf extract for one month.

Histological and Histochemical techniques

The rats of the control and treated groups were sacrificed after one month and small pieces of the liver were taken for the histological and histochemical studies. Specimens were prepared via fixation in 10% neutral buffered formalin solution and Carnoy's fluid. Paraffin sections of 5µm thickness were prepared and stained with Harris haematoxylin and eosin (HandE) (Bancroft and Gamble, 2008). The collagen fibres were stained by using Mallory's trichrome stain. The intracellular polysaccharides were detected by Periodic Acid-Schiff Reagent (PAS) method (Bancroft and Gamble, 2008).

Morphometric Analysis

Morphometric data was obtained by using an image analyser (ImageJ 1.46r). The quantitative percentage of collagen in the liver was calculated by using the sections stained with Mallory Trichrome at magnification of 400x. The hepatocytic content of polysaccharide was studied by using PAS-stained section at 400x magnification. The apoptotic changes in the hepatocytes for different groups of study using caspase -3 immnuo stained sections at 400x magnification. All these observations were carried in five non-overlapping field / section in five serial sections from each animal in each group.

Statistical analysis:

All statistical analyses were performed via P Aleontological Statistics Version 3.0 (PAST 3.0) statistical software. The obtained data were expressed as mean± standard deviation (SD) and analysed using analysis of variance (ANOVA)-Bonferroni with $p<0.05$ considered statistically significant.

RESULTS

Histological and Histochemical Results

The liver sections from control group stained with Haematoxylin and eosin showed hepatic lobules composed of central vein lined by endothelial cells. The hepatocytes are arranged in one cell thick plates radiating fashion from central vein with irregular sinusoidal spaces in between (Fig.1A). Hepatocytes are polyhedral with abundant eosinophilic cytoplasm, basophilic granules and little glycogenous material. Hepatocytic nuclei are large, vesicular. The portal area is composed of portal vein, hepatic artery, bile duct embedded in small amount of connective tissue with few lymphocytes (Fig.1A). The sinusoids are lined by endothelial cells and Kupffer cells.

In irradiated group, liver sections of rats showed dilated hepatic sinusoids. Affected hepatocytes lost their nuclei with vacuolated cytoplasm and some other shad pyknotic nuclei (Fig. 1B). Widened and dilated hepatic portal veins filled with amyloidal substances as well as erythrocytes were seen and its wall was thickened and invaded with many fibroblasts. Many pyknotic nuclei were also noticed surrounding the portal vein (Fig.1B). Also, progressive wideness of hepatic sinusoids as well as irregular central veins was detected. Furthermore, there was a remarkable proliferation in the cellular wall of a ruptured bile duct (Fig. 1B). Hepatocellular necrosis appeared lobular inflammation with lymphocytic infiltration. In Group 3, received olive extract concomitant with irradiation, considerable degree of improvement on the level of hepatic cellular structure, hepatic sinusoids and portal tracts was detected (Figs.1C and 2C). In Group1, investigations of normal liver sections stained with Mallory's trichrome stain showed the appearance of little amounts of collagenous fibres around the hepatocytes and hepatic sinusoids in the form of fine threads of collagen fibres around central vein (Fig.2A). Also, fine threads of collagen fibres were detected in the portal area surround the hepatic portal vein and bile duct (Fig.2A). In Group 2, liver sections of irradiated rats showed an obvious significant increase in the collagenous fibres deposition around hepatic sinusoids, central vein and portal tract structures in comparison to the control group (Figs.2B). In Groups 3 the amount of collagenous fibres deposition was around hepatic sinusoids, central vein and portal tract structures was significantly decreased to the levels observed in this group (Figs.2C and 2C).

Investigations of normal liver sections stained with PAS revealed PAS+ve reaction (Fig.3A, Table 1). However, carbohydrates were not uniformly distributed within the cytoplasm of these cells. Carbohydrates were found in the majority of the hepatocytes as coarse and fine pink granules. The nuclei showed negative affinity to the reaction (Fig.3A). In Group 2, liver sections of irradiated rats showed that the amount of liver carbohydrates was significantly decreased in comparison to the control group (weak PAS+ve reaction) and carbohydrates were mainly concentrated near the basement membrane of some hepatocytes. Moreover, the most destructed hepatocytes showed weak reaction (Fig.3B). In Groups 3, most of the hepatocytes revealed significant improvement of PAS+ve reaction in comparison to the irradiated group (Fig. 3C, Table 1).

Table 1: Average area percentage of the collagen fibres surrounding the hepatocytes cells, and PAS^{+ve} expressions in the hepatocyte's cells for the different groups of the study expressed as mean ± SD.

Study Groups	Optical Density of the PAS^{+ve} expression in the hepatocytes cells	Average area percentage of the collagen fibers surrounding the hepatocytes cells
Group 1	132.02±9.31	112.8±9.33
Group 2	169.32±9.80	51.3±11.30
Group 3	130.79±7.20	119.9±8.80

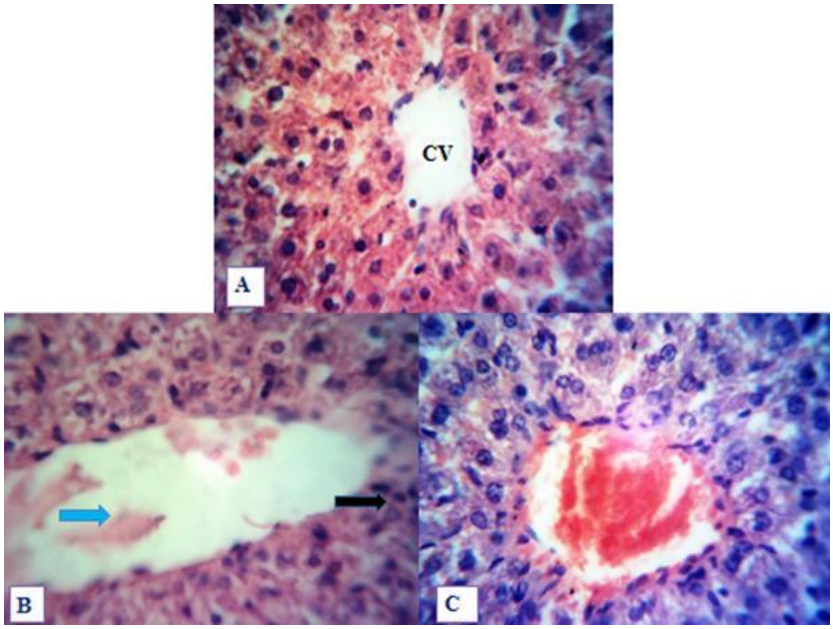

Figure1:(A) Histological light photomicrograph (H and E 400×) of the liver of control group showing normal lobular pattern with a centrilobular vein (CV) and radiating irregular branching and anastomosing plates of hepatocytes with intervening sinusoids lined with endothelial cells. (B) A photomicrograph of the liver of irradiated group 2 adult albino rat showing dilatation of central vein, hepatocytes necrotic changes such as marked hepatocytic ballooning, small pyknotic nuclei and fatty degeneration (black arrows) around the central vein. Congestion of central vein with blood (blue arrow) was detected. (C) Light photomicrograph of a section in a rat liver treated with olive extract showing that most of hepatic cellular structure and hepatic sinusoids are almost similar to that of the control group. (Hx. and E.x400)

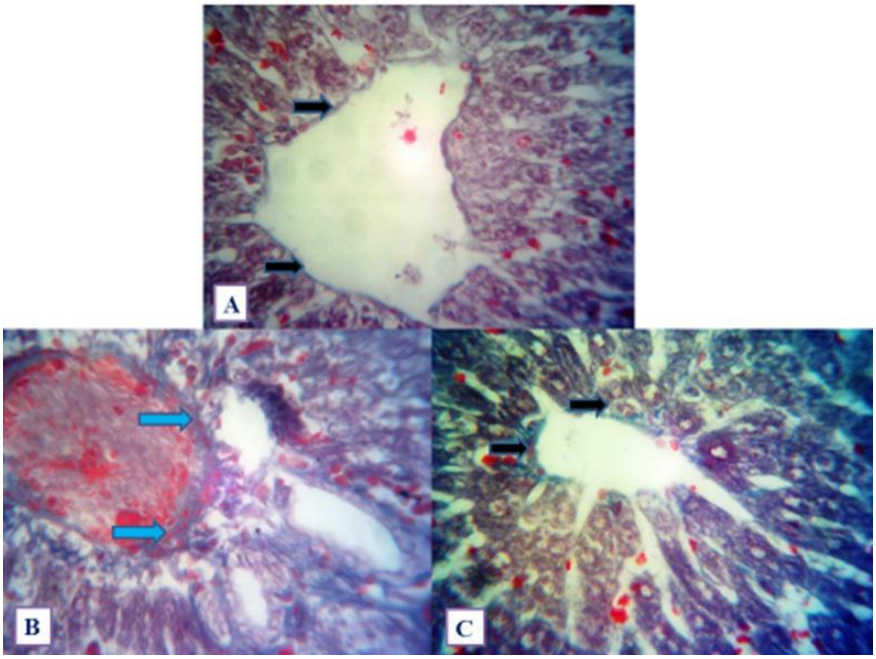

Figure2: (A) A photomicrograph of the control liver of adult albino rat showing normal distribution of fine threads of collagen fibres around central vein (black arrows). (B) A photomicrograph of the itrradiated liver of group 2 showing appearance of collagen fibres around central vein (blue arrows). (C) A photomicrograph of group 3 treated with olive extract showing distribution of fine threads of collagen fibres around central vein (black arrows). (Mallory's trichrome. x400).

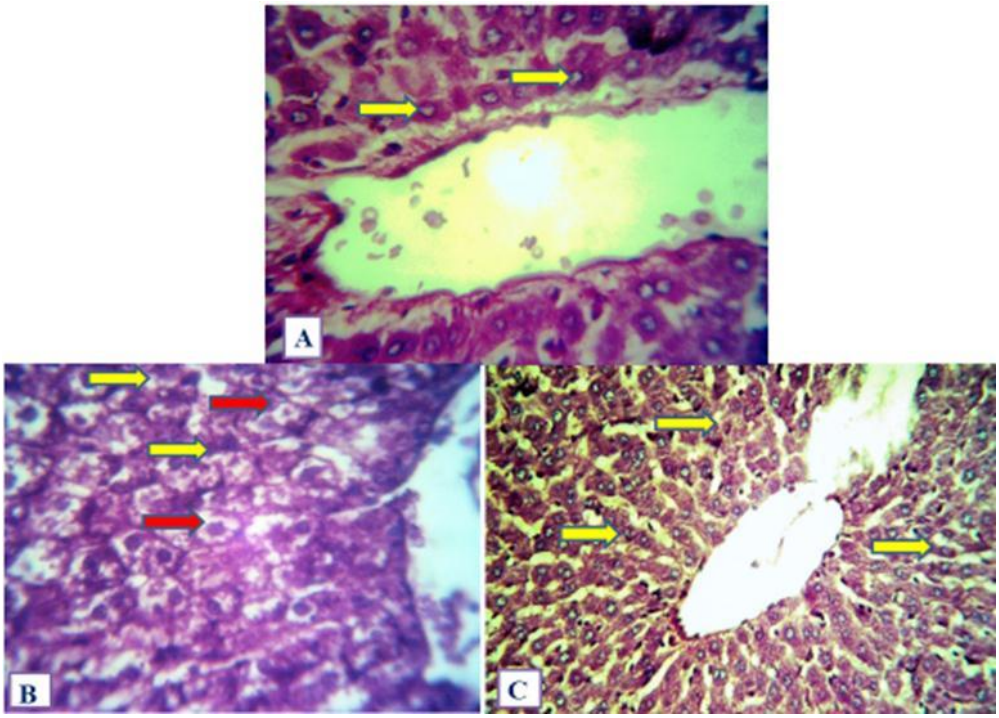

Figure 3: (A) A photomicrograph of the control liver of adult albino rat stained with Periodic Acid Schiff (PAS. A and B x400 C x200) showing strong PAS+ve granules in most of the hepatocytes. Carbohydrate granules appeared as coarse and fine pink granules in cytoplasm of hepatocytes (yellow arrows). (B) A photomicrograph of the liver of group 2) showing decreased amount of dispersed carbohydrates (red arrows). The amount of carbohydrates was mainly concentrated near the basement membrane of some hepatocytes (yellow arrows). (C) A photomicrograph of group 3 treated with olive extract showing marked increase in carbohydrates content of hepatocytes (yellow arrows)

DISCUSSION

The results of the current study showed that olive leaves extract has considerable protective effects against morphological changes of hepatic tissues. The antioxidant properties of olive extract may be responsible for this protection.

The harmful effects of the ionizing radiation produced on the human body should be taken into consideration as it's used on a large scale in diagnosis, therapy and industry (Jagetia, 2007; Kunwar et al. 2010).

Experimental studies on animals have shown that exposure to ionizing radiation induces oxidative stress in different tissues. The interaction of ionizing radiation with the biological system results in the generation of reactive oxygen species (ROS) (Saada, et al. 2003; Azab, 2007; Ammar, 2009).

Similar to our study results (Soliman et al. 2007; Abdelhafez and Kandeal, 2018) postulated that liver of rat irradiated one and three days at 5 Gy gamma irradiation showed post-irradiation destructed areas, infiltration with inflammatory cells in the portal area and a number of necrotic cells with pyknotic and karyolitic nuclei. (Kafafy et al. 2001) found that the most remarkable effect after gamma irradiation is the loss of the normal configuration of liver cell strands radiating around the central vein in many areas. Severe damage to hepatic parenchyma and focal necrosis were seen 10 days post irradiation. (Saada et al. 2003) results suggested that the histological damage induced in the liver of irradiated rats was associated with an increase in the content of lipid peroxides and a decrease in the activity of the antioxidant enzymes SOD and catalase. In the present study, sections of the liver of irradiated rats showed different histological changes and loss of normal hepatic architecture. Where, the radiation effects began with dilated central vein with an obvious congestion, dilated hepatic sinusoids, loss of hepatocytes nuclei, vacuolated, degenerated cytoplasm and dilated hepatic portal vein and additional inflammatory cells (fibroblasts and lymphocytes) aggregated around the blood vessels and in the sinusoids. The same results were reported by (Zavodnik et al. 2003) and (Guryev, 2005). The present results concerning carbohydrate contents (glycogen) in

liver of rats exposed to gamma-irradiation revealed that most hepatocytes showed depletion in carbohydrate contents and few hepatocytes appeared nearly normal. The decrease of glycogen contents in liver induced hyperglycaemia. These observations are in agreement with those reported by earlier studies who found that radiation induced hyperglycaemia (El Maguid and El Gharib, 2004). So, the decrease in glycogen content in liver of irradiated rats of the present work induced hyperglycaemia in these animals. Our study results revealed more protective role for the olive leaf extract against the radiation apoptotic effects on hepatocytes. This may be due to that the active medical constituents of the olive leaf extract have antioxidant and anti-inflammatory properties (Visioli et al. 2002; Andreadou, et al. 2006; Ali, 2018; Abu-Amara and Meselhy, 2016)

Finally, our present study discovered, in group III, markedly significant structural hepatic amelioration and protection from the previously detected severe degenerative changes seen in group II. Furthermore, in that group there was an apparent improvement of liver architecture with some mild morphological changes like a mild dilatation of portal vein, a mild periportal mononuclear cellular infiltration, and a very few pervious and portal collagen fibres formation.

CONCLUSION

These results provide evidence that both olive leaf extract and stem cell therapy have radio protective effect as they reduced the pathological cellular injuries in the liver cells induced by accumulated doses of radiation exposure further studies may be done to confirm these findings.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

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AUTHOR CONTRIBUTIONS

All authors contributed to the research and/or preparation of the manuscript. Ali Hassan A. Ali, Obaid A M Alhajri and Alhaytham M Z Almuaddi participated in the study design and wrote the first draft of the manuscript. Abdulrahman Abdullah S Altamimi, Abdulhakim Alqahtani and Abdullah Ahmad A Twair collected and processed the samples. Abdullah Zaid J Alnefea and Nawaf Ali Saeed Alqahtani participated in the study design and performed the statistical analyses. All of the authors read and approved the final manuscript.

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