AMELIORATIVE EFFECT OF GINKGO BILOBA EXTRACT ON GAMMA RADIATION INJURY: HISTOLOGICAL AND HISTOCHEMICAL EVALUATION IN RATS

BY

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ABSTRACT

The present study aimed to evaluate the impact of exposure to ionizing radiation and the effect of pre-exposure treatment with extract of Ginkgo Biloba (EGB) on hepatic tissue architecture and hepatocyte cytoplasmic organelles and DNA. The study comprised of 30 Wistar-Albino rats (2.5 month old) separated into 3 equal groups (n=10). Group I: received EGB at a daily dose of 50-mg/kg by intraperitoneal injection for 8 weeks. Group II: received whole-body Gamma irradiation performed by a Cesium-137 ventilated Gamma Cell-40 at a dose rate 1 Gy/1.5 min.- once a week for 8 weeks and Group III: received EGB at a daily dose of 50-mg/kg by intraperitoneal injection for 2 weeks before and for 8 weeks during irradiation in a dose similar to Group II. Liver section in groupI showed polyhedral hepatocytes with no or mildly vacuolated cytoplasm, containing basophilic granules and central rounded vesicular nuclei and showed moderate PAS+ve reaction. Some hepatocytes contained more glycogen content than the others, by using Pritchard's technique and fat droplets appeared black in color by using Sudan black stain. Hepatocytes were studded with mitochondria, significant increase in DNA content with evident mitotic figures in some cells by using Feulgen reaction. In Group II, liver section showed that most of the hepatocytes were compacted and vacuolated with pyknotic nuclei and large area of cellular infiltration with significant decrease of the +ve PAS reaction and most of the hepatocytes lacked their glycogen content than the others with marked decrease in the fat content (Sudan black stain). Also liver section showed marked decrease in the mitochondrial content (Pritchard's technique) and marked decrease in the DNA content shown as faintly stained nuclei (Feulgen reaction). Most of the hepatocytes in Group III liver were in mitotic state, stages of division, polyploidy could be observed and the cytoplasm became condensed and granulated with significant increase of +ve PAS reaction and near normal black-stained fat droplets (Sudan black stain). By using Pritchard's technique, the liver of Group III showed apparent increase in the

mitochondrial content and Feulgen reaction identified increased DNA content which manifested as darkening of the nuclei and appearance of mitotic figures. It could be concluded that antioxidants EGB confers a beneficial radio-preventive effect against irradiation damage induced in rat liver and a significant prophylactic effect of EGB was observed when EGB was administered 2 weeks before irradiation and for 8 weeks during irradiation.

INTRODUCTION

The standardized extract of Ginkgo Biloba (EGB) has antioxidant properties as a free radical scavenger (Bridi et al., 2001). EGB, the standardized extract of Ginkgo biloba leaves is a well defined product and contains approximately 24% flavone glycosides (primarily quercetin, kaempferol and isorhamnetin) and 6% terpene lactones (2.8-3.4% ginkgolides A, B and C, and 2.6-3.2% bilobalide) (Kleijen & Knipschild, 1992). Ginkgolide B and bilobalide account for about 0.8% and 3% of the total extract, respectively. Other constituents include proanthocyanadins, glucose, rhamnose, organic acids, D-glucaric and ginkgolic acids (Pietri et al., 1997). EGB promotes vasodilation and improves blood flow through arteries, veins and capillaries. It inhibits platelet aggregation and prolongs bleeding time (Hu et al., 2002).

Oxygen free radicals are normally neutralized by very efficient systems in the body including antioxidant enzymes like super oxide dismutase (Aricioglu et al., 2001). In a healthy subject, there is a balance between free radicals and the levels of antioxidants. In some pathological conditions such as oxidative stress, the level of antioxidants is significantly reduced (Matkovics et al., 1997). Schindowski et al. (2001), studied changes of susceptibility to apoptotic cell death by oxidative stress in aging and its inhibition by the antioxidant Ginkgo Biloba extract EGB and found that apoptosis could be reduced in vitro by treatment with EGB.

Treatment with EGB has shown beneficial effects in reducing reperfusion injury, a classic model of injurious effect of accumulated free oxygen radicals. Akgül et al. (2008), investigated the effect of ginkgo biloba on testicular ischemia-reperfusion injury and found that malondialdehyde, nitrate and nitrite levels in testicular tissue were increased after unilateral testicular torsion and EGB-761 has a protective effect on testicular injury induced by ischemia-reperfusion. As another form of protective effect of ginkgo biloba against oxidative free radical injury, Pierre et al. (2008), reported that the standardized preparation of Ginkgo biloba EGB-761 totally protected adhesive properties and endothelial lipoperoxide levels. Moreover, it limited the decrease in Na, K-ATPase

activity induced by oxidized-LDL to levels similar to natural-LDL. This suggests that EGB-761 protects endothelial adhesive properties and helps prevent the disruption of ionic homeostasis. The EGB-761-mediated inhibition of ox-LDL-induced lipoperoxide levels in endothelial cells appears to be an important mechanism by which EGB protects endothelial properties.

The toxic effect of ionizing radiation is related to the ionization. Ionization of tissues, composed mainly of water, generates H_2O {sup+} and H_2O {sup-} ions, which in turn form H and OH free radicals and because oxygen free radicals are very reactive chemically, biological damage, such as attacks on DNA and proteins results (Carmon et al., 2000). The present study was designed to evaluate the impact of exposure to ionizing radiation and the effect of pre-exposure treatment with EGB-761 on hepatic tissue architecture and hepatocyte cytoplasmic organelles and DNA.

MATERIAL AND METHODS

The study was conducted in Departments of Forensic Medicine, Anatomy and Pathology, Faculty of Medicine, Benha University in conjunction with Radiation Oncology and Nuclear Medicine department, Tanta University. The study comprised 30 Wistar-Albino rats (2.5 month old, 200-250mg). Rats were kept under standard conditions, temperature, humidity and 12-hs day/night cycle, and maintained on standard diet and free water supply till the start of study regimens. Rats were gathered and separated into 3 equal groups (n=10):

1. Group I: received an intraperitoneal injection of EGB (Tanakan tablets; Amriya for Pharmaceutical Industries; Egypt; 40 mg pure extract/tab) at a dose of 50 mg/kg/day for 8 weeks (Louajri et al., 2001).

2. Group II: received whole-body Gamma irradiation performed by a Cesium-137 ventilated Gamma Cell-40 at a dose rate 1 Gy/1.5 min once a week for 8 weeks (Cameron et al., 1968).

3. Group III: received an intraperitoneal injection of EGB at a dose of 50 mg/kg/day for two weeks before and during the 8 weeks of irradiation (total duration 10 weeks since start of EGB administration). Irradiation was performed in a dose similar to Group II.

Animals of group one and two were sacrificed 8 weeks at the end of EGB administration or irradiation, respectively. Animals of Group III were sacrificed 10 weeks since start of prophylactic EGB administration; i.e. 8 weeks after irradiation. Samples were prepared for histological and histochemical examination under the light microscope.

Preparation of histological and histochemical samples:

Animals' sacrificing was followed by rapid dissection; a biopsy was taken from the liver, subjected to fixation in 10% formalin for at least 24 hrs, then washed and dehydrated in an ascending series of alcohols. Samples were cleared in terpiniol for 2 days, then embedded in paraffin wax, and prepared for sectioning under the usual microtome. Sections of the liver were stained with the following: Haematoxylin and Eosin stain, P.A.S reaction (Drury & Wallington, 1980), Feulgen reaction (Bancroft & Stevens, 1990), Prichard's technique (Malaty, 1972) and Sudan black stain (Bancroft & Stevens, 1990).

RESULTS

Examination of the liver sections of Group I animals showed that the hepatocytes appeared polyhedral in shape with no or mildly vacuolated cytoplasm, containing basophilic granules and central rounded vesicular nuclei. Some hepatocytes appeared to be bi-nucleated. The hepatocytes were arranged in the form of branching and anastomosing cords separated by dilated blood sinusoids and radiated from the central veins (Fig.1).

Most of the hepatocytes of Group II animals were compacted and vacuolated with pyknotic nuclei and large area of cellular infiltration (Fig. 2). However, most of the hepatocytes of Group III were in mitotic state, stages of division, polyploidy could be observed and the cytoplasm became condensed and granulated (Fig. 3). Other sections that cells retained their polyhedral shape, with apparent increase in the number of bi-nucleated hepatocytes as well as dilated sinusoids and fatty change of the cytoplasm and many of the cells lining the sinusoid had bulky nuclei (Fig. 4).

PAS stained liver section of Group I showed a moderate PAS+ve reaction and some hepatocytes contained more glycogen content than the others (Fig. 5). Hepatocytes of the liver of Group II showed significant decrease of the +ve PAS reaction. It could be observed that most of the hepatocytes lacked their glycogen content than the others (Fig 6). PAS stained liver section of Group III showed a significant increase of PAS+ve reaction (Fig. 7).

Pritchard's technique showed that hepatocytes of Group I were studied with mitochondria in the form of fine granules distributed throughout the cytoplasm (Fig. 8). Hepatocytes of Group II showed marked decrease in the mitochondrial content (Fig. 9), while showed apparent increase in the mitochondrial content in Group III (Figs. 10 & 11).

Feulgen reaction showed the nuclear DNA content of the hepatocytes and the cells lining the sinusoids. It appeared

magenta red in color. Hepatocytes of Group I showed significant increase in DNA content with evident mitotic figures in some cells (Fig. 12). Hepatocytes of Group II showed marked decrease in the DNA content in the form of faintly stained nuclei (Fig. 13). Hepatocytes of Group III animals showed that DNA content gradually increased (Figs. 14 & 15).

Sudan black stained sections showed that fat droplets in hepatocytes of Group I appeared black in color (Fig. 16), while hepatocytes of Group II showed marked decrease in the fat content (Fig. 17) but in Group III hepatocytes showed near normal black-stained fat droplets (Fig. 18).

DISCUSSION

EGB, an extract from green leaves of the Ginkgo biloba tree, is a natural antioxidant. The main ingredients of EGB contain 24% ginkgo-flavone glycosides and 6% terpenoids, (Pehlivan et al., 2002). It is well known for its cheap prices and negligible side effects. EGB has various biological activities and different pharmacologic effects, including antioxidation, anti-inflammatory and modulation of immune response (Kusmic et al., 2004). For its few side effects, EGB is extensively used in the therapy of central neural system disorders, acute pancreatitis, myocardial and intestinal ischemia / reperfusion injury which are associated with inflammatory mediators (Zeybek et al., 2003).

The present study showed that the accumulative dose of gamma radiation up to 1 Gy weekly for 8 weeks greatly affect hepatocytes, most of hepatocytes were compacted and vacuolated, the nuclei become pyknotic and large area of cellular infiltration could be detected. Decrease of glycogen content in the irradiated cells may be due to atrophy of the cell. The current study also showed that antioxidant supplementation before radiation exposure may prevent the expected cell damage induced by radiation as most of the hepatocytes were in mitiotic state and stages of division and polyploidy could observed with condensed and granulated cytoplasm. Also there was apparent increase in the number of binucleated hepatocytes as well as dilated sinusoids. Many of the cells lining the sinusoids had bulky nuclei.

These findings agreed with Mustafa et al. (2006) who evaluated the effects of EGB on the extent and severity of rat model of ulcerative colitis suggested that it may be effective in the treatment of ulcerative colitis through its scavenging effect on oxygen-derived free radicals. Sener et al. (2006) tried to determine the possible protective effects of EGB against oxidative organ damage induced by irradiation and reported that in irradiation groups,

glutathione levels were decreased significantly, while the malondialdehyde levels, myeloperoxidase activity and collagen content were significantly increased in the various studied tissues, however, in the EGB treated irradiation groups (IR), all of these oxidant responses were prevented significantly. Lactate dehydrogenase (an indicator of tissue damage) and tumour necrosis factor (TNF)-alpha levels were increased significantly following irradiation but were significantly decreased with EGB treatment. Sener et al. (2006) concluded that EGB, through its free radical scavenging and antioxidant properties, attenuates irradiation-induced oxidative organ injury, suggesting that EGB may have a potential benefit in enhancing the success of radiotherapy. The glycogen content is increased; this may due to the hypertrophy of the cells. DNA and fat contents increased. This increase is a dose dependent manner. Theses data are additional to the antioxidant properties of EGB reported in the literature and indicate a possible role for the extract in the treatment of diseases involving free radicals and oxidative damage. This may be due to the effectiveness of EGB extract on the enzyme activity. In accordance with these findings; Zhou et al. (2007) reported that the treatment of Ginkgo biloba extract significantly decreased the serum concentration of ALT and AST and the expressions of TNF-alpha and IL-1 in the hepatic tissue in EGB group and relieved the hepatocyte swelling and necrosis by inhibiting activation of Kupffer cells and regulate the cell factors to protect the liver.

Moreover, Yao et al. (2007) explored the hepatoprotective effect and underlying mechanism of EGB against ethanol-induced oxidative damage; results showed that EGB, especially at high dose, ameliorated ethanol-induced macrovesicular steatosis and parenchymatous degeneration in hepatocytes and decreased serum aminotransferases level. Furthermore, it reduced ethanol-derived glutathione depletion and lipid peroxidation and inhibited the inactivation of superoxide dismutase; glutathione peroxidase and catalase and importantly, it induced hepatic microsomal HO-1 on mRNA, protein expression and enzymatic activity, which is paralleled to the EGB-derived hepatoprotective effect. Dias et al. (2008) reported that in the short-term study, pretreatment of rats with 1000 ppm EGB significantly reduced the rates of cell proliferation, apoptosis and p53, TGF-alpha immunoreactivity and the number of glutathione S-transferase P-form-positive hepatocytes.

As regards the mitochondrial content, there was an apparent increase in the mitochondrial content 8 and 10 weeks after treatment compared to the irradiated; this may refer to the prolonged phase of regressive regeneration of the liver. These data go in hand with Welt et al. (2004)

who reported in experimentally induced diabetes and acute hypoxia model that the volume fraction of mitochondria was significantly increased after induction of diabetes but less increased in the EGB protected group and the volume density of mitochondrial cristae was significantly diminished in all diabetic groups; EGB could improve this parameter in the diabetic-hypoxic group. More recently, Yeh et al. (2009) investigated whether EGB could protect testes from Doxorubicin-induced testicular tissues oxidative stress and cell apoptosis in experimental rats and reported that EGB pretreatment effectively alleviated the doxorubicin-induced impaired spermatogenesis, depleted haploid germ cell subpopulations, increased lipid peroxidation products, depressed antioxidant enzyme activities, reduced antioxidant enzyme expression and elevated apoptotic indexes.

The present study reported that the nuclear DNA content of the hepatocytes and the cells lining the sinusoids using Feulgen reaction appeared magenta red in color and sections examined 8 weeks after irradiation showed a marked decrease in the DNA content. These findings was previously reported by Lee and Park (2003), who found that at lower doses of irradiation there will be no obvious injury but a number of the cells that survive will have incorrectly repaired the DNA damage so that they carry mutations, while at doses high enough, cells may be killed by damage to DNA and other parts of the cell to cause great injury to the body and even rapid death.

Treatment with EGB ameliorated the toxic effect of irradiation, where examination of liver section 8 and 10 weeks after irradiation and treatment with it showed that DNA content gradually increased. This protective effect agreed with Moreno et al. (2004) who assessed the effect of different concentrations of EGB on the labeling of blood constituents with Tc-99m and on the mobility of a plasmid DNA treated with stannous chloride at room temperature and reported that EGB decreased the labeling of RBC. The supercoiled form of the plasmid was modified by treatment with stannous chloride (SnCl2) and protected by 40 mg/ml EGB and attributed the effect of EGB on the tested systems to its chelating action with the stannous ions and/or pertechnetate or to the capability to generate reactive oxygen species that could oxidize the stannous ion.

It could be concluded that antioxidants EGB confers a beneficial radio-preventive effect against irradiation damage induced in rat liver and a significant prophylactic effect of EGB was observed when EGB was administered 2 weeks before irradiation and for 8 weeks during irradiation.

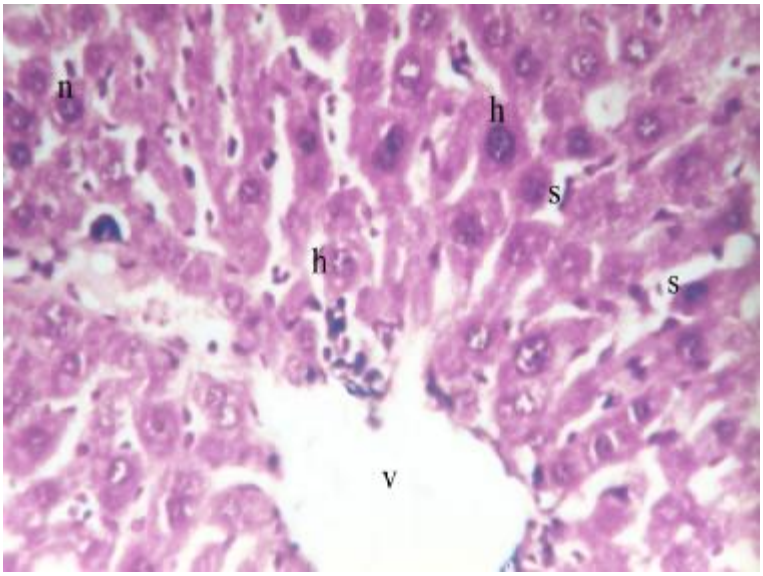

Fig. (1) : A light micrograph of a cross section in the liver of Group I (received EGB only for 8 wks) showed that the hepatocytes (h) appeared polyhedral in shape with no or mildly vacuolated cytoplasm, containing basophilic granules and central rounded vesicular nuclei. Some hepatocytes appeared to be bi-nucleated. The hepatocytes were arranged in the form of branching and anastomosing cords separated by dilated blood sinusoids (s) and radiated from the central veins (v). (Hx. & E. stain 200x)

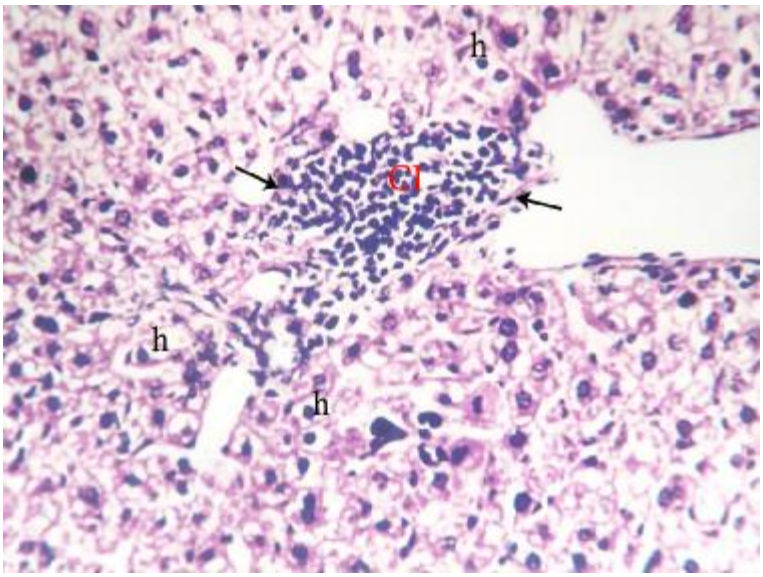

Fig. (2) : A Light micrograph of a cross section in the liver of Group II (received irradiation only for 8 wks) showing that most of the hepatocytes (h) were compacted and vacuolated with pyknotic nuclei and large area (arrowed) of cellular infiltration (CI).
(Hx. & E. stain 200x)

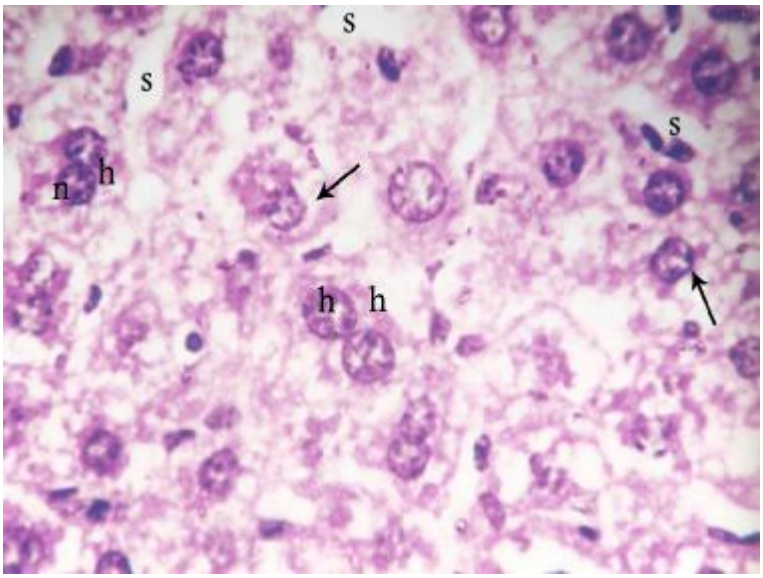

Fig. (3) : A Light micrograph of a cross section in the liver of Group III animals (received EGB 2-wks prior to and 8-wks during irradiation) showing that most of the hepatocytes were in mitotic (M) state, stages of division, polyploidy (P) could be observed and the chromatin (C) became condensed and the cytoplasm granulated (arrow).
(Hx. & E. stain 400x)

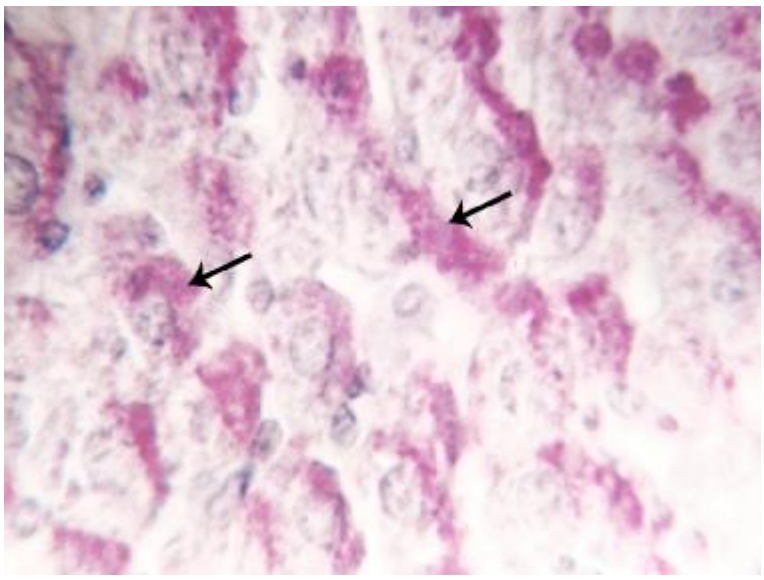

Fig. (4) : A Light micrograph of a cross section in the liver of Group III (received EGB 2-wks prior to and 8-wks during irradiation) showing that hepatocytes (h) retained their polyhedral shape, with apparent increase in the number of bi-nucleated hepatocytes as well as dilated sinusoids (s) and fatty change of the cytoplasm. Many of the cells lining the sinusoid had bulky nuclei (n). (Hx. & E. stain x400)

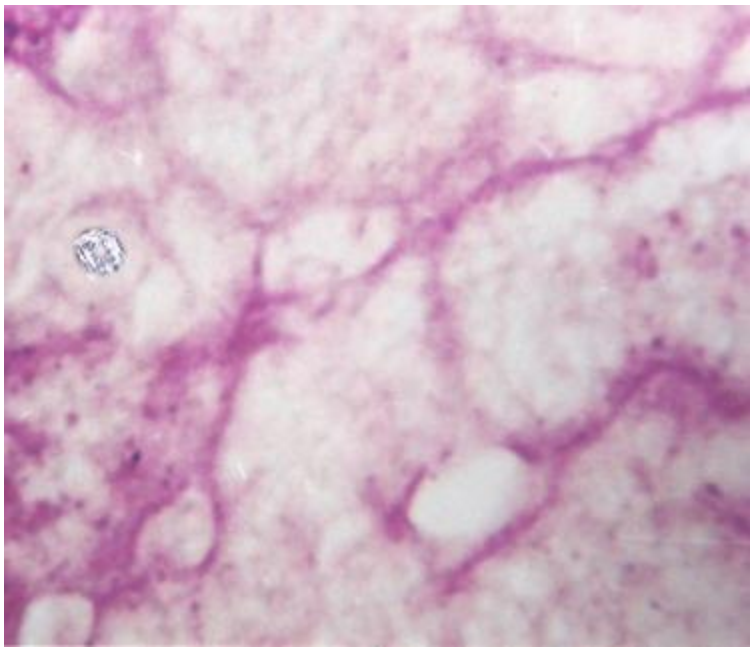

Fig. (5) : A Light micrograph of a cross section in liver of Group I (received EGB only for 8 wks) showing a moderate PAS +ve reaction. Some hepatocytes contained more glycogen (arrows) content than the others. (PAS stain x400)

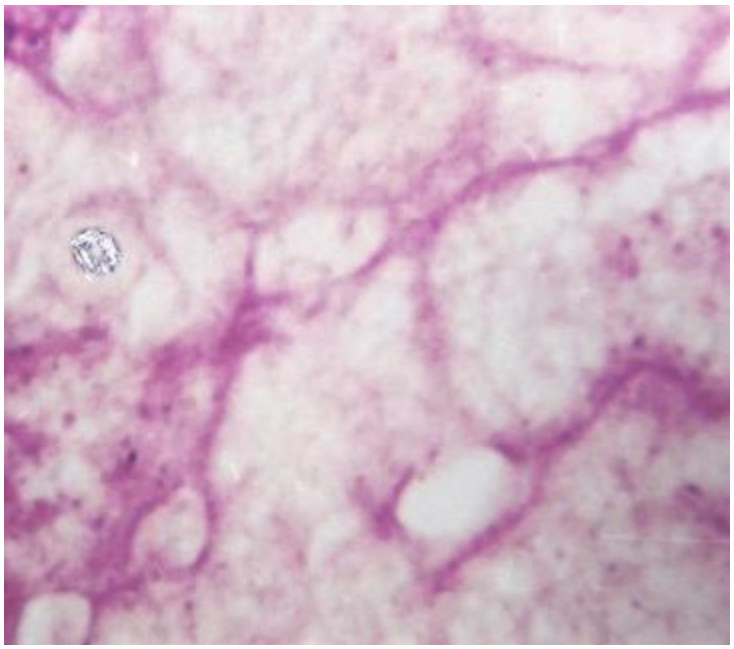

Fig. (6) : A Light micrograph of a cross section in the liver of Group II (received irradiation only for 8 wks) showing significant decrease of the +ve PAS reaction. It could be observed that most of the hepatocytes lacked their glycogen content than the others. (PAS stain x400)

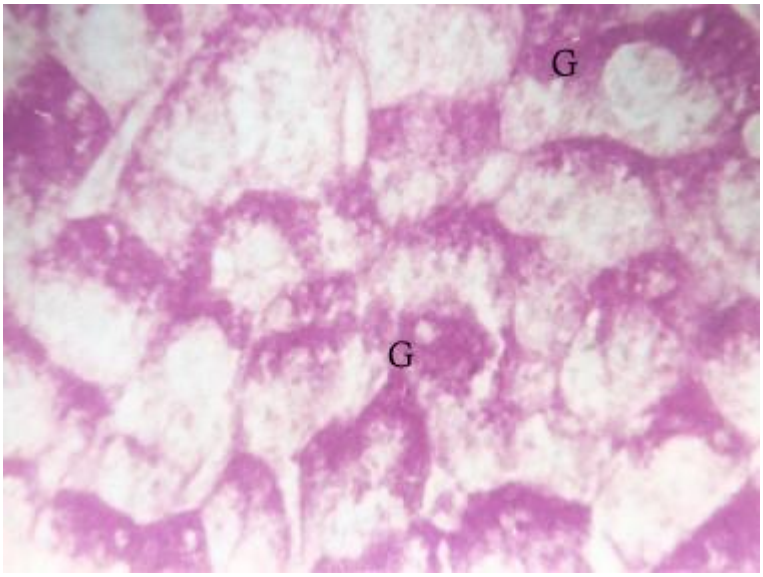

Fig. (7) : A Light micrograph of a cross section in the liver of Group III animals (received EGB 2-wks prior to and 8-wks during irradiation) showing significant increase of +ve PAS reaction indicating increased glycogen (G) content. (PAS stain x400)

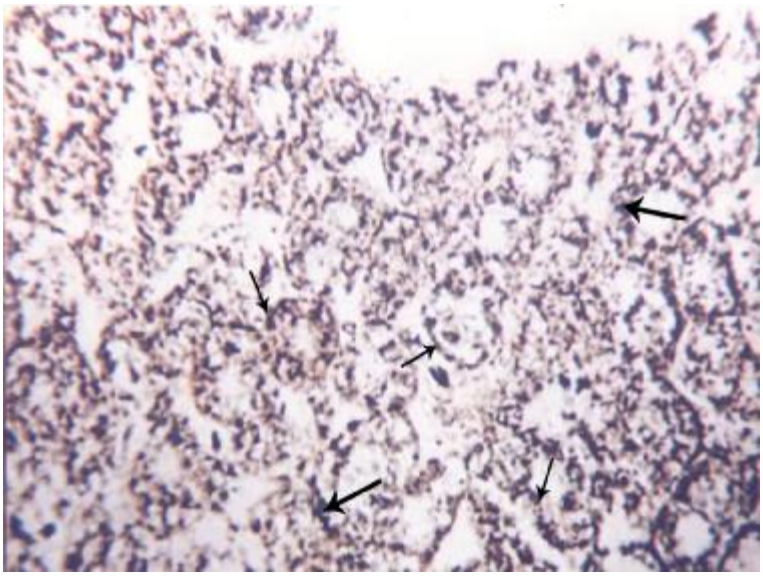

Fig. (8) : A Light micrograph of a cross section in the liver of Group I (received EGB only for 8 wks) showing fine granules of mitochondria distributed throughout the cytoplasm. Hepatocytes were studded with mitochondria (arrows). (Pritchard's technique x200)

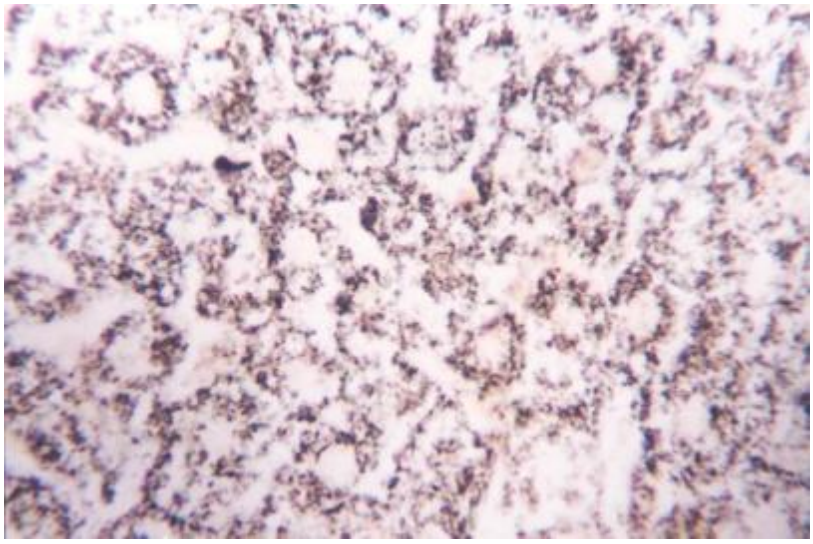

Fig. (9) : A Light micrograph of a cross section in the liver of Group II (received irradiation only for 8 wks) showed marked decrease in the mitochondrial content.
(Pritchard's technique x200)

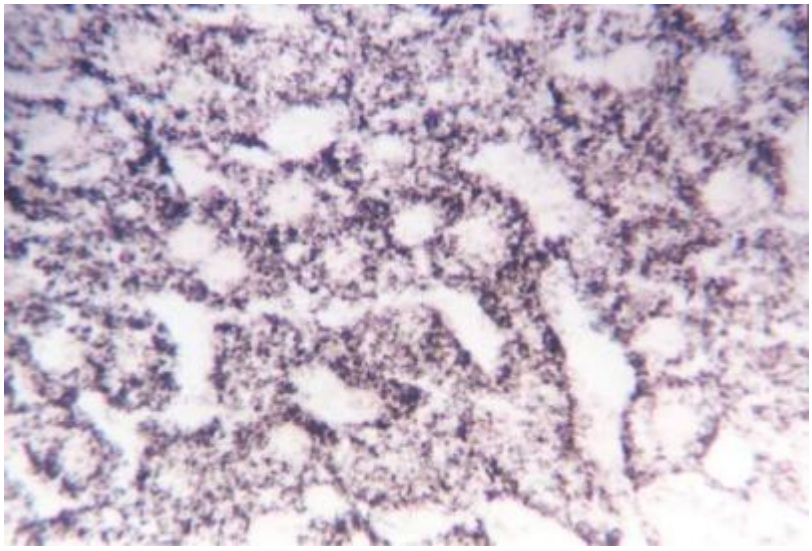

Fig. (10) : A Light micrograph of a cross section in the liver of Group III (received EGB 2-wks prior to and 8-wks during irradiation) showing apparent increase in the mitochondrial content. (Pritchard's technique x200)

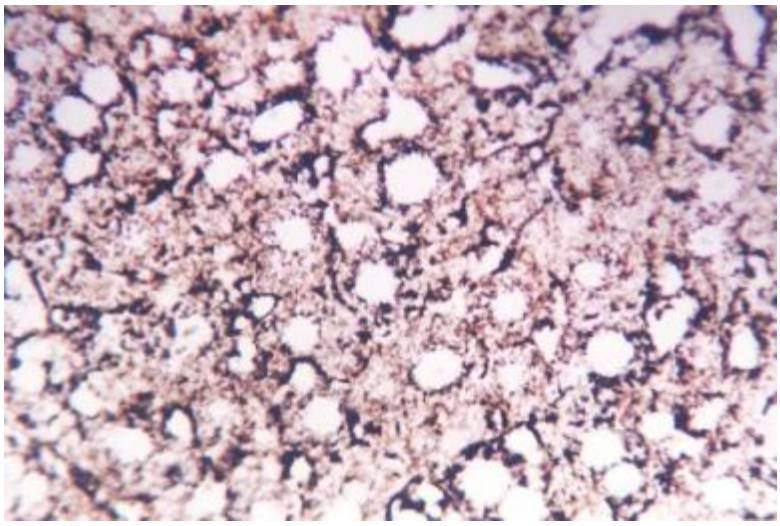

Fig. (11) : A Light micrograph of a cross section in the liver of Group III (received EGB 2-wks prior to and 8-wks during irradiation) showing apparent increase in the mitochondrial content. (Pritchard's technique x200)

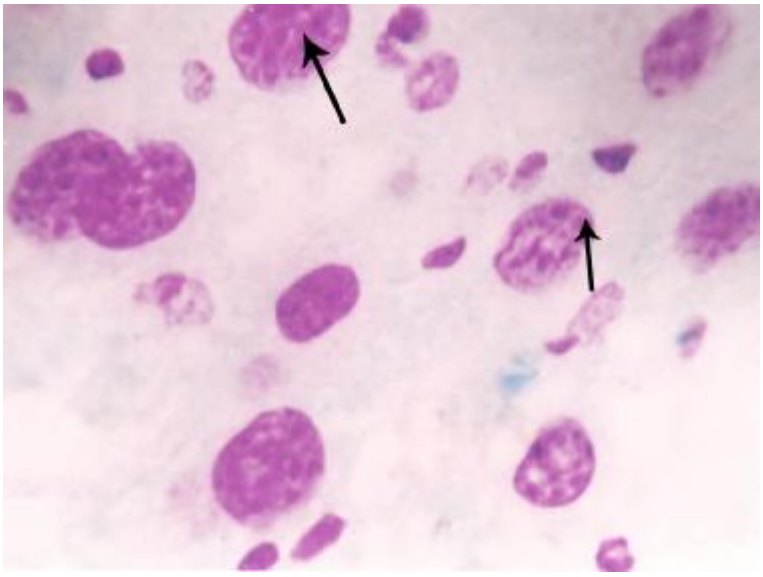

Fig. (12) : A Light micrograph of a cross section in the liver of Group I (received EGB only for 8 wks) showing significant increase in DNA content with evident mitotic figures (arrows) in some cells. (Feulgen reaction x400)

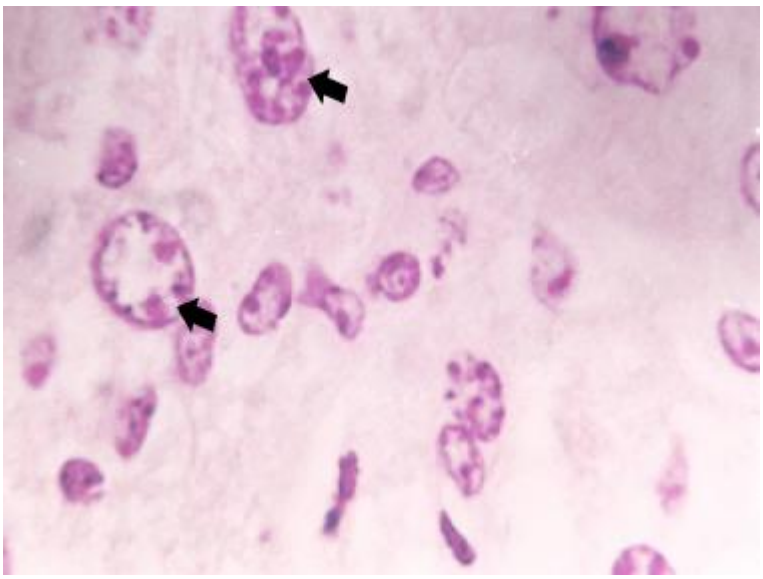

Fig. (13) : A Light micrograph of a cross section in the liver of Group II (received irradiation only for 8 wks) showing marked decrease in the DNA content in the form of faintly stained nuclei (arrows). (Feulgen reaction x400)

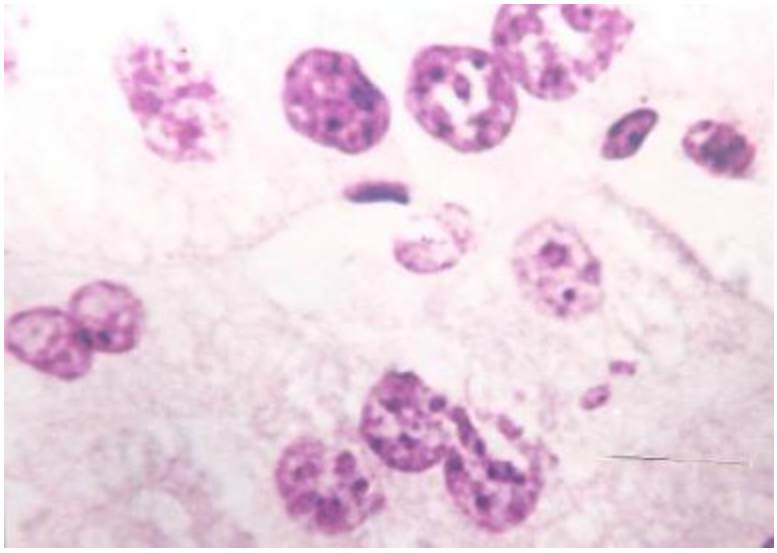

Fig. (14) : A Light micrograph of a cross section in the liver of Group III (received EGB 2-wks prior to and 8-wks during irradiation) showing that DNA content gradually increased manifested by darkening of the nuclei. (Feulgen reaction x400)

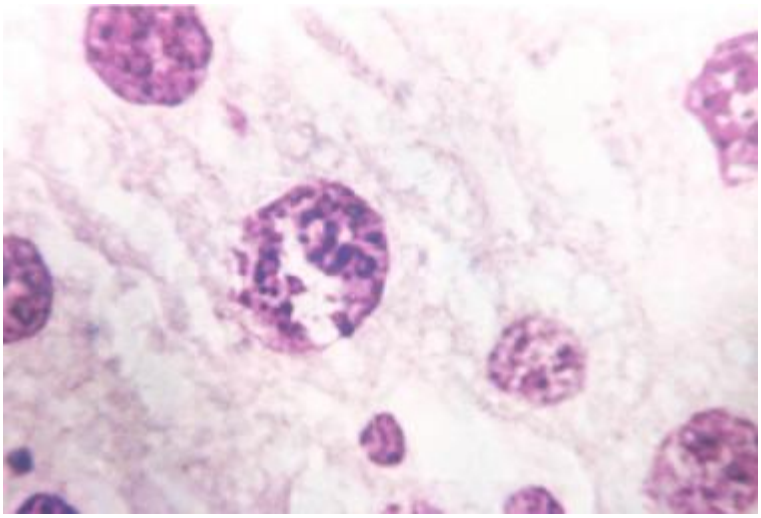

Fig. (15) : A Light micrograph of a cross section in the liver of Group III (received EGB 2-wks prior to and 8-wks during irradiation) showing increased DNA content manifested as darkening of the nuclei and appearance of mitotic figures. (Feulgen reaction x400)

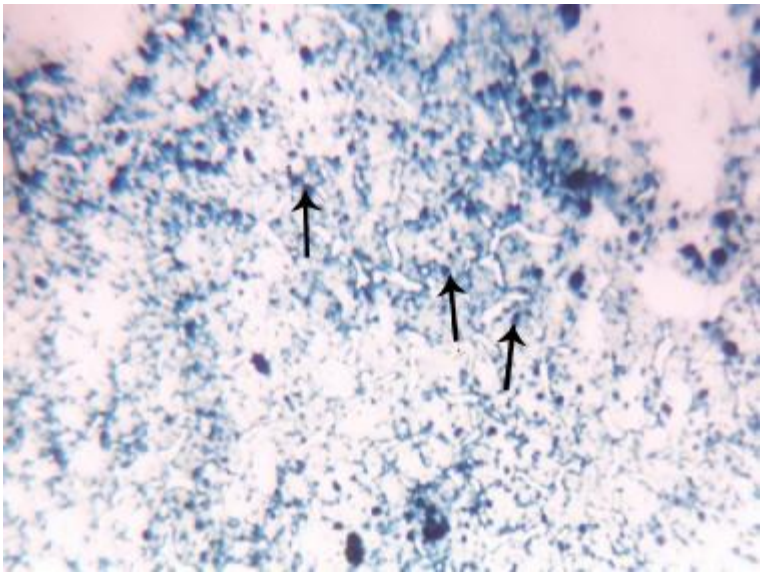

Fig. (16) : A Light micrograph of a cross section in the liver of Group I (received EGB only for 8 wks) showed that fat droplets in hepatocytes appeared black in color (arrows). (Sudan black stain x200).

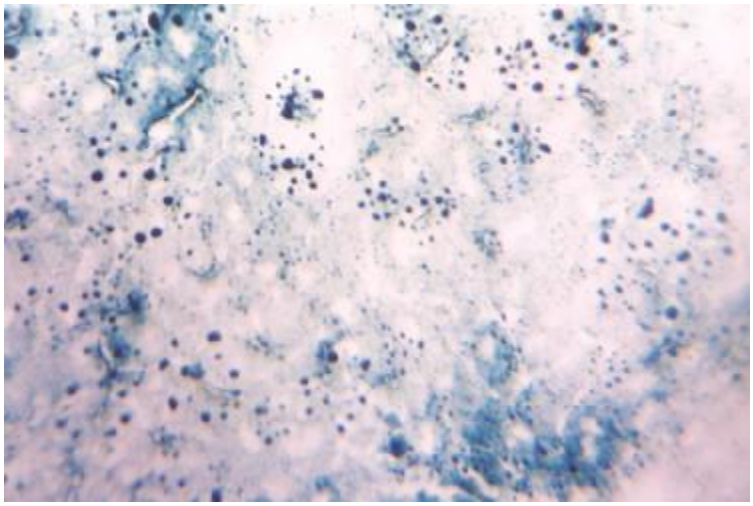

Fig. (17) : A Light micrograph of a cross section in the liver of Group II (received irradiation only for 8 wks) showing marked decrease in the fat content. (Sudan black stain x200)

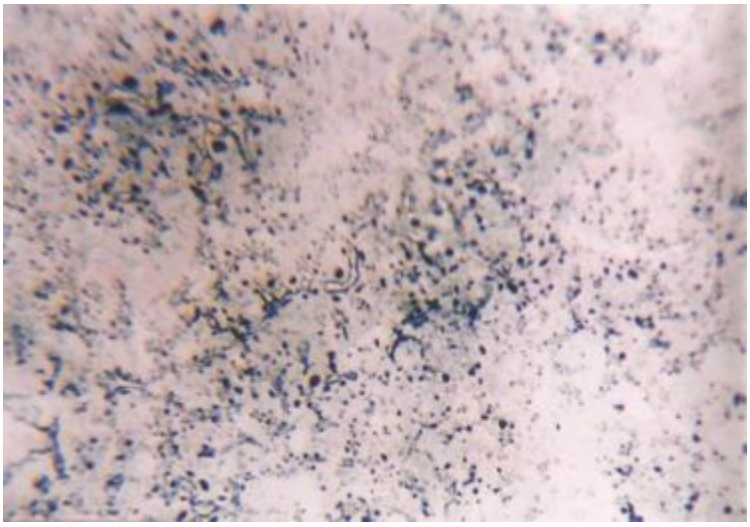

Fig. (18) : A Light micrograph of a cross section in the liver of Group III (received EGB 2-wks prior to and 8-wks during irradiation) showed near normal black-stained fat droplets. (Sudan black stain x200)

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تقييم تأثير خلاصة نبات الچينكوبيلوبا على الأضرار الناجمة عن أشعة جاما من خلال الفحص النسيجى والكيميائى النسيجى فى الجرذان

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تهدف هذه الدراسة لتقييم وتقدير أثر التعرض للإشعاع المؤين. فعندما تتأين الأنسجة يولد أكسجين حر نشط كيميائياً مسبباً ضرراً للأنسجة الحيوية، كما تهدف هذه الدراسة لتقييم أثر إعطاء علاج مستخرج من نبات الچينكوبيلوبا قبل التعرض للإشعاع وأثناء فترة التعرض وتأثير ذلك على أنسجة الكبد ومحتوياتها.

أجريت الدراسة على ثلاثون جرذ أبيض عمرها شهران ونصف وقسمت هذه الجرذان إلى ثلاث مجموعات كل مجموعة عبارة عن عشرة جرذان.

المجموعة الأولى : أعطيت الجرذان جرعة (50 كجم/كجم) من خلاصة نبات الچينكوبيلوبا بواسطة الحقن البيريتونى لمدة 8 أسابيع.

المجموعة الثانية : تعرضت الجرذان لأشعة جاما بمعدل جرعة (1Gy/1.5min) 1 چى لكل 1.5 دقيقة مرة واحدة إسبوعياً لمدة 8 أسابيع.

المجموعة الثالثة : أعطيت الجرذان بنفس الجرعة المعطاة للمجموعة الأولى وذلك لمدة إسبوعين قبل إعطاء المادة المشعة وكذلك لمدة 8 أسابيع التى أعطيت الجرذان فيها المادة المشعة بنفس الجرعة التى أعطيت للمجموعة الثانية.

- تم ذبح جرذان المجموعة الأولى والثانية بعد 8 أسابيع أما جرذان المجموعة الثالثة فقد تم ذبحها بعد 10 أسابيع من إعطاء الجرعة والإشعاع.
- هذا وقد أظهرت نتائج تلك الدراسة أن إعطاء مادة الچينكوبيلوبا قبل وأثناء العلاج بأشعة جاما أحدث تحسناً ملحوظاً فى أنسجة الكبد وذلك على عكس الأثير السلبى الذى ظهر فى جرذان المجموعة الثانية التى أعطيت المادة المشعة فقط، ويمكن الاستنتاج بأن المواد المضادة للأكسدة والچينكوبيلوبا لها أثر وقائى ضد العلاج بالإشعاع والأضرار التى يسببها فى الكبد وخاصة عندما تعطى قبل التعرض للإشعاع وأثناء فترة التعرض له.