

## Ameliorative Effect of Two Antioxidants on The Liver of Male Albino Rats Exposed to Electromagnetic Field

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### ABSTRACT

**Aim of the work-**This study aimed to determine the ameliorative effect of silymarin (SIL) and vitamin E (Vit.E) against changes induced by mobile phone radiation in the liver of male albino rats. **Material and methods-**Total of 48 adult male albino rats were assigned for this study. The 1<sup>st</sup> group served as control (n=6); the 2<sup>nd</sup> group exposed to mobile phone generator radiation (900MHz) for 2hr/day 3days/week for two months, 3<sup>rd</sup> group (+ve control) supplemented with SIL, 4<sup>th</sup> group (+ve control) supplemented with Vit.E, 5<sup>th</sup> group (+ve control) supplemented with SIL and Vit.E, 6<sup>th</sup> group: exposed group supplemented with SIL, 7<sup>th</sup> group : exposed group supplemented with Vit.E and 8<sup>th</sup> group exposed group supplemented with SIL and Vit.E. Physiological, histopathological and histochemical changes were studied. **Results-** Exposure to mobile phone causes reduction in RBCs, Hb, Hct, MCV, MCH and MCHC. However, WBCs count, platelets count, lymphocytes % and neutrophil % were increased. Also, there were increases in liver enzyme activities ALAT, ASAT and ALP in serum and liver tissue significantly and increased oxidative stress markers (MDA and H<sub>2</sub>O<sub>2</sub>). While, antioxidants (CAT and GSH) were decreased in serum and liver tissue. Numerous histopathological changes were detected in the liver tissue of rats of the irradiated group with altered collagen fibres, polysaccharides and total protein in hepatocytes of the central and portal areas of the liver tissue in the exposed group. These changes manifested good amelioration in the exposed groups that supplemented with SIL and/or Vit.E.

**Conclusion-** Treatment of rats with SIL and/or Vit.E ameliorated the dangerous effect of mobile phone radiation.

**Key words:** Mobile phone radiation - Albino rats - Liver -Silymarin - vitamin E.

### INTRODUCTION

The evolution of mobile phones is one of the fastest technology in the history of innovation.<sup>1</sup> The number of mobile phone users is constantly increasing. Mobile phone becomes a frequent source of contamination of the human environment by producing non-ionizing radiation (NIR).<sup>2</sup> This phenomenon has raised concerns about the possible hazards of the electromagnetic field radiation emitted by mobile phones which can affect people's health.<sup>3,4</sup> The close proximity of the antenna of this device to the abdominal organ's when carried on the belt has raised concerns about the biological interactions between electromagnetic radiation and internal organs.<sup>5,6</sup> Mobile phones are mainly placed in the front or side pockets (close to the liver); the mobile phone emitting 900 MHz electromagnetic radiation which may be absorbed by various body organs according to the places where they are carried.<sup>7</sup> It is possible to say that the deleterious effects of electromagnetic microwaves are generally exerted through elevation of body temperature<sup>8</sup>, creation of free radicals<sup>9</sup> and disruption of oxidant/antioxidant balance in various tissues

exposed to EMF that has been shown in the experimental studies.<sup>10,6</sup>

Liver was used in this study because it's high iron content. This makes the liver more susceptible to the effects of the magnetic fields.<sup>11</sup>

Recent scientific studies have been focusing on the use of plant products as therapeutic agents.<sup>12,13</sup>

El Banna *et al.*<sup>14</sup> demonstrated that extract of *Silybium marianum* have a significant hepatoprotective and antioxidant activity and may be useful for patients who suffer from liver diseases, as it increase the activities of antioxidant enzyme SOD, CAT activities and GSH level. The protective effect of this extract may be attributed to the presence of flavonoid compounds and their antioxidant effects and free radical scavenging properties.<sup>15,13</sup> Silymarin (SIL) effects have also been indicated in various illnesses of different organs such as prostate, lung, kidneys, pancreas and skin.<sup>16</sup>

Vitamin E (Vit.E) is an important lipid-soluble antioxidant. It functions through the glutathione peroxidase pathway protecting cell membranes from oxidation by reacting with

lipid radicals, there by preventing lipid oxidation (LPO) and the initiation of oxidative tissue damage.<sup>4,17</sup>

### **Material and methods**

#### **Experimental design**

A total of 48 male albino rats about 8-10 weeks old and weighing 110–120 grams. They were maintained on standard normal diet and water *ad libitum*, The animals were housed in clean cages and maintained under controlled conditions of temperature ( $25 \pm 1.5^\circ\text{C}$ ), light (12 hours light: 12 hours dark) and good ventilation.

#### **Exposure system and application of electromagnetic field**

Albino rats (n=48) were divided into eight groups (n=6). G1- the control group; G2- EMF group: rats exposed to 900 MHz (2hrs/day, 3times/week) for 2 months. G3- positive control received SIL only 70mg/kg b.wt. G4- positive control received Vit. E only (60mg/kg b.wt. G5- positive control received SIL and Vit. E, G 6- irradiated group plus SIL. G7- irradiated group plus Vit.E. G8- irradiated group plus SIL and Vit.E.

A specially designated electromagnetic field (EMF) exposure system, with a round plastic tube cage and a dipole exposure antenna was used and it produced EMF equals to mobile phone radiation with frequency equals 900 MHz<sup>18</sup>, with a specific absorption rate (SAR) equals 1.2 w/kg and constant power about 1.4 mW/cm (Holiday Industries Inc., UK).

#### **Chemicals**

Vitamin E is used as alpha-tocopherol acetate. It is purchased from Sigma Company, dissolved in corn oil and given orally at a dose level of 60 mg/kg. b.wt dissolved in corn oil.<sup>19</sup>

Silymarin purchased from South-Egypt Drug Industries Company (SEDICO) was given orally in a dose of 70 mg/kg.b.wt.<sup>20</sup> dissolved in distilled water all over the experimental period by gastric tube

#### **Preparation of samples and biochemical analysis:**

Animals of all groups were sacrificed at the end of the experiment. Blood samples were put in tubes containing ethylene diamminetetraacetic acid (EDTA) as anticoagulant for haematological parameters. RBCs, Hb, WBCs, Hct, PLTs and blood indices (MCV, MCH and MCHC) were carried out using an automated 18-parameter

hematology analyzer (ABX Micros 60, Horiba ABX, France) and serum obtained by centrifugation at 3000 rpm for 10 min. For the assessment of liver enzymes, ALAT and ASAT were measured<sup>21</sup> and ALP<sup>22</sup> in serum and liver tissue. Antioxidants such as catalase (CAT) enzyme<sup>23</sup> and glutathione (GSH) were detected.<sup>24</sup> Oxidative stress marker, lipid peroxidation was evaluated by measuring malondialdehyde (MDA)<sup>25</sup> and hydrogen peroxide ( $\text{H}_2\text{O}_2$ )<sup>26</sup> were assayed in serum and liver tissue.

#### **Histological and histochemical techniques**

The liver was immediately excised and fixed in 10% neutral formalin. Paraffin sections (5µm in thickness) were prepared for processing the histological and histochemical studies. For general histology, sections were stained with Harris' haematoxylin and eosin.<sup>27</sup> Total proteins were detected by using mercuric bromophenol blue method<sup>28</sup> polysaccharides were detected by using periodic acid Schiff's (PAS) reagent<sup>29</sup> and collagen fibres were stained by using Mallory's trichrome stain.<sup>30</sup>

#### **Statistical analyses**

Data were represented as means, standard error of mean (SEM) percentage of change. Significant differences between the mean values were statistically analyzed using simple one way analysis of variance (**SPSS program, version 16, Duncan's multiple range test**) for the significant interrelation between the various groups.<sup>31</sup>

### **RESULTS**

Table (1) showed that SIL and/or Vit.E treated groups had normal RBCs, Hb, Hct, MCV, MCH and MCHC when compared to the normal control group ( $P > 0.05$ ). It was noticed that RBCs, Hb, Hct, MCV, MCH and MCHC decreased significantly ( $P < 0.05$ ) in rats exposed to EMF for two months in comparison with the control group. However, these parameters increased in the irradiated group supplemented with SIL and/or Vit.E treated groups when compared to the irradiated group.

Table (2) showed that SIL and/or Vit.E didn't affect WBCs count, lymphocyte percent, neutrophil percent and platelets count when compared to normal control group ( $P > 0.05$ ). It was noticed that WBCs, lymphocyte, neutrophil and platelets increased significantly ( $P < 0.05$ ) in rats exposed to EMF for two months in comparison with normal control group. However, these parameters decreased in the irradiated group supplemented with SIL and/or Vit.E

treated groups compared to the irradiated group with significant difference ( $P < 0.01$ ).

Tables (3 and 4) showed that SIL and/or Vit.E didn't affect ALAT, ASAT and ALP activities in serum and liver tissue when compared to the normal control group. ALAT, ASAT and ALP activities increased significantly ( $P < 0.05$ ) in rats exposed to EMF for two months in comparison with normal control group. However, ALAT, ASAT and ALP activities decreased in irradiated group supplemented with SIL and/or Vit.E in comparison with that obtained in the irradiated group. Serum ALAT activities in the irradiated rats treated with antioxidants still had higher activities than that in the normal control group.

Tables (5 and 6) showed that SIL and/or Vit.E didn't affect the CAT enzyme activities of GSH, MDA and  $H_2O_2$  levels in serum and liver tissue. The CAT activity and GSH levels decreased significantly ( $P < 0.05$ ) in rats exposed to EMF for two months in comparison with normal control group. Whereas, CAT activity and GSH levels in serum and liver tissue increased in irradiated group supplemented with SIL and/or Vit.E in comparison with the irradiated group. While, MDA and  $H_2O_2$  increased in serum and liver tissue of rats that exposed to EMF when compared to the control group. Administration of SIL and/or Vit.E to the exposed rats showed good amelioration.

#### **The histopathological changes**

Several histopathological changes were observed in liver tissue of the irradiated; these changes include: corrugated and ruptured endothelial lining of the central vein which contained haemolysed blood cells, nearly all nuclei of the hepatocyte showed pyknosis (Figure 3). The portal area showed highly dilated and congested hepatic portal vein, thickened arterial wall and malformed bile ducts with some vacuolated hepatocytes of irradiated group (R) (Figure 4). Somewhat normal appearance of liver tissue of the exposed group that supplemented with SIL (RS) (Figures 5,6). Somewhat normal appearance of the exposed group supplemented with Vit.E (RE) but, the portal area contained few aggregation of lymphocytes (Figures 7,8). Somewhat normal appearance of the central and portal areas of the liver tissue of the exposed group supplemented SIL and Vit.E (RSE) (Figures 9, 10).

**Collagen fibres:** normal distribution of collagen fibres was observed in liver tissue of a rat of the control group (Figure 11). Increased collagen fibres were realized inside the highly dilated hepatic portal vein and in the detached endothelial lining walls of the bile ducts and arterial walls; some arterial walls were fibrotic with numerous scattered collagen fibers in between hepatocytes of group R (Figure 12). Decreased collagen fibres were noticed in the liver tissue of rats of group RS (Figures 13,14) and in groups RE and RSE (Figures 15,16).

**Polysaccharides :** normal distribution of PAS +ve materials is detected in liver tissue of a rat of the control group (Figure 17). Highly decreased polysaccharides were realized in hepatocytes of the central and portal areas, but they are increased in walls of the hepatic portal veins, bile ducts and arterial walls of the portal area and in the haemolysed RBCs inside the hepatic portal vein of the liver tissue of R group (Figures 18,19). Reduced PAS +ve materials were demonstrated in some hepatocytes of liver tissue of rats of groups RS, RE and RSE respectively (Figures 20,21,22).

**Total proteins:** normal distribution of total proteins in the central and portal areas of liver tissue of a rat of the control group was detected in Figure (23). Reduced total protein was noticed in most hepatocytes of group R, but they increased in the thickened walls of the blood vessels and bile ducts with mild staining affinity in the hemolysed blood cells (Figure 24, 25). Somewhat normal distribution of total proteins was demonstrated in liver tissue of rats of groups RS, RE (Figures 26, 27) and RSE (Figures 28, 29).

## **DISCUSSION**

### **Complete blood count**

In the present study, there were decreases in RBCs count, Hb, and Hct, RBCs indices (MCV, MCH and MCHC) upon exposure of rats to 900MHz for 2 months. The depletion in the values of hematological parameters following EMF radiation exposure may be attributed to direct damage caused by radiation and due to overproduction of ROS by microwave radiation interaction.

It has also been reported that the production of free radicals cause hemolysis. <sup>32</sup> **Abdel-Rassoul *et al.***<sup>33</sup> agreed with that information on the potential effects of RF radiations especially effects such as anemia that can arise as a result of long term exposure to non-ionizing

radiations from mobile phone base stations. The combined effects of free radicals on the red blood cell membrane and cytoskeleton may contribute to the leak of hemoglobin out of the cells. The hemolysis of the red blood cells reflects the loss of integrity of the cells which can lead to the liberation of intracellular hemoglobin. In addition, radiation was reported to cause oxidation of the sulphhydryl groups and induce conformational changes of membrane proteins.<sup>34</sup> The depletion in the values of hematological parameters following EMF radiation exposure may be attributed to (a) direct damage caused by lethal dose of radiation (b) due to overproduction of ROS by EMF interactions.<sup>35</sup>

In the present study Supplementations of the exposed group with SIL and/or Vit.E ameliorated the changes in the Hb, RBCs count, Hct and RBCs indices.

Improvements in RBCs, Hb, Hct and WBCs in the exposed group supplemented with SIL is in a greement with the work which was done by **El-Gabry et al.**<sup>36</sup>. They found that whole body  $\gamma$ -irradiated female rats with two doses 1 Gy and 6 Gy for one week induced significant declines in RBCs, Hb and Hct. SIL manifested good amelioration in the radiation-induced changes in the studied parameters when orally administered (10 mg /100g b.wt) twice daily.

Silymarin and silibinin exert antioxidant activity and support redox homeostasis in several *in vitro* and *in vivo* models. **Kiruthiga et al.**<sup>37</sup> have shown that administration of SIL increased the activities of antioxidant enzymes like SOD, CAT, GPx, GR and GST together with decreased levels of MDA in erythrocytes exposed to H<sub>2</sub>O<sub>2</sub>.<sup>38</sup> The present results showed that serum H<sub>2</sub>O<sub>2</sub> was increased (47%) in the irradiated group. This result may support the hemolysis of RBCs.

#### **White blood cells count and platelets**

In the present study, there was an elevation in WBCs count , lymphocyte % and neutrophil %.

The increase in lymphocytes may be due to the harmful action of EMF exposure that stimulates the hematopoietic system to release more lymphocytes causing an increase in their number in the blood stream.<sup>39</sup> **Hsu et al.**<sup>40</sup> demonstrated that radiation exposure was a significant risk factor for elevated WBCs and differential WBC counts over time. A significant increase of WBCs count among survivors with radiation dose 2 Gy was

detected in men. Elevated WBC count might reflect inflammation.

**Horvath et al.**<sup>41</sup> showed that both silybinin and Vit. E synergistically restored the lymphocyte proliferation, cytokine activity, total free radical scavenger capacity in partially hepatectomized rats. They concluded that preoperative treatment with silibinin and/or Vit. E modulated the cellular immunoresponse. Vit.E could enhance immunity by maintaining the functional and structural integrity of important immune cells.<sup>42</sup>

In the present study, there was an elevation in platelets (PLTs) count. Increased megakaryocytes in irradiated rats were detected by **Abd Rabou et al.**<sup>43</sup> Their increase may lead to increased blood platelets which were observed in the present study.

In the present investigation supplementation of the exposed group with SIL and/or Vit.E ameliorated the increases in WBCs count, lymphocyte and neutrophil percent and platelets count.

**Psotova et al.**<sup>44</sup> demonstrated that SIL increased the lag time of hemolysis and stabilized the cell membrane by reducing the rate of glutathione loss in erythrocytes. It also decreased the concentration of peroxy radicals derived from Amidinopropane (AAPH) as a chain breaking antioxidant and radical scavenger.<sup>45</sup> Additionally, SIL significantly reduced PLTs activation (adhesion and aggregation) in rats.<sup>46</sup>

Supplementation of Vit.E (60mg/kg) to the exposed group showed amelioration of the WBCs count, lymphocyte percent and PLTs count. Changes in hematological parameters may be attributed to increased of free radicals produced by EMF interactions, while the antioxidants SIL and Vit.E have suppressed these harmful effects by scavenging some of the free radicals.

#### **Liver**

Liver is a very important organ for the healthy and lasting life of mammals. EMF induced liberation of free radicals and oxygen species can induce liver disease and the main organ of detoxification. Another reason for selection of liver was its sensitivity to waste products.<sup>47</sup>

The present findings showed increased activity of liver enzyme ALAT, ASAT and ALP in serum and liver tissue homogenate of the exposed group. While, the activities of these enzymes were reduced after the treatment of the exposed groups with SIL and/or Vit.E.

However, results of the present study were in disagreement with those described by **Achudume *et al.***<sup>48</sup> who stated that exposure of male wister rats to EMF base station 900 MHz for 60 days continuously causes significantly decreases in ALAT and ASAT activities. They attributed the decreased activities of both transferases to liver parenchymal injury as a result of radiation emitted by base station, indicating liver dysfunction.

In this respect, **Ramadan *et al.***<sup>20</sup> stated the protective effect of silymarin was attributed to its antioxidant and free radicals scavenging properties.<sup>49</sup> Silymarin prevents liver damage by maintaining the integrity of the plasma membrane, thereby suppressing the leakage of enzymes.<sup>50</sup> Silymarin appears to act as an antioxidant not only because it acts as a scavenger of the free radicals that induce lipid peroxidation, but also because it influences enzyme systems associated with glutathione and superoxide dismutase.<sup>51</sup> Also, SIL suppressed the apoptosis in hepatocytes<sup>52</sup> as shown in the present study.

Silymarin can alleviate hepatocyte membrane and thus prevent the xenobiotics from going into the cell via enterohepatic circulation. Silymarin can slightly bind to the iron and inhibit human hepatocyte glutathione reduction.<sup>53</sup> Silymarin can enter inside the nucleus and act on RNA polymerase enzymes resulting in increased ribosomal formation. This in turn hastens protein and DNA synthesis.<sup>12</sup> This action has important therapeutic implications in the repair of damaged hepatocytes and restoration of normal functions of liver.<sup>54</sup>

**Dindic *et al.***<sup>2</sup> noticed that the activity of ALAT and MDA concentration in serum and liver tissue were significantly higher in the exposed rats. Increased ALAT activity indicated cytotoxic effect of non-ionizing radiation on hepatocytes, inducing apoptosis and necrosis. An elevated oxyradical generation and subsequent cell membrane disruptions were reported to be the reasons for electromagnetic field-induced cell damage.

The present study investigated a significant decrease in serum CAT activity and GSH. However, MDA and H<sub>2</sub>O<sub>2</sub> were increased in serum and liver tissue homogenate of rats of the exposed group. Supplementation of SIL and/or Vit.E showed good amelioration in the levels of these parameters.

SIL has been reported to maintain the GSH homeostasis. This might be the reason for

elevated glutathione levels observed during SIL treatment.<sup>47</sup> Also, with the work which was done by **El-Gabry *et al.***<sup>36</sup> who found that whole body  $\gamma$ -irradiated female rats with two doses 1 Gy and 6 Gy for one week showed higher levels of plasma MDA, reduced levels of blood glutathione (GSH). While, SIL administration (10 mg / 100 g b. wt) twice daily manifested good amelioration in the radiation-induced changes in MDA and GSH levels. Results of the present study were supported by a work done by **Koc *et al.***<sup>55</sup> who observed that MDA content was increased and CAT activity was decreased in testis tissue of rats which were exposed to EMR emitted by a mobile phone during calling for 10 min every 1 h for 8 h each day for one month. Antioxidants were given daily as Vit.E (50 mg/kg intramuscularly) and vitamin C (20 mg/kg, intraperitoneally) to the exposed rats, these antioxidants ameliorated the previous changes.

Results of the present study come in agreement with the result of **Abd El Rahman *et al.***<sup>56</sup> who reported that SIL, Vit.E and their co-administration in rats exposed to EMF (900 MHz) showed increase in oxidative stress identified by increases in serum MDA and thiobarbituric acid reactive substances (TBARS), associated with decreases in SOD, CAT, GSH-Px activities and GSH content.

Silymarin and silibinin exert antioxidant activity and support redox homeostasis either *in vitro* or *in vivo* models. **Kiruthiga *et al.***<sup>37</sup> have shown that administration of SIL increased the activities of antioxidant enzyme like SOD, CAT and GPx together with a decrease in the level of MDA marker for lipid peroxidation. In erythrocytes exposed to H<sub>2</sub>O<sub>2</sub> and SIL, they reduced GSH depletion and ROS production.

#### **The histopathological changes**

The present study showed corrugated and ruptured endothelial lining of the central vein which contained haemolysed blood cells, nearly all nuclei of the hepatocyte showed pyknosis. Highly dilated and congested hepatic portal vein, thickened arterial wall and malformed bile ducts with some vacuolated hepatocytes in the exposed group. Administration of SIL and/or Vit.E manifested good amelioration in these changes.

The present findings come in agreement with those of **Eid *et al.***<sup>57</sup> who revealed that newly born mice exposed to RF-EMF from mobile

phone (45min/day) for one month liver showed dilated and congested blood sinusoids with prominent Kupffer cells, debris of degenerated cytoplasmic organoids with pyknotic and karyolotic nuclei, some haemorrhagic areas, dilated and congested hepatic portal vein, hemolyzed blood cells and lymphocytic infiltration. **Topali et al.**<sup>6</sup> revealed marked hydropic degeneration in the parenchyma, particularly in pericentral regions, vacuolization in the mitochondria, expansion in the endoplasmic reticulum and necrotic hepatocytes in pups of pregnant rats that exposed to 900MHz for 1h daily during days 13–20 of pregnancy. **Mohamed**<sup>58</sup> revealed that SIL and/or Vit.E showed anti-damaging effect in lung tissue of rats that exposed to mobile phone radiation for 2 months. The present study come in agreement with the work carried by **Nassar et al.**<sup>59</sup> who demonstrated the radioprotective role of SIL in the hepatic tissue of rats by administration of SIL by oral gavage, at a dose of 70mg/kg, one hour before  $\gamma$ -irradiation (5Gy). The histological examination of liver sections of  $\gamma$ -irradiated rats resulted in many pathological criteria such as hydropic degeneration, cytoplasmic vacuolation, pyknosis, karyolysis, nucleoli disappearance, necrosis, leucocytic infiltration and liver cell degeneration. SIL treatment prior to  $\gamma$ -irradiation succeeded to minimize the deleterious effects of  $\gamma$ -irradiation in the hepatic tissue and supported the liver to initiate a phase of recovery, regeneration and tissue healing. **Mohamed**<sup>58</sup> revealed that SIL and/or Vit.E showed anti-damaging effect in lung tissue of rats that exposed to mobile phone radiation for 2 months.

#### **Collagen fibres**

The present study showed increased collagen fibers inside the highly dilated hepatic portal vein and in the detached endothelial lining and in walls of the bile duct, arterial walls, some arterial walls are fibrotic with numerous scattered collagen fibers in between the hepatocytes. Administration of SIL and/or Vit.E ameliorated these changes.

The present results are in agreement with those of **Zaghloul**<sup>60</sup> who observed pronounced sinusoidal fibrosis around the ductular-like structures, where a considerable amount of collagen fibres was seen in the intercellular spaces, adjacent to the basal lamina that surrounds the biliary ductular structures in hepatic lobules of the liver of rats after seven

days following the end of exposure to EMF. Highly increased collagen fibres was detected in the liver and lung tissues of the pregnant rats and their fetuses exposed to 2Gy gamma rays on day 7 or day 14 of gestation.<sup>61</sup> Increased collagen post-radiation exposure in the different tissues was detected by several authors.<sup>62,63,64</sup>

The present investigation is supported by the work done by those of **Eid et al.**<sup>57</sup> who revealed that newly born mice exposed to RF-EMF from mobile phone 45min/day for one month showed increased collagen fibres in hepatocytes of the exposed group when compared to the control group.

Administration of SIL and/or Vit.E showed slightly decreased collagen fibres in the central and portal areas of liver tissue.

The present investigation is supported by the work done by **Mohamed**<sup>58</sup> who revealed that SIL and Vit. E could protect the lung tissue from the damage produced after the exposure of rats to mobile phone radiation 900MHz.

#### **Polysaccharide**

The present study revealed highly decreased polysaccharides in the hepatocytes of the central and portal areas of the liver tissue of the irradiated group, but they were increased in walls of the hepatic portal veins, bile ducts, arterial walls of the portal areas and in the haemolysed RBCs inside the hepatic portal vein.

The reduction of PAS+ve materials was noticed by **Saeid et al.**<sup>65</sup> who observed reduction of limitation of PAS +ve materials around the central vein of liver of the white rabbits and they concluded that EMFs can decrease liver glycogen stores. Reduced glycogen in cells post-irradiation may be due to decreased T3 and T4 hormones of the thyroid glands, which lessen entrance of glucose to the cells.<sup>61</sup>

The reduction of PAS +ve materials was also noticed by **Eid et al.**<sup>57</sup> who observed a significant decrease of PAS +ve materials in the central and portal areas in liver of newly born mice exposed to RF-EMF from mobile phone (45min/day) for one month.

In the present study administration of SIL to the exposed group showed reduced PAS +ve materials in some hepatocytes of liver tissue. Vit.E administration showed decreased polysaccharides in the hepatocytes, but they were increased in and around the hepatic portal vein of the liver tissue. However, co-administration of SIL and Vit.E showed

somewhat normal appearance of PAS +ve materials in the liver tissue. SIL pre-treatment succeeded in restoring the glycogen stores within the cytoplasm of liver cells. It could be proposed that SIL ameliorates the principal functions of the liver which are related to the regulation of carbohydrate metabolism and blood glucose homeostasis.<sup>66</sup>

It can be proposed that decreased glycogen stores observed in liver sections of the exposed group because more energy was needed to detoxify and overcome EMF induced stress. Thus, the next alternative source of energy to meet the increased energy demand is proteins.<sup>67</sup> Post administration of SIL increased the cytoplasmic glycogen particles, compared to CDDP (Cisplatin) group, where granules appeared moderately stained. However, the marked reduction in glycogen stores induced by CDDP was improved after pretreatment with SIL.<sup>68</sup> **Mohamed**<sup>69</sup> demonstrated that pregnant mice exposed to EMF (900-1800 MHz) from day 0 until day 21, then the resulting pups continue to receive irradiation for the following 4 months 1h/day showed reduced PAS+ve materials in the neurons of hippocampus of the newly born mice.

#### **Total protein**

The present results revealed reduced total protein in most hepatocytes of the liver tissue of the irradiated group, but they increased in the thickened walls of the blood vessels and bile ducts with mild staining affinity in the hemolysed blood cells. Increased malondialdehyde (MDA) in liver tissue of the exposed rats in this study may be responsible of damaging protein. The present findings showed that administration of SIL and/or Vit.E showed somewhat normal restored total proteins in the central and portal areas of liver tissue to their normal level. According to **Abouzeinab**<sup>68</sup> pretreatment with SIL, recovered total protein content to normal appearance where, protein granules were associated with the nucleoli and concentrated adjacent to the nuclear envelope of most nuclei. Besides, the cytoplasm of hepatocytes possessed large-sized intensely stained protein granules similar to those recorded in the control groups. Using different hepatotoxic substances showed that silymarin has multiple actions as a hepatoprotective agent. The antioxidant property and cell-regenerating functions led to increased protein synthesis.<sup>70</sup> Irradiation of animals at 900-1800 MHz resulted in a marked reduction in the total

protein content giving weak to moderate reaction in some hippocampal areas.<sup>69</sup>

Proteins are mainly involved in the architecture of the cell.<sup>71</sup> **Abdelmeguid *et al.***<sup>72</sup> stated that the decrease in protein could be attributed to the disruption of lysosomal membranes under the effects of various toxicants; thus leading to the liberation of their hydrolytic enzymes in the cytoplasm. Additionally, the presence of hydrolytic enzymes could cause the lysis and dissolution of the target material within the cytoplasm.

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**Table (1)** shows red blood cells (RBCs), Hb, Hct and blood indices in the control and different treated groups.

Groups Parameters	Normal control	Positive control			Treatment			
		SIL	Vit.E	SIL.+ Vit.E	Irrad.	Irrad.+ SIL.	Irrad.+ Vit.E	Irrad.+ SIL+ Vit.E
RBCs (x10 <sup>6</sup> cell/mm <sup>3</sup> )	4.71± 0.14	4.63± 0.18	4.87 ± 0.19	5.03± 0.17	4.2± 0.11a	4.68 ± 0.17	4.62± 0.18	4.7± 0.19
% of changes vs normal cont. % of changes vsIrrad.group		-1.70	+3.4 0	+6.79	-10.83	-0.64 +11.4 3	-1.91 +10.0 0	-0.21 +11.90
Hb (g/dl)	13.97± 0.42	13.85± 0.33	14.2± 0.25	14.93± 0.34	10.70± 0.33a	12.56 ± 0.41 b	12.85± 0.42b	12.98± 0.37b
% of changes vs normal cont. % of changes vsIrrad.group		-0.86	+1.65	+6.87	-23.41	-10.09 +17.3 8	-8.02 +20.09	-7.09 +21.31
Hct (%)	42.66± 1.30	41.55± 1.20	42.80± 1.40	44.90± 1.50	36.70± 1.30a	41.80 ± 1.50	41.54± 1.40	42.48± 1.20b
% of changes vs normal cont. % of changes vsIrrad.group		-2.60	+0.33	+5.25	-13.97	-2.02 +13.90	-2.63 +13.19	-0.42 +15.75
MCV(fl)	90.57± 0.16	89.40± 0.18	87.80± 0.11a	89.40± 0.15	87.38± 0.15a	89.31± 0.19b	90.00± 0.18b	90.03± 0.14b
% of changes vs normal cont. % of changes vsIrrad.group		-1.29	-3.06	-0.58	-3.52	-1.39 +2.2 1	-0.63 +3.00	-0.60 +3.03
MCH (pg)	29.60± 0.18	29.91± 0.19	29.20± 0.1	29.70± 0.15	25.47± 0.14a	26.83± 0.13ab	27.61± 0.15ab	27.81± 0.12ab
% of changes vs normal cont. % of changes vsIrrad.group		+1.05	-1.35	+0.34	-13.95	-9.36 +5.3 4	-6.72 +8.41	-6.05 +9.19
MCHC (%)	33.21± 0.15	33.45± 0.14	33.19 ± 0.16	33.25± 0.17	29.15± 0.17a	30.04 ± 0.14a b	30.90 ± 0.16a b	30.55 ± 0.15a b
% of changes vs normal cont. % of changes vsIrrad.group		+0.72	-0.06	+0.12	-12.2	-9.55 +3.0 5	-6.96 +6.00	-8.01 +4.80

All results represent Mean ±SE

a : significant in comparison with the normal control group.

b: significant in comparison with the irradiated group.

**Table (2) shows white blood cells (WBCs) count, lymphocyte%, neutrophil% and platelets count in the control and different treated groups.**

Groups Parameters	Normal control	Positive control			Treatment			
		SIL	Vit.E	SIL.+ Vit.E	Irrad.	Irrad. + Sily.	Irrad. + Vit.E	Irrad. + SILy+ Vit.E
WBCs ( $\times 10^3$ cell/mm <sup>3</sup> )	6.10± 0.29	6.06± 0.31	5.98± 0.22	5.93± 0.26	9.70± 0.32a	7.80± 0.35ab	7.50± 0.27ab	6.50± 0.25b
% of changes vs normal cont. % of changes vsIrrad. group		-0.66	-1.97	-2.79	+59.02	+27.87 -19.59	+22.95 -22.68	+6.56 -32.99
Lymphocyte (%)	32.75± 1.20	32.64± 1.50	32.88± 1.40	32.3±0 1.30	38.72± 1.3a	34.28± 1.50	34.65± 1.40	34.03± 1.30
% of changes vs normal cont. % of changes vsIrrad. group		-0.34	+0.40	-1.37	+18.22	+4.67 -11.46	+5.48 -10.51	+3.76 -12.11
Neutrophil (%)	53.25± 0.26	53.36± 0.72	53.12± 0.24	53.7± 0.25	55.28± 0.29a	53.72± 0.25b	53.35± 0.24b	53.97± 0.26b
% of changes vs normal cont. % of changes vsIrrad. group		+0.21	-0.24	+0.85	+3.47	+0.88 -2.82	+0.19 -3.49	+1.35 -2.37
Platelets ( $\times 10^3$ /mm <sup>3</sup> )	312.70± 2.50	312.49± 2.30	312.92± 2.01	312.74± 2.70	350.10± 2.40a	342.50± 2.13a	333.30± 2.14ab	323.50± 2.56ab
% of changes vs normal cont. % of changes vsIrrad. Group		-0.07	+0.07	+0.01	+11.96	+9.53 -2.17	+6.59 -4.80	+3.45 -7.60

All results represent Mean  $\pm$ SE

**a** : significant in comparison with the normal control group.

**b**: significant in comparison with the irradiated group.

Table(3) shows serum liver enzyme activities ALAT, ASAT and ALP in the control and different treated groups

Groups Parameters	Normal control	Positive control			Treatment			
		SIL	Vit.E	SIL.+ Vit.E	Irrad.	Irrad.+ SIL.	Irrad.+ Vit.E	Irrad.+ SIL + Vit.E
ALAT (U/L)	66.71± 2.35	65.05± 2.31	66.30± 2.33	61.31± 2.31	114.00± 2.29 a	84.50± 2.31ab	82.30± 2.13ab	80.90± 2.14ab
% of changes vs normal cont. % of changes vs Irrad. group		-2.49	-0.61	-8.09%	+70.80	+26.60 -25.88	+23.37 -27.81	+21.27 -29.04
ASAT (U/L)	99.57± 3.72	104.4± 3.53	101.2± 3.77	109.0± 3.87	180.3± 3.90a	150.76± 3.88ab	149.01± 3.90ab	148.13± 3.92ab
% of changes vs normal cont. % of changes vs Irrad. group		+4.88	+1.67	+9.50	+81.08	+51.41 -16.38	+49.65 -17.35	+48.77 -17.84
ALP (U/L)	181.97± 3.51	189.36± 3.62	182.75± 3.52	181.41± 3.81	224.5± 3.89a	191.55± 3.77b	189.5± 3.67b	182.05± 3.78b
% of changes vs normal cont. % of changes vsIrrad. group		+4.06	+0.43	-0.31	+23.37	+5.01 -14.68	+4.14 -15.59	+0.04 -18.91

All results represent Mean ±SE a : significant in comparison with the normal control group. b: significant in comparison with the irradiated group.

Table(4) Shows liver tissue enzyme activities ALAT, ASAT and ALP in the control and different treated groups

Groups Parameters	Normal control	Positive control			Treatment			
		SIL	Vit.E	SIL.+ Vit.E	Irrad.	Irrad.+SIL.	Irrad.+ Vit.E	Irrad.+ SIL+ Vit.E
ALAT (U/g.tissue)	80.50± 2.52	83.50± 2.55	85.70± 2.62	77.90± 2.53	162.65± 2.53a	99.60± 2.45ab	93.20± 2.40ab	82.10± 2.50b
% of changes vs normal cont. % of changes vsIrrad. group		+3.70	+6.46	-3.23	+139.32	+23.73 -48.30	+15.78 -51.62	+1.99 -57.38
ASAT (U/g. tissue)	163.50± 3.7	162.2± 3.67	161.50± 3.78	160.50± 3.67	227.20± 3.77a	170.10± 3.89b	185.90± 3.91ab	166.10± 3.92b
% of changes vs normal cont. % of changes vsIrrad. Group		-0.78	-1.22	-1.83	+38.90	+4.04 -25.13	+13.70 -18.18	+1.59 -26.89
ALP (U/g. tissue)	200.30± 4.5	201.30± 4.52	204.30± 4.63	201.42± 4.67	254.80± 4.73a	215.20± 4.50b	223.80± 4.67ab	216.20± 4.81b
% of changes vs normal cont. % of changes vs. Irrad. group		+0.51	+2.00	+0.56	+27.20	+7.44 -15.54	+11.73 -12.17	+7.94 -15.15

All results represent Mean ±SE a : significant in comparison with the normal control group. b: significant in comparison with the irradiated group.

Table(5) shows serum catalase (CAT) activity, GSH, MDA and H<sub>2</sub>O<sub>2</sub> levels in the control and different treated groups.

Groups Parameters	Normal control	Positive control			Treatment			
	Normal control	SIL	Vit.E	SIL+ Vit.E	Irrad.	Irrad.+ SIL	Irrad.+ Vit.E	Irrad.+ SIL + Vit.E
<b>CAT (U/L)</b>	<b>70.10± 1.70</b>	<b>69.92± 1.70</b>	<b>69.9± 1.67</b>	<b>70.13± 1.70</b>	<b>52.30± 1.67a</b>	<b>63.70± 1.66b</b>	<b>62.51± 1.56ab</b>	<b>68.61± 1.64ab</b>
% of changes vs normal cont. % of changes vs Irrad. group		-0.13	-0.16	+0.10	+25.30	-9.01 +21.80	-10.80 +19.52	-2.00 +31.19
<b>GSH (mmol/L)</b>	<b>14.01± 0.40</b>	<b>14.25± 0.50</b>	<b>13.91± 0.52</b>	<b>16.03± 0.56a</b>	<b>8.98± 0.53a</b>	<b>13.91± 0.59b</b>	<b>13.51± 0.52b</b>	<b>14.10± 0.51b</b>
% of changes vs normal cont. % of changes vs Irrad. Group		+1.70	+ 0.71	+14.42	- 35.90	-0.71 +54.90	-3.57 +50.45	+0.64 +57.02
<b>MDA (nmol/ml)</b>	<b>27.25± 0.50</b>	<b>27.01± 0.48</b>	<b>27.03± 0.49</b>	<b>26.93± 0.46</b>	<b>32.63± 0.47a</b>	<b>28.4± 0.46b</b>	<b>28.6± 0.48b</b>	<b>28.00± 0.45b</b>
% of changes vs normal cont. % of changes vs Irrad. group		-0.88	-0.81	-1.17	+19.74	+4.22 -12.90	+4.95 -12.35	+2.75 -14.19
<b>H<sub>2</sub>O<sub>2</sub> (U/L)</b>	<b>0.84± 0.08</b>	<b>0.76± 0.073</b>	<b>0.79± 0.078</b>	<b>0.75± 0.077</b>	<b>1.91± 0.06a</b>	<b>0.88± 0.06b</b>	<b>0.85± 0.061b</b>	<b>0.86± 0.05b</b>
% of changes vs normal cont. % of changes vs Irrad. group		-9.52	-5.95	-10.70	+127.80	+4.76 -53.90	+1.19 -55.50	+2.38 -54.97

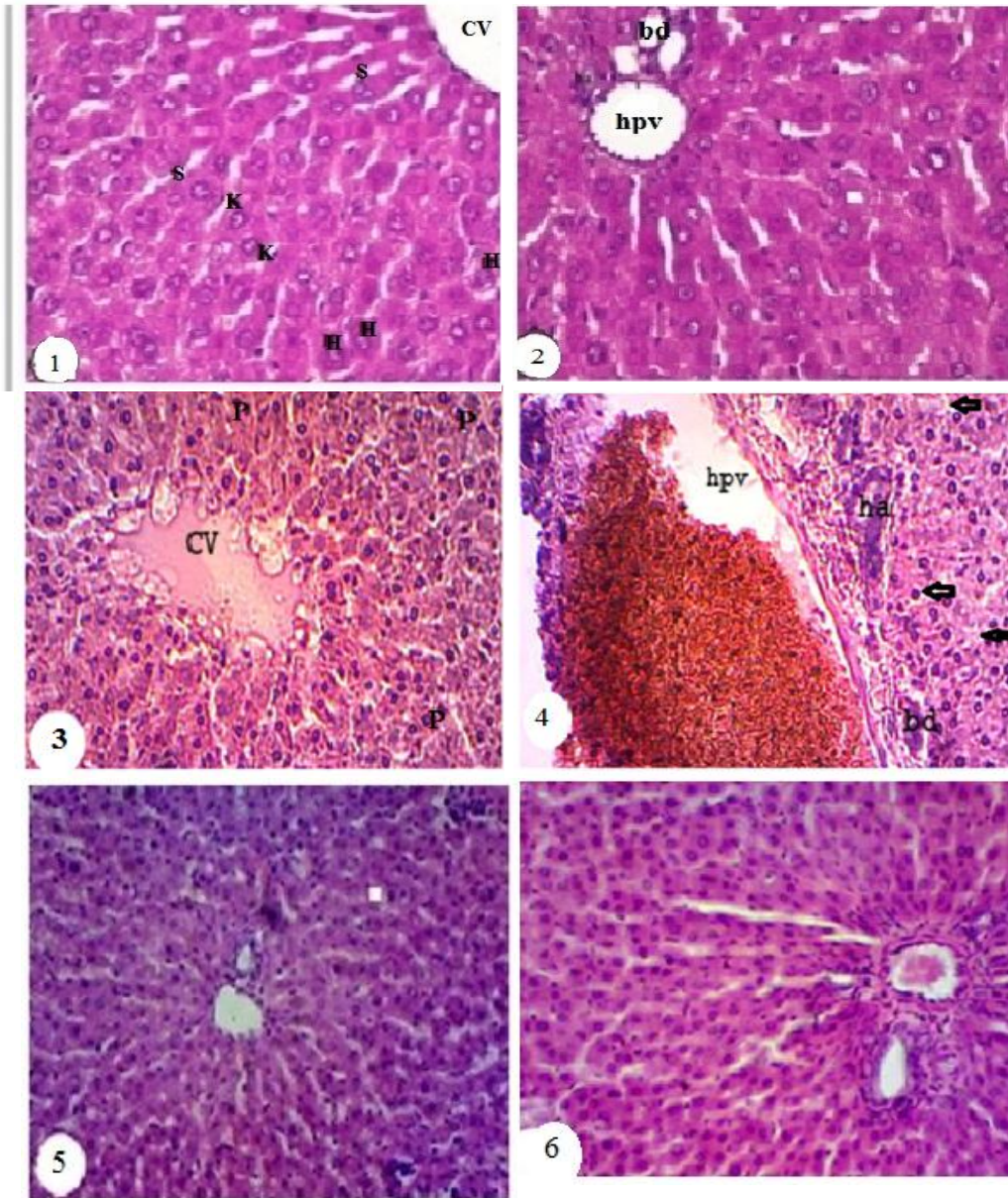
All results represent Mean ±SE **a** : significant in comparison with the normal control group. **b**: significant in comparison with the irradiated group.

**Table(6) Shows liver tissue catalase (CAT) activity, GSH, MDA and H<sub>2</sub>O<sub>2</sub> contents in the control and different treated groups.**

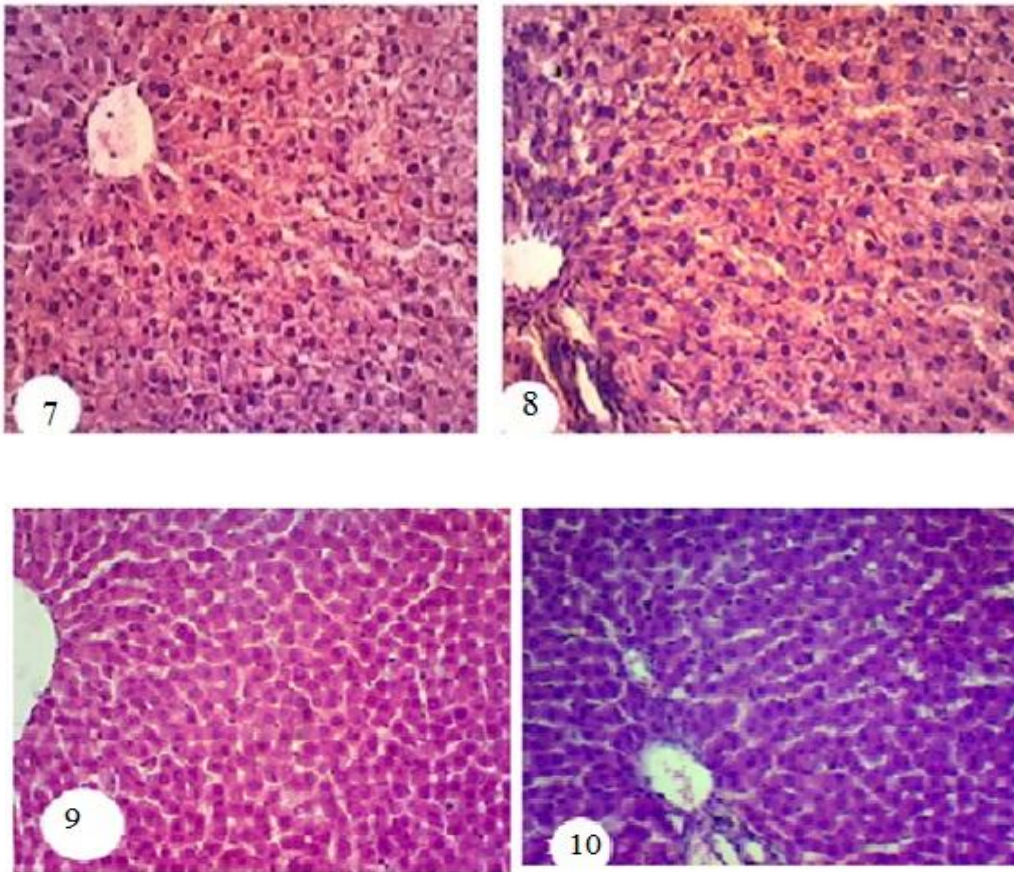
Groups Parameters	Normal control	Positive control			Treatment\			
		SIL	Vit. E	SIL+ Vit.E	Irrad.	Irradi. + SIL	Irrad. + Vit.E	Irrad. + SIL + Vit.E
<b>CAT</b> (U/g. tissue))	85.40± 2.00	88.90± 1.90	87.50± 1.80	91.40± 1.79	55.76± 2.50a	76.30± 1.90ab	80.80± 1.50b	82.30± 1.98ab
% of changes vs normal cont. % of changes vsIrrad. group		+4.10	+2.40	+7.03	-34.71	-10.66 +36.84	-5.39 +44.91	-3.63 +47.60
<b>GSH</b> (mmol/g.tissue)	29.88± 1.50	27.75± 1.40	29.11± 1.30	30.11± 1.10	18.43± 1.90a	24.21± 1.20a	23.21± 1.50	25.1± 1.10b
% of changes vs normal cont. % of changes vsIrrad. group		-7.13	-2.58	+0.77	-38.32	-18.98 +31.36	-22.32 +5.94	-16.00 +6.19
<b>MDA</b> (nmol/g. tissue))	33.80± 1.80	32.05± 1.70	33.00± 1.60	31.13± 1.80	45.05± 2.10a	34.61± 1.78b	35.30± 1.40b	33.60± 1.30b
% of changes vs normal cont. % of changes vsIrrad. Group		-5.18	-2.37	-7.90	+33.2	+2.40 -23.17	+4.44 -21.64	-0.59 -25.42
<b>H2O2</b> (U/g. tissue))	1.30± 0.08	1.10± 0.073	1.230± 0.078	0.90± 0.077	3.08± 0.10a	1.85± 0.08ab	1.79± 0.09ab	1.58± 0.07b
% of changes vs normal cont. % of changes vsIrrad. group		-15.38	-5.38	-30.77	+136.0 0	+42.31 -39.94	+37.69 -41.88	+21.54 -48.70

All results represent Mean ±SE a : significant in comparison with the normal control group. b: significant in comparison with the irradiated group.

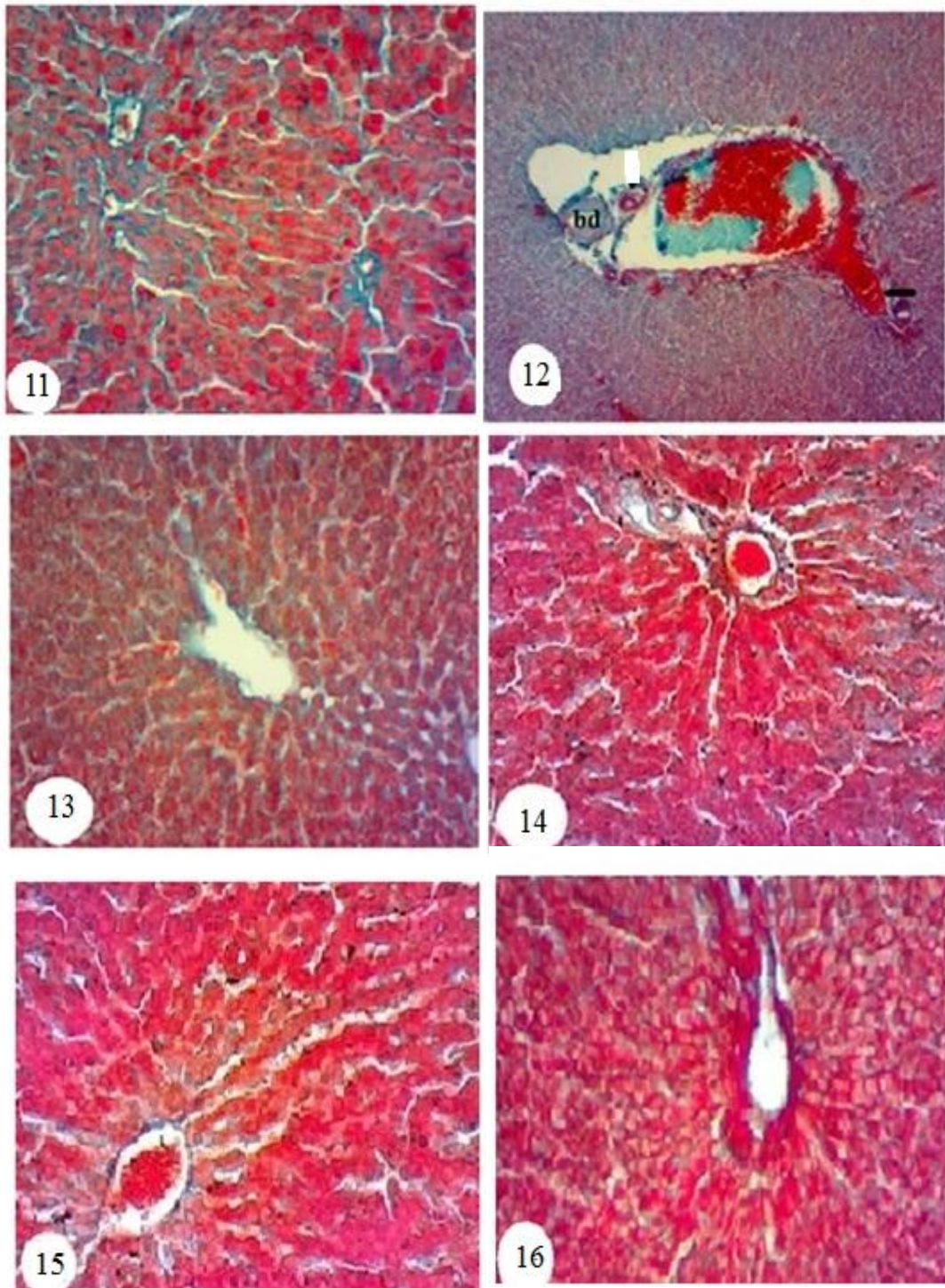
Histopathological changes





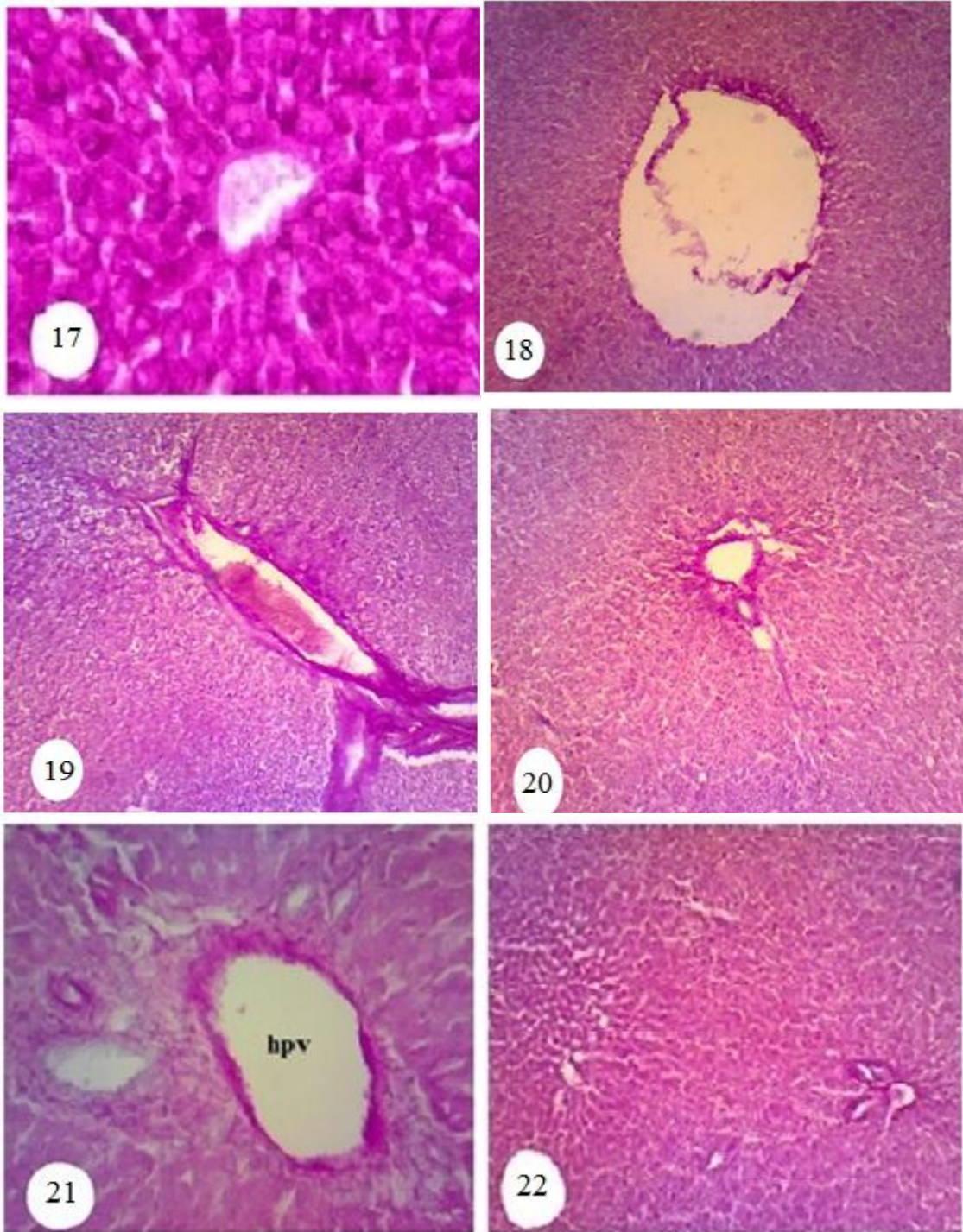


**Figs.(1-10)**► Photomicrographs showing liver tissue of the control and treated groups (H&Ex200).**1&2**:control group which contains the central vein (CV) , sinusoidal spaces (s), Kupffer cells (K) ,cords of hepatocytes (H) and the portal area which contains branch of the hepatic portal vein (**hpv**), branch of the hepatic artery (**ha**) and bile duct (**bd**).**3&4** -showing liver of an irradiated rat (R) with corrugated and ruptured endothelial lining of the central vein (CV) which contains haemolysed blood cells, nearly all nuclei of the hepatocyte showed pyknosis (P);the portal area of R group with highly dilated and congested hepatic portal vein (**hpv**), thickened arterial wall (**ha**) and malformed bile ducts (**bd**) with some vacuolated hepatocytes (→ ). **5& 6** showing somewhat normal appearance of the central and portal areas of the irradiated group which treated with silymarin (RS).**7&8** showing somewhat normal appearance of the central and portal areas of the irradiated group which treated with Vit.E (RE) but, the portal areas showed few aggregations of lymphocytes.**9&10** showing normal appearance of the central and portal areas of the liver tissue of group RSE.



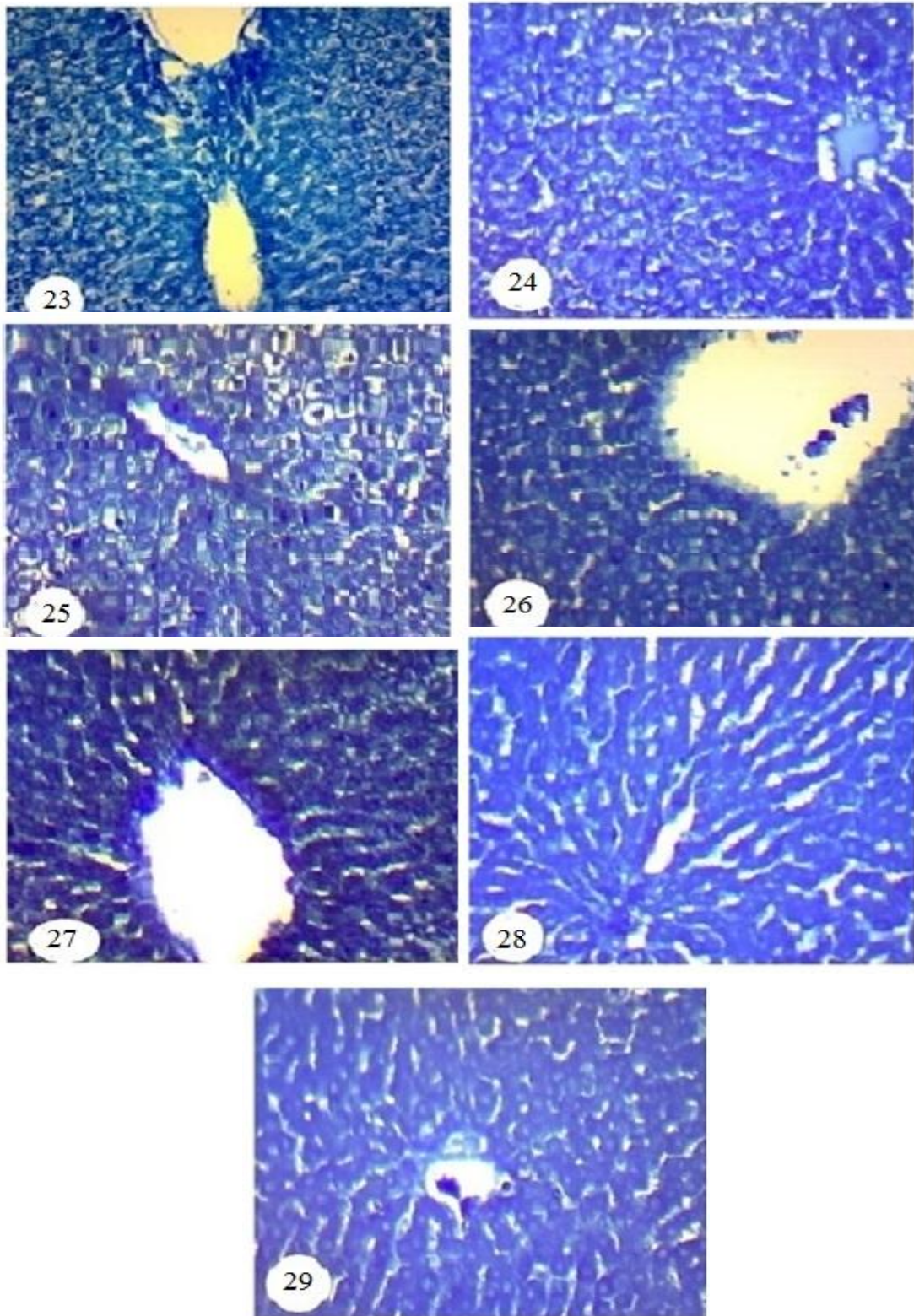
**Figs.(11-16)** Photomicrographs showing distribution of collagen fibres in liver of the control and treated groups(Mallory's trichrome, x200).11-Liver of the control group with thin scattered collagen bundles in the central and portal areas, sinusoidal spaces, around hepatocytes, walls of the blood vessels and bile ducts.12-**R** group showing increased collagen fibres inside the highly dilated hepatic portal vein and in the detached endothelial lining and in walls of the bile duct (bd), arterial walls, some arterial walls are fibrotic ( ) with numerous scattered collagen fibres in between hepatocytes of the liver.13&14 group **RS** showing slightly decreased collagen fibres in the central and portal areas.15-group **RE** showing decreased collagen fibres in the liver tissue.16-group **RSE** showing decreased collagen fibres in the liver tissue .





**Figs.(17-22)** Photomicrographs showing distribution of PAS +ve materials in liver tissue of rats of the control and treated groups (PAS x200).17- showing normal distribution of PAS +ve materials in liver tissue of rats of the control group 18-19-showing highly decreased polysaccharides in hepatocytes of the central and portal areas of the liver tissue of group R, but they are increased in walls of the hepatic portal vein , bile ducts and arterial walls of the portal area and in the haemolysed RBCs inside the hepatic portal vein (hpv).20- showing reduced PAS +ve materials in some hepatocytes of liver tissue of rats of group RS.21- showing decreased polysaccharides in the hepatocytes, but they increased in and around the hepatic portal vein (hpv) of the liver tissue of rats of groups RE.22-showing somewhat normal appearance of PAS +ve materials in the liver tissue of group RSE.





**Figs.(23-29)** Photomicrographs showing distribution of total proteins in the central and portal areas of liver tissue of rats of the control and treated groups(**Bromophenol blue x200**).23- Showing normal distribution of total proteins in the central and portal areas of liver tissue of a rat of the control group .24-25-showing reduced total protein in most hepatocytes, but they increased in the thickened walls of the blood vessels ; bile ducts of group **R** with mild staining affinity in the hemolysed blood cells.26-Showing, somewhat normal distribution of total proteins in the liver tissue of a rat of group **RS**.27-showing somewhat normal distribution of total proteins in the liver tissue of a rat of group **RE**.28&29-Showing normal distribution of total protein in the central and portal areas of liver tissue of group **RSE**.