The Protective Effect of Ginger (*Zingiber Officinale*) Against Adriamycin- Induced Hepatotoxicity in Rats: Histological Study

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Abstract: Aim: The present study aimed to show the histopathological and ultrastructural changes occurred in the rat liver post-application of adriamycin and to evaluate the possible protection of ginger against adriamycin induced hepatic toxicity. Material And Methods: Animals were divided into four groups. The first group was given saline orally. The second group was given ginger extract orally with a single daily dose 250 mg/kg b.wt. for two weeks. The third group was treated with adriamycin in six intraperitoneal injections (each containing 2.5 mg/kg/b.wt) over a period of two weeks. The fourth group was treated with adriamycin at the same dose level as those of group III and by oral administration of ginger extract (250 mg/kg/b.wt) daily for two weeks. Livers specimens from all groups were examined by light and electron microscopies. Results: Adriamycin treated group revealed destruction of the hepatic cords, cytoplasmic vacuolization, cellular degeneration, pyknotic and atrophic nuclei, damaged mitochondria and accumulation of lipid droplets in the hepatocytes and deposition of a collagen-like fibrous material in the blood sinusoids and space of Disse with dilated intercellular spaces. Dilated blood sinusoids and dilated bile canaliculi with atrophy of its microvilli were also noticed. Damaged Kupffer cells with vacuoles were prominent. The hepatotoxic effect of adriamycin was ameliorated with partial disappearance of pathological hepatic damage when treatment was combined with ginger. The results of the present work indicated that ginger had protective effect against liver damage induced by adriamycin.Conclusion: The present results provide in vivo evidence of direct chemotherapeutic hepatotoxicity caused by adriamycin. Appropriate protective measures as ginger must be applied with anticancer treatment for improving liver structure. The results can further suggest the possible use of ginger against oxidative stress induced organ toxicities.

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1. Introduction

Adriamycin is an anthracyclic antibiotic with broad spectrum of anti-neoplastic activity that has been found to be highly effective against tumors.[1] The clinical usefulness of adriamycin is limited by its cardiotoxicity and hepatotoxicity.[2] Adriamycin can have serious side effects in liver.[2] Some researchers proposed that adriamycin-induced toxicity is most likely mediated by diverse oxidative damage on cellular components like membrane lipids in the plasma membranes and mitochondria.[3] Oxidative damage occurs in a cell when the concentration of reactive oxygen species generated, exceeds the antioxidant capability of the cell.

Research on ginger, the spice from rhizomes of *Zingiber officinale* reveals a number of analeptic properties as an antioxidant, anti-lipidemic, anti-hyperglycemic, anti-inflammatory and anti-cancer agent.[4,5,6] This medicinal herb is excellent for oral therapy as it is effective, non-toxic and without serious side effects.

Free radicals scavengers are known to reduce adriamycin-induced toxic effects.[7] Ginger extracts scavenge superoxide anion and hydroxyl radicals. It reveals significant inhibitory properties against free radical attack and lipid peroxidation in mouse liver microsomes, as well as potent protective effects on primary rat hepatocytes against oxidative damage.[8] Ginger offer palliative and curative properties via an antioxidant effect. Though, the antioxidant activity of ginger is well known. Its hepatoprotective effect of ginger against ethanol, carbon tetrachloride and acetaminophen-induced hepatotoxicity in rats are also previously documented.[9,10,11]

Hepatic metabolism converts drugs into products that are more easily excreted.[12] The central role of liver in drug metabolism predisposes them to toxic injury.

For the previous mentioned reasons, the liver was choosen to focus in the present study of the pathological effects of adriamycin. Combination of phytochemicals and dietary supplements with anticancer drugs offers some promise.[13] To the best our knowledge, the possible modulating structure effect of ginger extract in the presence of adriamycin has not been yet investigated. Hence, the present study aimed to show the histopathological with ultrastructural changes occurred post-application of

adriamycin on the rat liver and to evaluate the possible protection of ginger against adriamycin induced hepatic injury to build a foundation for later extension of ginger cytoprotection against adriamycin toxicity to liver.

2. Materials and Methods

Chemicals

Adriamycin (10 mg adriamycin hydrochloride) was obtained from Pharmacia Italia (Milan, Italy). All other chemicals of reagent were obtained from Sigma (MO, USA).

Ginger extract

Fresh ginger (*Zingiber Officinale*) rhizome (purchased from local market in Abha) was washed several times with water. Aqueous ethanol extract of rhizome of ginger was prepared by the method as previously described.[11] In brief, rhizome of ginger (500 g), were cut into small pieces and homogenized in a mixer using 50% ethanol. The homogenate was centrifuged at 2500 rpm for 10 min and the supernatant was collected. Solvent in the pooled supernatant was completely evaporated at low temperature with the aid of a rotary vacuum evaporator. The residue thus obtained was designated as ethanol extract and was made a fine suspension in distilled water for the study.

Experimental animals

All experiments were approved by the Ethics Committee of King Khaled University. The animals were housed in an air-conditioned room and were kept in standard laboratory conditions under natural light and dark cycle (12 h light/dark) maintained at an ambient temperature 25 ± 2 °C. The animals were fed with standard pellet diet and water *ad libitum*.

Experimental design

Male adult albino rats (Wistar strain) weighing 280 g ± 8 g were used in the present study. After acclimatization, the rats were assigned randomly to four groups, each with 10 rats, as follow:

Group I: Control rats and was given saline orally.

Group II: Rats were administered ginger extract orally with a single daily dose of 250 mg/kg/b.wt. using special gavage needle for 14 days.

Group III: Rats were treated with adriamycin (doxorubicin hydrochloride) in six equal intraperitoneal injections (each containing 2.5mg/kg/b.wt) over a period of 2weeks for a total cumulative dose of 15mg/kg /b.wt.[14]

Group IV: Rats were orally treated with ginger extract 250 mg/kg/b.wt. daily together with adriamycin (2.5mg/kg/b.wt) for a total cumulative dose of 15mg/kg/b.wt. over a period of 2weeks.

Specimen preparations

At the end of the experiment, the rats were weighed and recorded then killed by cervical dislocation under diethyl ether anesthesia. Liver was then dissected out and weighed on an electrical balance and weight was recorded. Liver was observed for any gross morphological changes. The liver was fixed in 10% formalin. For light microscope preparations, livers were cut into small slices, then dehydrated in gradual series of ethanol, embedded in paraffin wax and sectioned at 5 μm thickness. Slides were stained by hematoxylin and eosin and Masson´s trichrom stain for histological examination. A minimum of three sections per liver were examined and each section was examined two times in its entirety. Liver specimens from all animals were evaluated using light microscope (Nikon Eclipse E-200). For transmission electron microscopy, livers were immersed in 5% glutaraldehyde for two hours then washed in 0.1 M phosphate buffer at 4 °C and post-fixed in 1% osmium tetraoxide. After dehydration in gradual series of ethanol, the tissues were embedded in epon 812. Blocks with tissues were cut into semithin sections, then stained with toluidine blue and examined using a light microscope. Representative fields of semithin sections were selected. Ultrathin sections were stained with uranyl acetate and lead citrate, and examined with transmission electron microscope (Jeol 1200 EX, Japan), in the electron microscope unit at College of Medicine, King Khaled University.

A numerical scoring system assessed the extent of deposition of collagen fibers and fibrosis.[15,16] Deposition of collagen fibers was graded as: grade 0: no deposition; grade 1: enlarged portal tracts with mild deposition of collagen fibers; grade 2: periportal or portal-portal septa of collagen fibers, but intact architecture; grade 3: deposition of collagen fibers with architectural distortion; grade 4: probable or definite cirrhosis with marked deposition of collagen fibers. Hepatocyte necrosis or degeneration was also graded as: grade 0, no hepatocyte necrosis or degeneration; grade 1, focal necrosis or degeneration of hepatocytes (mild lesion); grade 2, multifocal necrosis or degeneration of hepatocytes (moderate lesion); grade 3, locally extensive or diffuse necrosis or degeneration of hepatocytes (severe lesion). [15,16]

Statistical analysis

Results are expressed as mean ± SEM. Mann–Whitney U-test for histopathological scores was used to analyze the significance of differences among groups. The differences were accepted as statistically significant when $P < 0.05$.

3. Results

Adriamycin-treated rats were obviously ill and unable to move readily, with fur raised, scruffy coupled with weight loss and decreased food intake. These rats also had red exudates around the nose and eyes, soft stool and enlargement of abdomen. Rats exposed to ginger alone appeared healthy in all

aspects. All rats in the ginger plus adriamycin combination treatment group appeared healthy with minimal signs and symptoms of illness. The average body weight of control and ginger alone groups showed normal weight, where's the ginger plus adriamycin combination treatment group exhibited a light decrease in body weight. After dissection the animals treated with adriamycin showed loss of skeletal muscles and adipose tissue. The gross morphology of the livers in rats receiving adriamycin alone showed enlargement, congestion, hemorrhage, heavy centrilobular spotting and ascitis. The animals receiving both ginger and adriamycin had livers appeared completely normal. In gross morphology, ginger treatment was effective in reducing adriamycin -induced injury to the liver.

Effect of adriamycin on liver weight, body weight, ratio of liver weight X100 to body weight and mortality is shown in (Table 1). The liver weight and ratio of liver weight to body weight in adriamycin treated group significantly increased as compared to control, and ginger and adriamycin.

Table 1. Effect of ginger and adriamycin on liver weight, body weight, ratio of liver weight X100 to body weight and mortality.

Treatment	Body wt (g)	liver wt (g)	Liver X100/ Body ratio	Mortality
Control	280 ± 8	10.65 ± 0.86	3.80	0/10
Ginger	277 ± 8	10.50 ± 0.88	3.79	0/10
Adriamycin	238 ± 7*	12.87±0.97*	5.41*	2/10*
Adriamycin & ginger	261 ± 8*	11.12 ± 0.93*	4.26*	0/10

*Significantly different from control and all other groups $P< 0.001$.

The toxicity of adriamycin was manifested by a 15% ± 2.5% loss of body weight at the end of the experiment. However, rat treated with ginger and adriamycin exhibited only a 7% ± 2.8 % loss of body weight. Rat treated with adriamycin displayed also increased in liver weight at the end of the experiment that was significantly decreased by the treatment of adriamycin with ginger. The survival rates of animals in the control group and ginger alone group were consistently 100% in all series of experiments, while it was 80 % in adriamycin treated group and 100% in adriamycin and ginger group IV. This data indicates that ginger displays a proper bioavailability.

Light microscopic examination of liver sections of control and ginger treated rats revealed that the hepatic parenchymal cells are arranged as sheets intimately associated with a venous portal system in an uncomplicated and consistent way throughout the organ. The hepatic lobules appeared to be formed of hepatocytes. The hepatocytes arranged in cords radiating from the central veins. The hepatocytes form columns of cells adherent to each other by one or more surfaces with well-preserved cytoplasm and prominent round nucleus with fine arrangement of Kupffer cells. The hepatic sinusoids were seen as narrow spaces in between the hepatic cords (Figs. 1, 2).

In contrast, rats receiving adriamycin showed massive pathological changes. The most pronounced pathological abnormalities observed involved dissolution and degeneration of hepatic cords, which appeared as empty vacuoles aligned by strands of necrotic hepatocytes. Several of the hepatocytes were fused together forming degenerated areas of destroyed cells that lost their normal characters. Also, nuclei of some hepatocytes were completely demolished leading to the existence of damaged cells (Fig. 3). Hepatocyte degeneration is mainly associated with cytoplasmic vacuolation with the nuclear contour generally intact, whereas the hepatocyte necrosis is associated with karyorrhexis, in addition to degenerative changes. Most injury was characterized by increased numbers of inflammatory cells, necrotic and apoptotic hepatocytes. The blood sinusoids were dilated and some of the hepatocytes showed cytoplasmic vacuoles with degenerated nuclei (Fig. 4). The striking sign of the liver tissue injury was well discerned at the central vein. Some central veins exhibited remarkable dilatation and congestion and its endothelium is eroded (Fig. 5).

For revealing the deposition of collagen fibers, the liver sections stained with Masson´s trichrome. Collagen fibers, as stained blue by Masson´s trichrome, were distinctly deposited in the liver of adriamycin treated rats, suggesting abundant accumulation of collagen in the liver of adriamycin treated rats (Fig. 6). Adriamycin treatment showed increase and expansion of collagen fibrous septa around the central vein and in the blood sinusoids (Fig. 7). The central vein was surrounded by an exuberant amount of the collagen fibers after adriamycin treatment (Fig. 8). Portal vein congestion with partial eroded lining was cleared. The portal tract showed abundant collagen fibers surrounding it with bile duct dilatation and proliferation and was generally associated with periductal collagen fibers (Fig. 9).

Sections from control and ginger alone groups were devoid of the previous mentioned features and demonstrated mild hepatocellular changes. Sections of animals treated with adriamycin and ginger showed that the liver tissue acquired improvement compared with adriamycin group. Mild dilatation and congestion of sinusoids were appeared. The repairing effects-up to

certain limits were noticed. Hepatocytes showed much improvement with large number of binucleated hepatocytes (Figure 10). There is reduction in deposition of collagen fibers around the portal tract, central veins (Fig. 11) and in between hepatocytes and sinusoids (Fig. 12).

Ultrastructure of sections of groups I and II were normal with nearly rounded nuclei with regular structural organization. The cytoplasm crowded with organelles, particularly rough endoplasmic reticulum, smooth endoplasmic reticulum, golgi apparatus, ribosome and mitochondria (Fig. 13).

In contrast, the hepatocytes of liver sections from group treated with adriamycin showed irregularity and degenerative changes in the nuclear membrane. Shrunken and pyknotic nucleus in the degenerated hepatocyte is seen. The cytoplasm was condensed and contained degenerative changes such as condensed, atrophied and damaged mitochondria losing its cristae (Fig.14). Dilated intercellular spaces observed between hepatocyes (Fig. 14). Early phases of apoptosis and atrophic nuclei were also detected (Fig. 15). Disrupted rough endoplasmic reticulum and numerous of different sizes lysosome were also seen (Fig. 15). Cytoplasmic vacuolization (Fig.15), accumulation of lipid droplets and cytoplasmic inclusion bodies were observed (Fig. 16).

Vacuoles were also seen in the heterochromain of the hepatocyte nucleus. Collagen-like fibrous septa deposition was seen in the space of Disse of blood sinusoid in between hepatocytes (Fig. 17). Dilated bile canaliculi with damage and atrophy of its microvilli were also seen (Fig. 18). Swollen and damaged Kupffer cell with irregular nuclear membrane. large autophagic vacuole was also seen (Fig. 19).

Cytoplasmic vacuolization, cristae loss in mitochondria, collagen fibers deposition and other degenerative changes were markedly attenuated in adriamycin with ginger treated group (Figs. 20 & 21). Most of the Hepatocytes nucleus, cytoplasmic organelles, Intercellular space, bile canaliculi and Kupffer cells are more or less normal in group IV (Figs. 20 & 21).

Morphometric studies

Histopathological evaluation was performed in three liver sections per rat from all rats in each group (Table 2).

Table 2. Histopathological scoring of liver sections from all groups (mean ± S.D.).

Treatment	Degeneration and necrosis	Deposition of collagen fibers
Group 1 control	0.2 ± 0.08	0.15 ± 0.07
Group 11 ginger	0.23 ± 0.08	0.19 ± 0.08
Group 111 Adriamycin	2.25 ± 0.23*	3.09 ± 0.25*
Group 1V Ginger & adriamycin	0.85 ± 0.16*	1.11 ± 0.18*

*= Significant different from others $P < 0.05$

Concurrent treatment with adriamycin and ginger significantly attenuated the extent and severity of the histological features of liver damage induced by adriamycin alone. Degeneration and necrosis scores in the liver sections from adriamycin treated rats group III were significantly reduced in rats treated with ginger and adriamycin group IV (Table 2). The degeneration and necrosis scores were reduced to about 62% by adding ginger. Hepatic collagen deposition were significantly decreased in group IV compared to group III ($p < 0.05$), suggesting that ginger ameliorated hepatic collagen deposition in adriamycin treated rats. The collagen fibers deposition scores were reduced to nearly 64% when the rats treated with ginger.

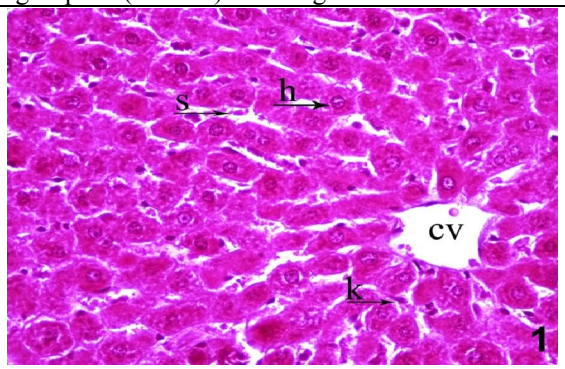

Figure (1): A photomicrograph of a section in the liver of the control rat showing radially arranged hepatocytes (h) around the central vein (cv) and blood sinusoids (s) clearly seen in between them. Notice the presence of kupffer cells (k). (H&E X 400).

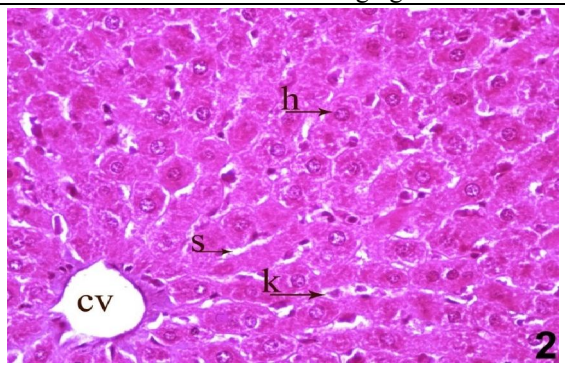

Figure (2): A photomicrograph of a section in the liver of the ginger treated rat showing radially arranged hepatocytes (h) around the central vein (cv) and blood sinusoids (s) clearly seen in between them. Notice the presence of kupffer cells (k). (H&E X 400).

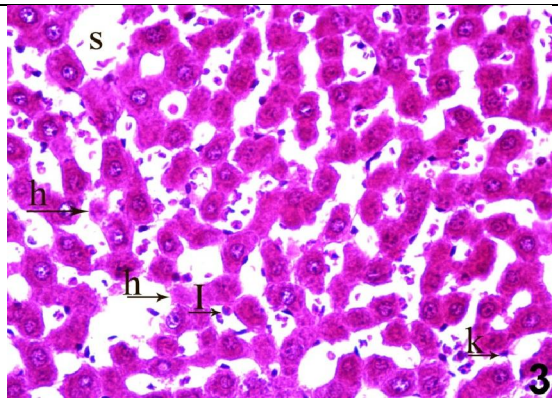

Figure (3): A photomicrograph of a section in the liver of adriamycin treated rat showing degenerated hepatocytes (h), destructed hepatic cords and in between them wide and dilated blood sinusoids (s). Notice the presence of many inflammatory cells (I) and kupffer cells (k). (H&E X 400).

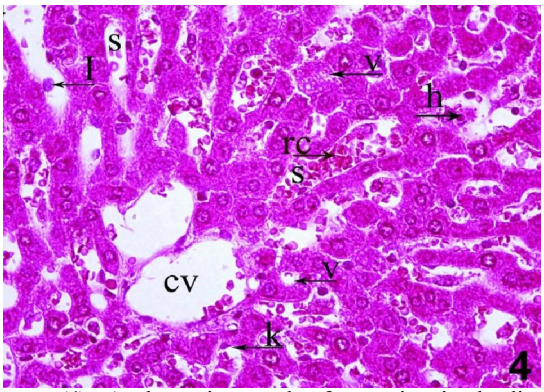

Figure (4): A photomicrograph of a section in the liver of adriamycin treated rat showing the central vein (cv) with degenerated hepatocytes (h) and many inflammatory cells (I) with dilated blood sinusoids (s) full by red blood corpuscles (rc) are clearly seen. Notice the presence of vacuoles (v) and kupfer cells (k). (H&E X 400).

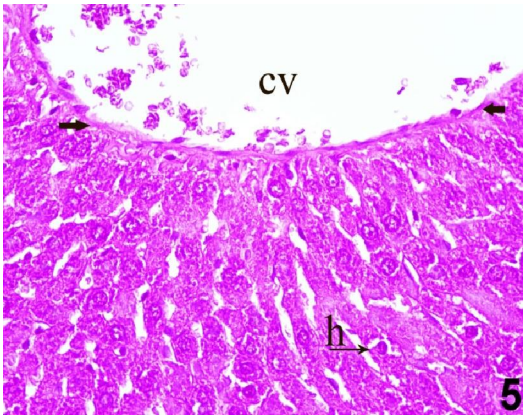

Figure (5): A photomicrograph of a section in the liver of adriamycin treated rat showing many degenerated and vacuolated hepatocytes (h). Notice the central vein (cv) is wide and dilated and its endothelium is eroded (thick arrow). (H&E X 400).

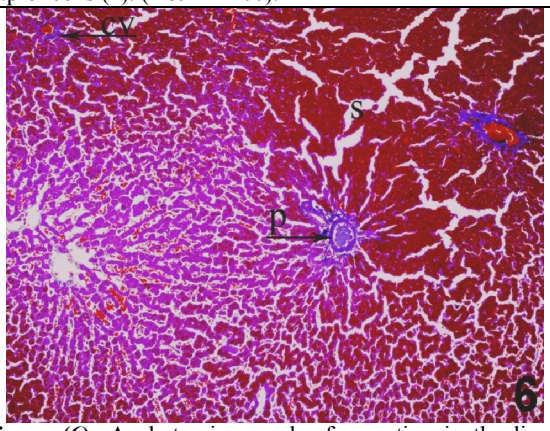

Figure (6): A photomicrograph of a section in the liver of adriamycin treated rat showing the central vein (cv) and portal tract (P) with marked deposition of collagen fibres surrounding them and dilated blood sinusoids (s) are clearly seen. (Masson´s trichrome X 100).

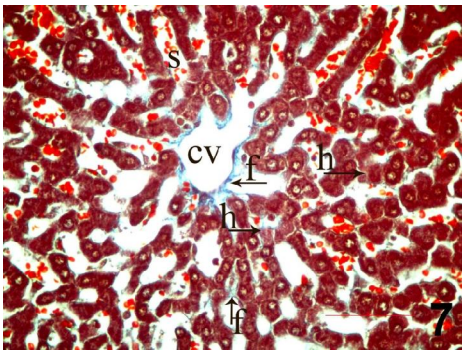

Figure (7): A photomicrograph of a section in the liver of adriamycin treated rat showing degenerated hepatocytes (h) with deposition of collagen fibres (f) around the central vein (cv) and in the dilated blood sinusoids (s). (Masson´s trichrome X 400).

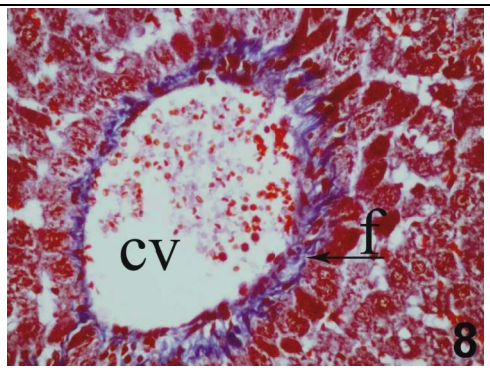

Figure (8): A photomicrograph of a section in the liver of adriamycin treated rat showing remarkable dilatation of the central vein (cv) with deposition of collagen fibres (f) around it . (Masson´s trichrome X 400).

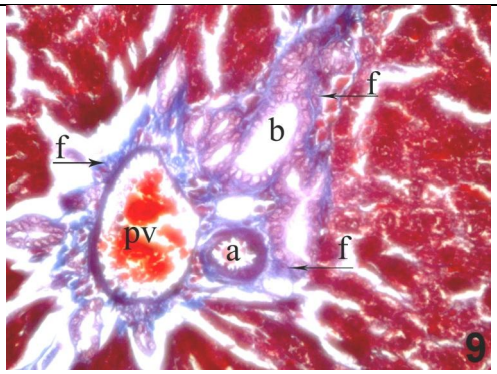

Figure (9): A photomicrograph of a section in the liver of adriamycin treated rat showing branches of portal vein (pv) with eroded lining, hepatic artery (a) and bile duct (b). Marked deposition of collagen fibres (f) is surrounding them. (Masson´s trichrome X 400).

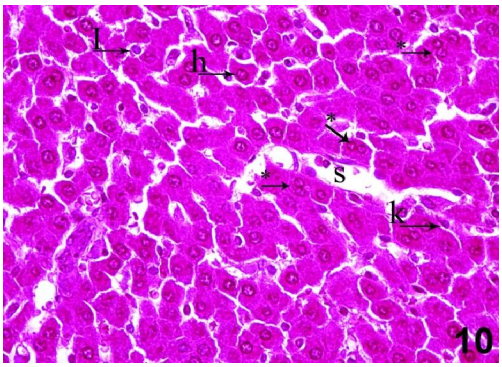

Figure (10): A photomicrograph of a section in the liver of adriamycin and ginger treated rat showing hepatocytes (h) more or less normal with large number of binucleated cells (*). Some inflammatory cells (I) are present. Kupffer cells (k) and mild dilated blood sinusoids (s) are noticed. (H&E X 400).

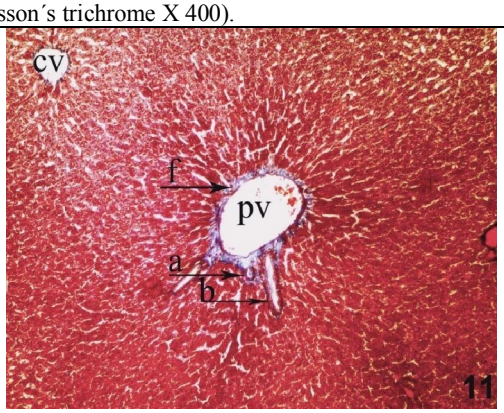

Figure (11): A photomicrograph of a section in the liver of adriamycin and ginger treated rat showing the central vein (cv) and branches of portal vein (pv), hepatic artery (a) and bile duct (b) with mild deposition of collagen fibres (f) surrounding them. (Masson´s trichrome X 100).

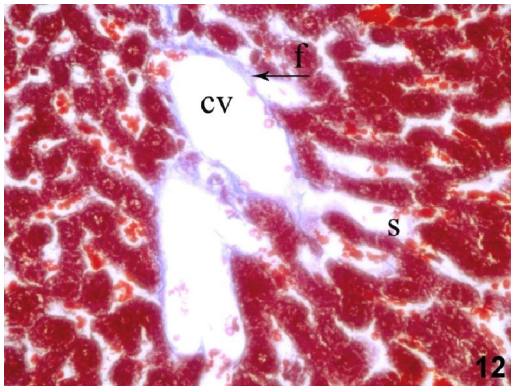

Figure (12): A photomicrograph of a section in the liver of adriamycin and ginger treated rat showing the central vein (cv) with mild deposition of collagen fibres (f) around them and around the blood sinusoids (s). (Masson´s trichrome X 400).

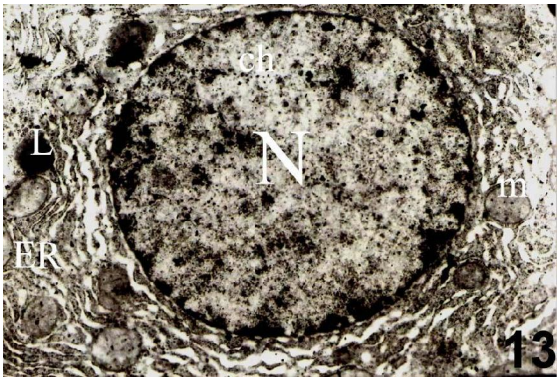

Figure (13): An electron micrograph of control rat´s liver showing a hepatocyte with an active nucleus (N) with euchromatin (ch), lysosome (L), rough endoplasmic reticulum (ER) and mitochondria (m). (X 8000).

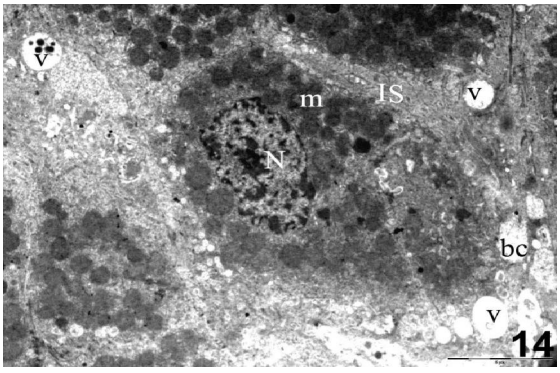

Figure (14): An electron micrograph of adriamycin treated rat´s liver showing degenerated hepatocyte with shrunken and pyknotic nucleus (N) and mitochondria (m) is losing its cristae. Dilated intercellular spaces (IS), vacuoles (v) and dilated bile canaliculus (bc) are also seen. (X 5000).

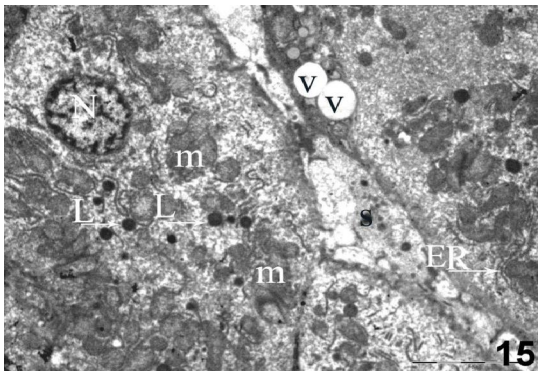

Figure (15): An electron micrograph of adriamycin treated rat´s liver showing degenerated hepatocytes with numerous lysosome (L) of different sizes, damaged mitochondria (m), vacuoles (v), atrophic nucleus (N) and disrupted rough endoplasmic reticulum (ER) are seen. Blood sinusoid (s) is dilated. (X 6000).

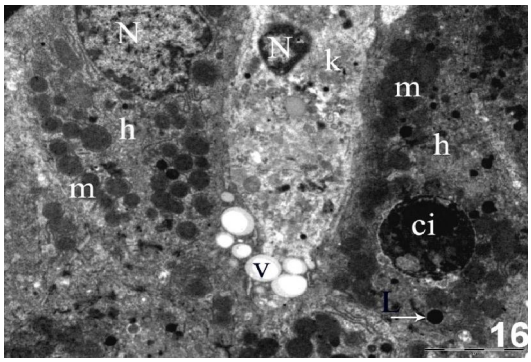

Figure (16): An electron micrograph of adriamycin treated rat´s liver showing degenerated hepatocytes with cytoplasmic inclusion body (ci). Lipid vacuoles (v) and mitochondria (m) losing its cristae are also seen. Notice the irregular nuclear membrane of the nucleus (N). There is also degenerated Kupffer cell (k) with a pyknotic and atrophic nucleus (N) . (X5000).

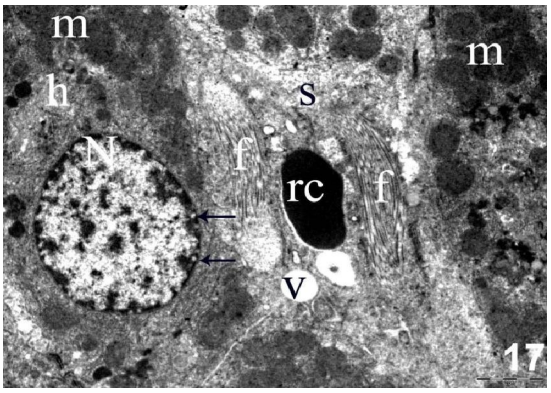

Figure (17): An electron micrograph of adriamycin treated rat´s liver showing degenerated hepatocyte (h) Mitochondria (m) losing its cristae and hepatocyte nucleus (N) with vacuoles in the heterochromatin (arrow). Deposition of collagen fibres (f), vacuoles (v) and red blood corpuscle (rc) in blood sinusoid (s) between hepatocytes (h) are also seen. (X 8000).

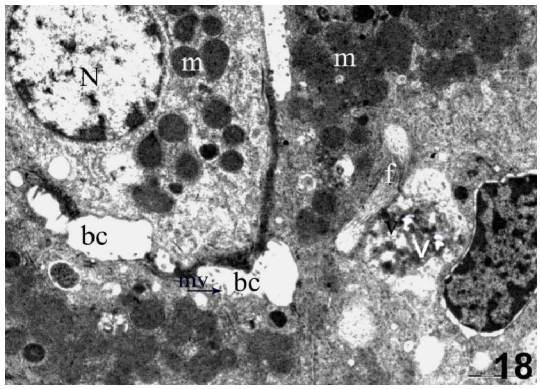

Figure (18): An electron micrograph of adriamycin treated rat´s liver showing hepatocyte with more or less normal nucleus (N) is seen. Dilated bile canaliculi (bc) with damaged and atrophy of its microvilli (mv). Deposition of collagen fibres (f) is observed. Large vacuole (v) and mitochondria (m) losing its cristae are also seen. (X 8000).

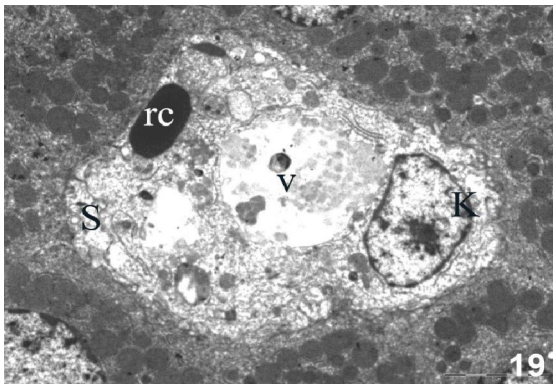

Figure (19): An electron micrograph of adriamycin treated rat´s liver showing swollen and damaged Kupffer cell (k) which contains large vacuole (v) and damaged cytoplasm with destructed of most organs. Red blood cell (rc) is seen in the blood sinusoid (s). (X 5000)

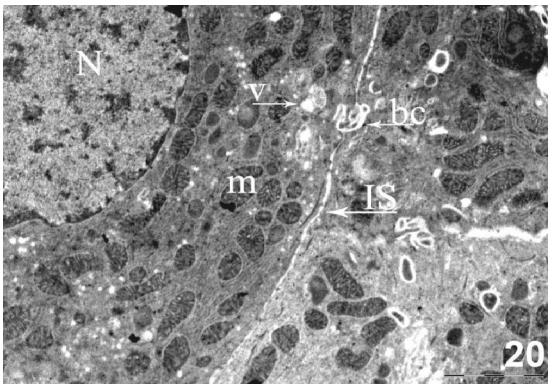

Figure (20): An electron micrograph of adriamycin and ginger treated rat´s liver showing more or less normal intercellular space (IS), bile canaliculi (bc) with some vacuoles (v). Notice the mitochondria (m) with normal cristae is present. (X 12000

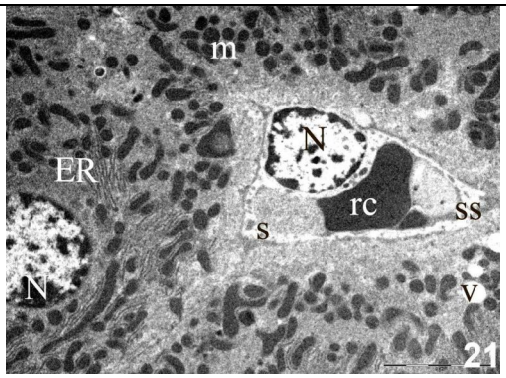

Figure (21): An electron micrograph of adriamycin and ginger treated rat´s liver showing a few vacuoles (v) are observed in the hepatocyte cytoplasm. Nucleus (N), mitochondria (m) and rough endoplasmic reticulum (ER) are more or less normal. Kupffer cell nucleus (N), space of Disse (ss) and red blood corpuscle (rc) in the blood sinusoid (s) are also seen. (X 6000).

4. Discussion

In this study, the health of animals was carefully monitored because of studies observing that animals sustaining liver damage can lose body weight and activity.[17] The result of administration of ginger was not associated with any mortalities or significant weight loss or changes in liver weight. Ginger did not induced any pathological abnormality or changes in general conditions, body weight, and food and water consumption.[18] Rats treated with the adriamycin alone (group III) showed a significant decrease in body weight gain, suggesting that hepatotoxicity might have contributed to this loss.[19] It has been shown that adriamycin induced damage in the liver.[20] Loss of the body weights was due to loss of skeletal muscles and adipose tissue.[21] The reduction in body weight is correlated also with the decreased food intake observed during the experimental period.

The dose of adriamycin used in this study corresponds to the dose that currently being used in clinical practice.[22] The present investigation showed many histopathological and ultrastructural abnormalities in the liver including marked disruption of hepatic cords, dilated central vein and blood sinusoids, inflammatory infiltration and deposition of collagen fibers. Adriamycin has been shown to induce accumulation of inflammatory cells indicating hepatic damage.[2] Periportal fibrosis were detected after adriamycin administration.[23]The present results is in agreement with the research of El-Sayyad *et al*.[23]

Many hepatocytes showed degeneration and necrosis with karyorrehexis indicating apoptosis. Adriamycin is thought to kill cells primarily by fragmenting DNA and causing cell death by apoptosis.[24] Apoptosis is a common feature of hepatotoxicity induced by many chemicals, as thioacetamide and acetaminophen.[25,26] Vacuole like defects appeared in the heterochromatin region of hepatocytes of adriamycin treated group. Compared with adriamycin and ginger group no heterochromatin vacuoles were found. Nuclear vacuolation in hepatocytes is a marker of senescence and likely to be a consequence of liver injury.

Adriamycin affects many intracellular targets in liver cells.[24] Mitochondria have been considered as one of the targets in adriamycin-induced subcellular damage. As an inhibitor of mitochondrial complex-I, adriamycin produces reactive oxygen species which can result in oxidative stress and subsequent apoptosis.[27]

Cytoplasmic inclusion bodies were observed in adriamycin treated group III. It was suggested that disruption of mitochondria liberates the inclusions into the cytoplasm.[28] Inclusion bodies induced by adriamycin have previously been reported as being homogenously distributed through the liver.[29]

The hepatocytes in adriamycin treated rats show widening and dilatation of the intercellular space. Acute stress can prompt intercellular space dilation in another study.[30]

The sinusoidal dilatation and lesions observed in group III were morphologically similar to those seen in veno-occlusive disease in humans.[31] The intensity of the sinusoidal dilatation and congestion was occasionally severe, and included the disruption of the sinusoidal wall integrity. It was illustrated by the extravasation of erythrocytes in Disse's space. Deposition of collagen-like fibrous materials also were seen in the present study in the space of Disse, which can inhibit the exchange between the sinusoids and parenchymal cells, ultimately producing liver cell injury. Structural integrity of the sinusoids is necessary for the maintenance of normal exchanges of fluids, particles, and metabolites. Impairment of substrate exchange is a major contributing factor of hepatic dysfunction in liver fibrosis.[32] Liver fibrosis is a response to various chronic liver injuries.[33] The process leading to liver fibrosis resembles the process of wound healing, including inflammation, synthesis of collagenous and noncollagenous extracellular matrix components, and tissue remodeling.[34] During the process of liver fibrosis, the collagen increase and form a basement-membrane-like structure within the space of Disse. At the cellular level,

origin of liver fibrogenesis is initiated by the damage of hepatocytes, resulting in the recruitment of inflammatory cells and platelets, and activation of Kupffer cells, with subsequent release of cytokines and growth factors.[34]

Electron microscopy study done by Kannan *et al* ,[35] as in the present study revealed the presence of lipid vacuoles in the liver of desethylaminodarone-treated rats. The clear cytoplasmic vacuoles contain predominantly electron-lucent material consistent with phospholipid.[36] Lysosomes are the primary site for phospholipid accumulation,[37] which results from an inability of the cell to catabolize this substrate.[38]

Damaged Kupffer cells which contains large vacuole were demonstrated in the present study. The liver is known to be a major immunological organ affecting systemic responses in animals because of the abundance of mononuclear phagocytes.[39] The Kupffer cells and liver phagocytic system produces an array of mediators which protect the body against invasion by xenobiotic chemicals and materials.[40]

Liver is the main site of adriamycin metabolism. A number of studies indicate that enzyme activation of adriamycin begins with the drug conversion to a semiquinone free radical.[41] Reduction of side chain carbonyl group yields a more toxic metabolite. Such metabolite accumulates in the heart and contributes to heart failure.[42] The liver may play an essential role in heart damage. Also the improvement of liver toxicity may prevent cardiac damage.

Some side-effects of the adriamycin in this study reveals drastic pathological features at both structural and ultrastructural levels, which could be used as the basis for determining the appropriate way to reduce their hepatotoxic effects.

The production of free radicals by adriamycin is proposed to be critical for cytotoxicity. Adriamycin metabolism triggered production of reactive oxygen species and reactive intermediates in liver resulting in oxidative stress followed by cell death.[43] Antioxidants prevent the adriamycin-induced toxicity in experimental animals as well as in human.[44,45] The study using ginger had reported the significant antioxidant activities.[46] It is likely that the preventive effects of ginger in adriamycin toxicity are based on its antioxidant activity. Ginger also inhibits NO synthesis in activated macrophages and prevents oxidation and nitration reactions induced by peroxynitrite.[47] Moreover, a recent study by El-Sharaky *et al*.[48] showed that treatment with ginger decreased the hepatic tissue content of NO induced by administration of bromobenzene.

Recent experimental observations reported that ginger is an effective anticancer agent.[6] Further, ginger can relieve the chemotherapy associated nausea and vomiting in patients.[49] The present study proved that the hepatic pathology caused by administration of adriamycin ameliorated by combined treatment with ginger.

Mitochondria are nearly normal in adriamycin and ginger treated group. Mitochondria are natural targets of phytochemical antioxidant protection.[27] Ginger could protect against adriamycin toxicity through a number of mitochondrial actions including up-regulation of specific anti-reactive oxygen species proteins, prevention of mtDNA damage, stimulation of replication, inhibition of membrane-active lipases, and protection of the electron transport chain.[50]

Recently, there are some interesting reports of plant-derived antifibrotic agent in experimental animals.[51] The percentage reduction in deposition of collagen fibers scores was about 64% in coadministration of ginger and adriamycin. Salvia miltiorrhiza, silymarin and Curcumin have been shown respectively to reduce the severity of hepatic fibrosis in treated rats.[52,53,54] The present study suggests the potential of ginger as anti-fibrotic agents.

In the present findings, the hepatotoxic pathological effect of adriamycin was ameliorated with partial disappearance of hepatic damage when treatment was combined with ginger. Degeneration and necrosis were decreased by 62% when ginger was combined with adriamycin. Thus, ginger appears to play a key role in the attenuation of hepatic injury, and then preserve the structural integrity of the hepatocellular structures.

More effective approaches are needed for increasing the therapeutic activity of anticancer drugs and decreasing systemic toxicity, with considerable interest focused on improvements through combination chemotherapy.[55,56] Ginger treatment afforded substantial protection against adriamycin liver damage, and merits careful evaluation for hepatoprotective potential. Combination chemo-therapy with this phytochemical would have the potential of expanding the therapeutic efficacy and possibly broaden the applications of adriamycin treatments. Appropriate protective measures as using ginger must be applied with anticancer treatment for improving liver structure.

Conclusion:

The present results provide in vivo evidence of direct chemotherapeutic hepatotoxicity caused by adriamycin. Histopathological evaluations showed that ginger protects and reduces most of the damage caused by adriamycin in the liver. The protective properties of ginger and the fact that ginger has been used by humans in food, makes it a potential therapy for adriamycin-induced hepatic toxicity. The results can further suggest the possible use of ginger against oxidative stress induced organ toxicities.

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