Aged Garlic Extract Protects Against Cisplatin-Induced Hepatotoxicity in Adult Male Rats: Biochemical and Ultrastructure Study

Ashraf Youssef Nasr

Anatomy Department, Faculty of Medicine, Zagazig University, Egypt
E-mail: ashrafnaeem2013@gmail.com; ashrafnaeem2013@yahoo.com

Abstract: Background: Cisplatin (CP) is one of the most active cytotoxic drugs. However, it has several side effects that are associated with increased oxidative stress. Aged garlic extract (AGE) is a natural product containing different compounds with antioxidant activity. **This study aimed** to evaluate the protective effects of AGE against cisplatin-induced hepatotoxicity. **Material & Methods:** Twenty four adult male Wistar rats were divided into four equal groups: control, AGE -treated, CP-treated, combined AGE and CP-treated. All rats were weighed once every three days. On the 22$^{th.}$ day, the rats were anesthetized by ether inhalation then blood samples were collected to assess liver biomarkers and the liver of each rat was excised, cleaned and weighed. Examination of liver was performed using light and electron microscopy. **Results**: CP-treated rats exhibited a significant increase in the serum levels of liver enzymes in addition to the significant reduction in the body and liver weights. Also, livers of CP-treated rats showed different histopathological and ultrastructural changes in the form of necrotic changes, dilatation and congestion of central vein, blood sinusoids and portal venules, hyperplasia of bile canaliculi, rarified cytoplasm containing dilated RER, pleomorphic mitochondria and many lysosomes and different apoptotic nuclear changes. However, animals pretreated with AGE showed significant decrease in the serum level of liver biomarkers and an improvement in the histopathological and ultrastructural changes of hepatocytes induced by CP. **Conclusion:** AGE has an ameliorative effect against cisplatin-induced hepatotoxicity and could be used as a dietary supplementation to reduce cisplatin toxic side effects.

Ashraf Youssef Nasr. **Aged Garlic Extract Protects Against Cisplatin-Induced Hepatotoxicity in Adult Male Rats: Biochemical and Ultrastructure Study.** *Life Sci J* 2014;11(12):92-101]. (ISSN:1097-8135). http://www.lifesciencesite.com. 16

Key words: Cisplatin, Aged garlic extract, Liver, Histopathology, Ultrastructure, Rat.

1. Introduction:

Cisplatin (CP), a potent antineoplastic drug, is widely used in the treatment of different solid-organ tumors. However, its clinical use is limited due to its toxic side effects including nephrotoxicity, neurotoxicity, ototoxicity and hepatotoxicity.[1,2] The toxicity of CP seems to be dose-dependent due to the cumulative effect of the drug[3], where its accumulation produces obvious necrotic changes within the tissues of the affected organs. The generation of reactive oxygen species (ROS) and nitrogen species (NS) is one of the possible mechanisms responsible for cisplatin toxicity through their oxidative stress injury and suppression of the antioxidant defense system.[4] To ameliorate the toxic effect of CP without inhibiting its antitumor effects, different experimental studies were carried out using a combination of CP with various radicular scavengers, enzyme inhibitors, sulfur-containing antioxidants and natural foods with antioxidant properties.[1,2]

Natural and herbal products have been used in traditional medicine to treat a variety of diseases including malignancies.[5] The anticancer activities of the extract from a number of herbal plants have been demonstrated. A number of previous studies concluded that herbal medicine might have anticancer effect by enhancing the immune system, including cell differentiation, inhibiting telomerase activities and inducing apoptosis of cancer cells.[6]

Garlic (Allium Sativum) is a worldwide traditional food and dietary supplement. Nowadays, many garlic preparations are used in the medical field including fresh garlic extract, garlic oil, aged garlic extract (AGE) and a number of organosulfur compounds. AGE is an odorless garlic preparation produced by prolonged extraction of fresh garlic at room temperature for at least 20 months. AGE contains mostly stable water-soluble organo-sulfur compounds which are responsible for the health benefits of AGE that have potent antioxidant and free radical scavenging activities, immune stimulation, anti-cancer and anti- infectious properties.[7]

Garlic and its derivatives have been previously used in the protection against drug-induced cardiotoxicity[8], nephrotoxicity[2,9] and cadmium-induced liver damage.[10]

The present study aimed to evaluate the possible morphological and biochemical protective effects of AGE on CP-induced hepatotoxicity in adult male rats, using light and electron microscopy in addition to the liver biomarkers.

2. Material and Methods

CP was obtained in the form of commercial Egyptian Unistin Vial (Egyptian International medical Company (EIMC) United Pharmaceuticals, Cairo, Egypt).

AGE (kyolic) was obtained from Wakunaga of America (Mission Viejo, CA).

Twenty-four adult male Wister albino rats (12-14 weeks of age) were supplied from the animal house, Faculty of Medicine, Zagazig University, Zagazig, Egypt. The rats were kept under appropriate conditions of temperature (25 ±2°C), humidity (60–70%) and light (12h dark/light cycle), free access of a commercial balanced diet and tap water *ad libitum*.

After one week of acclimatization, the animals were randomly divided into four equal groups in separate plastic cages, six rats each. Two groups (I and II) were used as control and received normal saline 0.5ml i.p. and distilled water P.O. (group I) and AGE 250mg/kg orally (group II) for 21 days. Groups (III and IV) received single i.p. dose of CP (7.5mg/kg) on day 16th, after successive administration of distilled water (0.5ml, orally, group III) or AGE (250 mg / kg orally, group IV). The initial and final body weights of rats were recorded.

On day 22$^{th.}$, the rats were anesthetized by ether inhalation and a vertical midline thoracic and abdominal incision was done to explore their viscera. Blood samples were collected in sterile labeled heparinized test tubes through a direct intracardiac puncture from each rat. The sere were separated and stored in a freezer at a -20°C for subsequent assessment of liver functions.

Assays for serum aspartate transaminase (AST) and alanine transaminase (ALT) were carried out using commercial kits (Roche Diagnostics, GmbH, D-68298, Mannheim, Germany) according to Reitman and Frankel.[11] Serum albumin and total bilirubin were determined using commercial kit supplied by Diamond, RA50, Ireland. Also, total bilirubin (TSB) in serum was assayed according to the method of Schmidt and Eisenburg.[12]

Liver of each animal was excised, cleaned, washed, blotted and weighed. The relative liver weight/body weight ratio (hepatosomatic index) was calculated according to the following formula, organ weight ratio (%) = organ weight X 100/ body weight.[13,14]

Tissue processing:

A. Light Microscopy: Specimens from each liver were fixed in 10% neutral-buffered formalin solution for 48 hours, dehydrated in ascending grades of ethyl alcohol, cleared in xylol and embedded in paraffin blocks. Serial sections (3-5µm) were cut using microtome (Leica RM 2125, Leica Biosystems Nussloch GmbH, Germany). The sections were washed in a water bath and left in the oven for dewaxing. Thereafter, the sections were stained with hematoxylin and eosin for general histological features determination[15] The stained tissue-slides were mounted with DPX (Di-N-Butyle Phthalate Xylene) and covered with cover slips. All slides were examined by a light microscope (Olympus BH-2, Olympus, Tokyo, Japan).

B. Electron microscopy: Specimens of 1mm^3-size from liver of each rat were immediately immersed in 2.5% glutaraldehyde buffered with 0.1 M phosphate buffer for 24-48 hours. Then, the specimens were washed in phosphate buffer (pH 7.2–7.4) 3–4 times for 20 min every time and post-fixed in a buffered solution of 1% osmium tetroxide for 2 h, after that washed in the same buffer 4 times for 20 min each. After fixation, the specimens were dehydrated in ascending grades of ethyl alcohol. Then, they were cleared in propylene oxide, embedded in a mixture of 1:1 of Epon resin - Araldite for 1 h. Polymerization was performed in the oven at 65°C for 24 hrs.[16]. Semi-thin sections (One µm-thick) were cut with a glass knife on LKB-2000S ultramicrotome, mounted on glass slides and stained with buffered toluidine blue. The appropriate areas were selected with the light microscope. The resin blocks were trimmed to get rid of the undesired tissue. Ultrathin sections (60-90 nm thick) were cut with a glass knife on a LKB ultramicrotome, then mounted on copper grids, double stained with uranyl acetate and lead citrate . The grids were examined and photographed using a transmission electron microscope (JEOL TEM-1200EX, Tokyo, Japan) operated at 60–70 kV, Faculty of Science, Ain Shams University.

Statistical analyses: Results were expressed as mean ± SEM. Comparison of means was done by the student's t-test (One way ANOVA) and the Mann-Whitney U test. Values of $P < 0.05$ were considered statistically significant. Statistical evaluation was conducted with SPSS version 16.0 (SPSS Inc., Chicago, IL, USA).

The study was performed after the approval of the Medical Ethical Committee of the Faculty of Medicine, Zagazig University, Zagazig, Egypt and followed the recommendations of the National Institutes of Health Guide for Care and Use of Laboratory Animals.

3.Results:

Body and liver weights of rats: A significant (P <0.005) decrease in the final body weight was observed in CP-treated rats as compared to the control rats (Figs. 1A & B). Also, there was a significant difference ($P.< 0.001$) in the final body weight in AGE+ CP-treated group with respect to control rats. On the other hand, the final body weight

of combined AGE and CP- treated rats revealed no significant ($P > 0.05$) difference compared to CP-treated rats. Also, the final body weight of AGE-treated rats showed no significant difference compared to control rats as well (**Table, 1**).

A significant ($P < 0.001$) decrease in absolute and relative liver weights was observed in CP-treated rats compared to control rats. However, a significant increase in both absolute ($P < 0.05$) and relative ($P < 0.001$) weights of liver was noticed in AGE + CP-treated rats compared to CP-treated rats (**Table, 1**).

Liver biomarkers of rats: Administration of CP induced a significant ($P < 0.01$) elevation in serum AST, ALT and TSB levels compared to those of the other three groups. However, a significant ($P < 0.01$) decrease in the serum levels of these biomarkers was observed in AGE+ CP- treated rats compared to CP-treated group. In addition, the serum albumin level showed a significant ($P < 0.01$) decrease in CP-treated rats as compared to control rats, meanwhile a significant ($P < 0.05$) increase in serum albumin level was noticed in AGE+CP-treated rats compared to CP-treated group (**Table 2**).

Table (1): Effect of cisplatin and / or aged garlic extract treatment on body and liver weights of rats

Animal group	Body weight			Liver Weight	
	Initial	Final	Changes	Absolute	Relative
Control	267.2±2.3	307.8±2.7^{b*}	(+) 40.7±1.9	11.1 ± 0.007^{b}	3.6±0.0002^{b**}
AGE-treated	268±2.2	306.3±2.3^{b*}	(+) 38.3±1.05	10.8 ±0.006^{ab}	3.5±0.0001^{ab**}
CP-treated	302.5±3.8	284.8±4.5^{a*}	(-) 17.3± 1.4	9.8 ± 0.13^{a}	3.4±0.0004^{a**}
AGE + CP	290.7±2.1	286.3±2.3^{a*}	(+) 4.3±0.33	10.2 ± 0.12^{ab}	3.6±0.0005^{ab**}

- Data are expressed as Mean ± SEM (n=6). $P < 0.01$ compared to control (^{a}) or cisplatin-treated (^{b}) rats at $P < 0.005$. (*): Significant difference between initial and final body weight (BW) of same group in all groups at $P. < 0.001$ except cisplatin-treated at $P. = 0.0295$ (< 0.05). (**):Significant difference between absolute and relative liver weights of same group at $P.< 0.0001$. CP: cisplatin; AGE: aged garlic extract.

Table (2): Effect of cisplatin and / or aged garlic extract treatment on liver biomarkers of rats

Animal group	ALT	AST	TSB	Albumin
Control	54.9 ± 1.2^{b}	42.0 ± 1.3^{b}	1.17 ± 0.006^{b}	4.55 ± 0.008^{b}
AGE-treated	59.5 ± 1.7^{b}	51.5 ± 1.99$^{a**, b}$	1.13 ± 0.006$^{a, b}$	4.32 ± 0.006$^{a, b}$
Cisplatin-treated	106.4 ± 3.7^{a}	91.8 ± 1.7^{a}	2.5 ± 0.009^{a}	3.3 ± 0.008^{a}
AGE + Cisplatin	74.3 ± 3.1$^{a*, b}$	64.9 ± 1.5$^{a, b}$	1.6 ± 0.007$^{a, b}$	3.9 ± 0.008$^{a, b}$

- Data are expressed as Mean ± SEM (n=6). $P < 0.001$ compared to control (^{a}) or cisplatin-treated (^{b}) rats. a*: $P.= 0.045$; a**: $P. = 0.0025$. TSB: total serum bilirubin; AST: Aspartate amino transferase enzyme; ALT: Alanine amino transferase enzyme.

Histopathological examination:

In the centrilobular hepatic parenchyma of control rats revealed normal architecture of radiating cords of hepatocytes from the central vein with blood sinusoids lined by flat endothelial and large kupffer cells in-between (**Figure 1A**). Mild congestion of the central vein were seen in the centrilobular zone of AGE-treated rats (**Figure 1B**). However, the liver parenchyma of CP-treated rats showed disorganized centrilobular hepatic cords, dilated and congested central vein and blood sinusoids, cellular necrosis, nuclear pyknosis with margination of its heterochromatin and destructed nuclear membrane (**Figure 1C**). On the other hand, mild dilatation and congestion of the central vein were observed in the hepatic parenchyma of the combined AGE+CP-treated rats (**Figure 1D**).

In the periportal area, the portal tracts were surrounded by condensed hepatic cords of small sized hepatocytes with irregular blood sinusoids in-between in control rats. Each portal tract consisted of bile ductule, hepatic arteriole and portal venule surrounded by collagen fibers **(Figure 2A)**. Dilated congested portal venules were seen within the portal tracts in both AGE-treated rats (**Figure 2B**). However, there were wide portal tracts with dilated and congested portal venule, proliferation and dilatation of bile ductules in the periportal area of CP-treated rats (**Figure 2C**). On other hand, normal parenchymal architecture with congested portal venules are observed in combined AGE+CP-treated rats (**Figure 2D**).

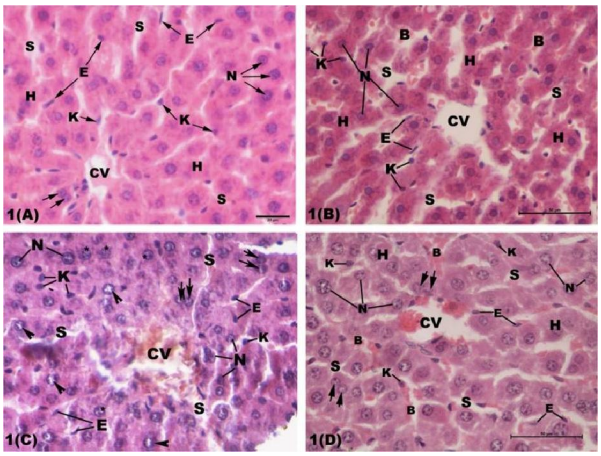

Figure (1): Light micrographs of the centrilobular zone of rats' livers showing; **1(A):** normal hepatic cords (H) radiating around the central vein (CV) with blood sinusoids (S) in-between. The hepatocytes exhibit central rounded nuclei (N). Few hepatocytes have two nuclei (double arrows). The sinusoids are lined by numerous flat endothelial (E) and few large kupffer (K) cells. **1(B):** Congested central vein (CV) is seen in aged garlic extract-treated rats. **1(C):** In cisplatin-treated rats, disorganized hepatic cords (H), centrilobular cellular degeneration with dilated and congested central vein (CV) and blood sinusoids (S) are observed. The nuclei (N) of some hepatocytes reveal peripheral condensation of heterochromatin (arrow head) and discontinuous nuclear envelope (*) with few binucleated cells (double arrows). **1(D):** Mild dilatation and congestion central vein (CV) is observed in the hepatic parenchyma of combined aged garlic extract and cisplatin-treated rats. B: blood cells.

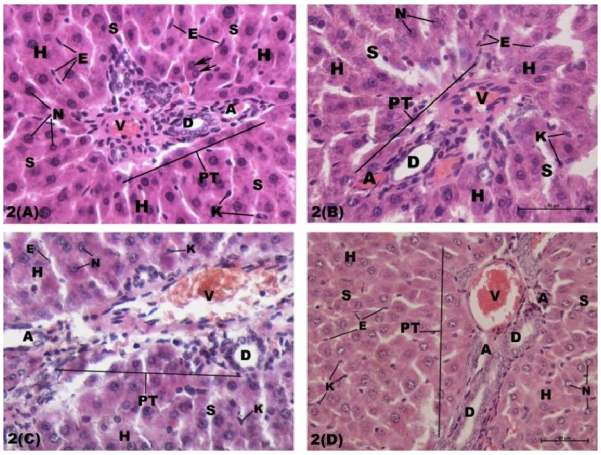

Figure (2): Light micrographs of the periportal zone of the hepatic lobule of the rats' livers showing **2(A):** normal portal tract (PT) consisting of bile ductule (D), hepatic arteriole (A) and portal venule (V) in control rats. The tracts are surrounded by a network of hepatic cords (H) with blood sinusoids (S) in-between. **2(B):** In aged garlic extract-treated rats; congested portal venules (V) are seen within the portal tract. **2(C):** In cisplatin-treated rats; wide portal tract (PT) with dilated congested portal venule (V), hyperplastic bile ductule (D) are seen within the periportal area. **2(D):** In combined aged garlic extract and cisplatin-treated rats; normal portal tract with congested portal venule (V) is observed in periportal area. E: endothelial cells; K: kupffer cells; N: nucleus.

Electron Microscopic Results:

Ultrastructure of the hepatocytes of control rat liver displayed regular oval-shaped heterochromatic nuclei with central electron-dense nucleoli. Their cytoplasm contained numerous round and oblong-shaped mitochondria, parallel flattened cisternae of rough endoplasmic reticulum, large scattered masses of glycogen granules, few lysosomes and lipid droplets (**Figure 3**). The biliary surface of the cell membranes of neighboring hepatocytes exhibited many long microvilli and tight junctions at the ends of the bile canaliculi (**Figure 4**). The blood sinusoids were lined by flat endothelial cells and large kupffer cells. The endothelial cells had long flat nuclei surrounded by little amount of cytoplasm containing a few organelles; while the kupffer cells showed large heterochromatic nuclei surrounded by excessive amount of cytoplasm. The sinusoidal surface of hepatocytes exhibited numerous microvilli projecting within the Disse space underneath the endothelial lining cells (**Figure 5**).

In AGE-treated rat liver, the hepatocytes showed normal central oval nuclei surrounded by granular cytoplasm. The cytoplasm contained numerous round-shaped mitochondria, groups of parallel rough endoplasmic reticulum cisternae, many lysosomes, small masses of glycogen granules and numerous free ribosomes (**Figures 6, 7**). Dilated bile canaliculi with many short microvilli projecting within their lumina were seen between the cell membranes of the neighboring hepatocytes as well (**Figure 7**). The blood sinusoids were lined by large kupffer cells and flat endothelial cells. Collagen bundles were present within the perisinusoidal space (**Figure 8**).

In CP-treated rat liver, most of the hepatocytes exhibited rarified cytoplasm containing compacted small-sized mitochondria with electron dense matrix and swollen destructed cristae, a few dilated cisternae of rough endoplasmic reticulum and many lysosomes. The cells had regular round-shaped euchromatic nuclei (**Figure 9**). The nuclei of some hepatocytes showed an irregular outline and marginal condensation of their heterochromatin with large electron-dense centrally located nucleoli. The cytoplasm of these cells exhibited dilated rough endoplasmic reticulum cisternae parallel to nuclear envelop and between the mitochondria, many small sized mitochondria with electron dense matrix and degenerated cristae, many lysosomes of different size and homogenous electron-dense microbodies (**Figure 10**). Wide intercellular spaces were observed among the parenchymal cells. Congested dilated blood sinusoids were seen between the hepatocytes. The sinusoidal surface of the hepatocytes exhibited few short microvilli projecting within the Disse space

underneath their lining endothelial cells (**Figure 11**). Wide bile canaliculi with short studded microvilli were observed between the cell membranes of the neighboring hepatocytes (**Figure 12**).

In combined AGE and CP-treated animals, the polygonal hepatocytes revealed oval heterochromatic nuclei with peripheral electron-dense nucleoli. The granular cytoplasm contained numerous round mitochondria, scattered single and groups of rough endoplasmic reticulum cisternae and a few lipid droplets (**Figures 13, 14**). Dilated bile canaliculi with short microvilli were seen between the hepatocytes with evident junctional complexes at their ends (**Figure 14**). The sinusoidal surface of the hepatocytes displayed numerous microvilli projecting within the Disse space underneath the endothelial lining cells (**Figure 15**).

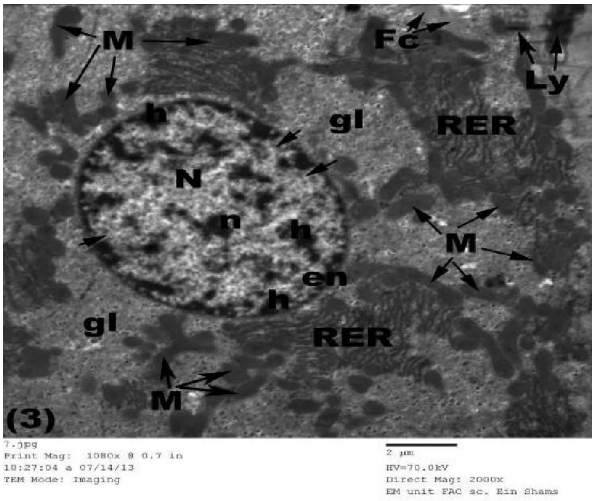

Figure (3): An electron micrograph of a control rat's liver showing regular oval-shaped nuclei (N) of the hepatocytes having variable-sized masses of the condensed heterochromatin (h) on the inner aspect of nuclear envelop (en) around the nuclear pores (arrow) and within the nucleoplasm. The cytoplasm contains numerous round and oval-shaped mitochondria (M), numerous rough endoplasmic reticulum cisternae (RER) in parallel groups of different number and singly encasing the mitochondria, scattered masses of glycogen granules (gl), few lysosomes (Ly) and fat globules (Fc). (Bar=2µm; Original mag.= x 2000; Printed mag.= x 10800)

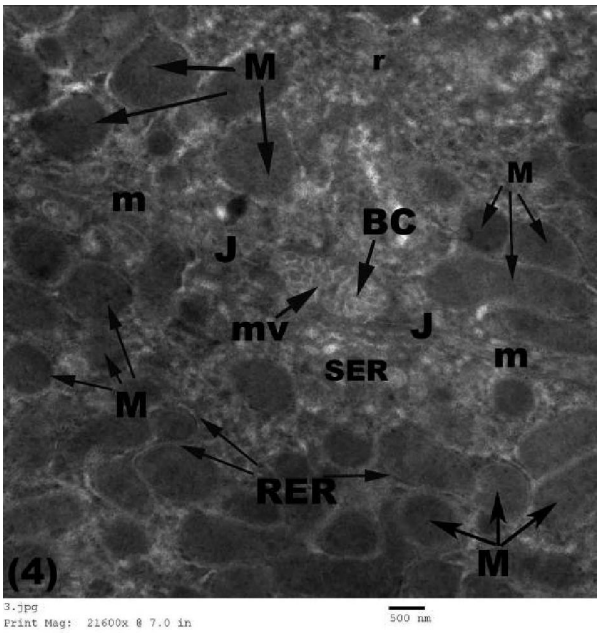

Figure (4): An electron micrograph of a control rat's liver showing the cell membranes (m) of the neighboring hepatocytes exhibit tight junctions (J) at the ends of the bile canaliculi (BC) and many long microvilli (mv) projecting within the biliary lumen. RER: rough endoplasmic reticulum; SER: smooth endoplasmic reticulum; M: mitochondria.
(Bar = 20 nm; Original Mag. = x 4000; Printed mag. = x 21600)

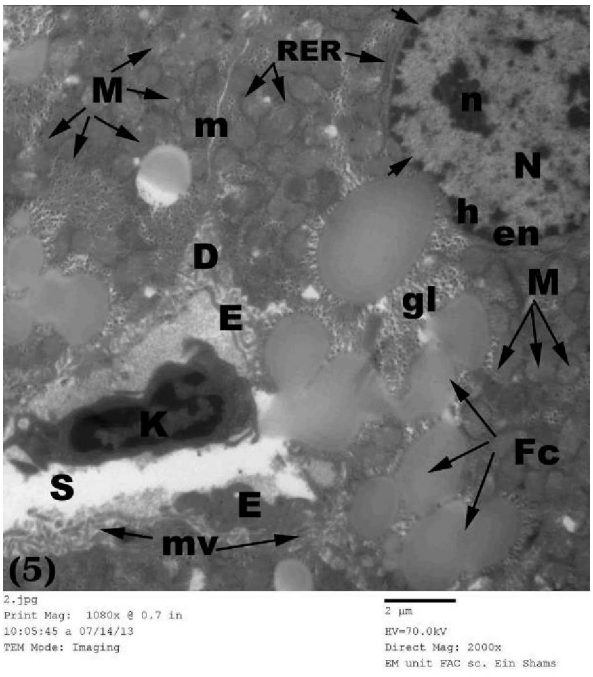

Figure (5): An electron micrograph of a control rat's liver showing the blood sinusoid (S) is lined by flat endothelial (E) and large kupffer (K) cells. The sinusoidal surface of hepatocytes shows numerous microvilli (mv) projecting within the space of Disse (D) underlying the endothelial cell. B: blood cells; Fc: fat globules; M: mitochondria; Gl: glycogen granules.
(Bar=2µm; Original mag.= x 2000; Printed mag.= x 10800)

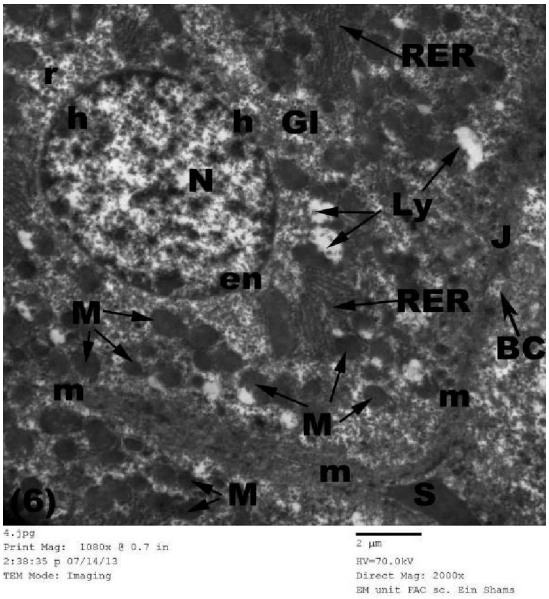

Figure (6): An electron micrograph of aged garlic extract-treated rat's liver showing the hepatocytes having oval-shaped nuclei (N) with regular nuclear envelope (en), peripheral nucleoli (n) and electron-dense masses of heterochromatin (h). The cytoplasm contains numerous round-shaped mitochondria, rough endoplasmic reticulum cisternae (RER), free ribosomes (r), glycogen masses (Gl) and few lysosomes (Ly). Bile canaliculi (BC) are seen between the cell membranes (m) of the neighboring hepatocytes with tight junctions (J) at their ends.
(Bar=2µm; Original mag.= x 2000; Printed mag.= x 10800)

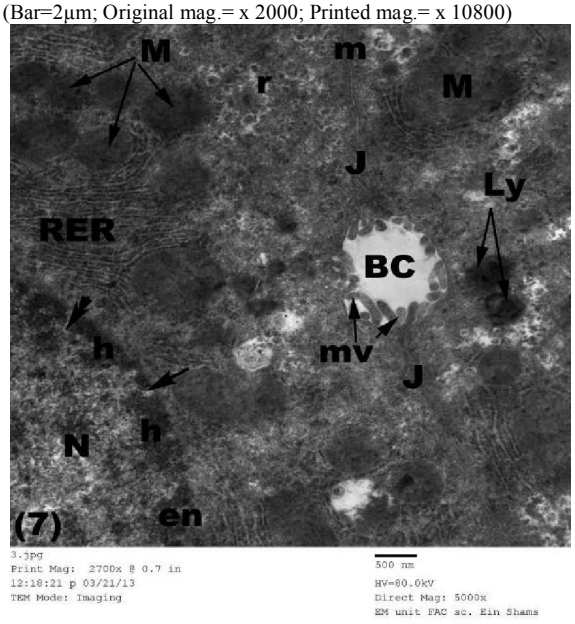

Figure (7): An electron micrograph of aged garlic extract-treated rat's liver showing wide bile canaliculi (BC) between the cell membranes (m) of the neighboring hepatocytes. Many short microvilli (mv) project within the biliary lumen. Tight junctions (J) are seen at their ends. many rounded mitochondria (M), rough endoplasmic reticulum cisternae (RER), free ribosomes (r) and few lysosomes (Ly) are seen within the pericanalicular cytoplasm. N: nucleus, h: heterochromatin; arrow: nuclear pores; en: nuclear envelop.
(Bar = 500 nm; Original mag. = x 5000; Printed mag. = 27000)

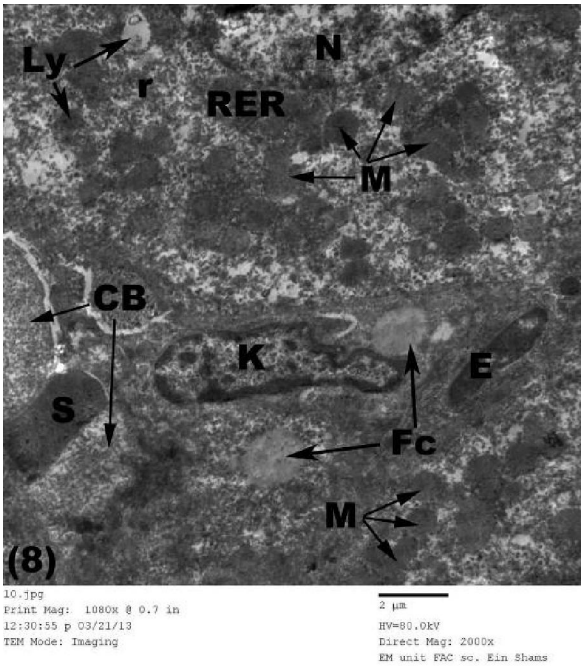

Figure (8): An electron micrograph of aged garlic extract-treated rat's liver showing the blood sinusoids (S) lining with the flat endothelial cells (E) and large kupffer cells (K). Many bundles of collagen fibers (CB) are seen within the perisinusoidal space. M: mitochondria; Fc: fat globules; RER: rough endoplasmic reticulum; r: free ribosomes; Ly: lysosomes; N: nucleus.
(Bar=2µm; Original mag.= x 2000; Printed mag.= x 10800)

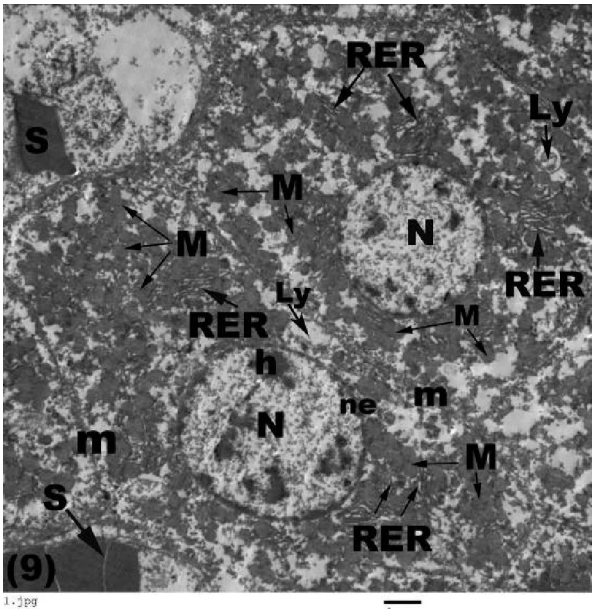

Figure (9): An electron micrograph of a cisplatin-treated rat's liver showing the hepatocytes having round nuclei (N) with little amount of peripherally condensed heterochromatin (h) on the inner aspect of nuclear envelop (en). The rarified cytoplasm contains many clumped round-shaped mitochondria (M), few dilated rough endoplasmic cisternae (RER) and many lysosomes (Ly). m: cell membrane.
(Bar=2µm; Original mag.= x 1000; Printed mag.= x 5400)

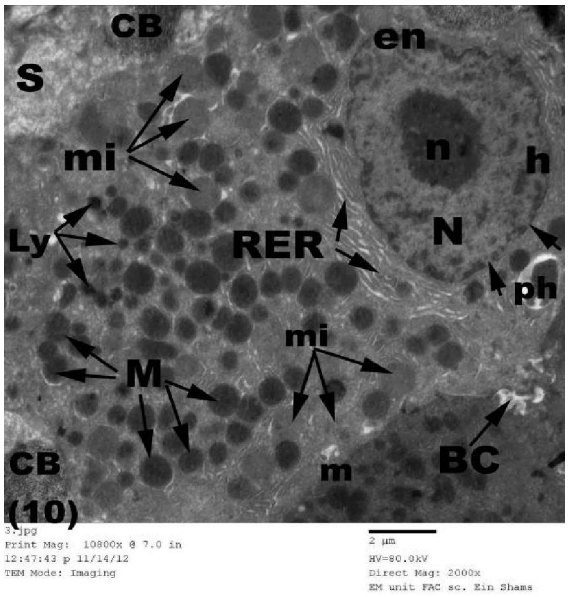

Figure (10): An electron micrograph of a cisplatin-treated rat's liver showing some hepatocytes having nuclei (N) with irregular nuclear envelop (en), little amount of peripheral condensed heterochromatin (h) and large electron dense centrally locating nucleoli (n). The cytoplasm contains many dilated rough endoplasmic reticulum cisternae (RER) around the nuclei and in-between the clumped electron-dense rounded mitochondria (M). Many homogenous electron dense microbodies (mi) and lysosomes are seen between the mitochondria as well. Collagen bundles (CB) are seen within the perisinusoidal space. S: blood sinusoid; m: cell membrane; BC: bile canaliculus; Ph: phagosome.
(Bar=2µm; Original mag.= x 2000; Printed mag.= x 10800)

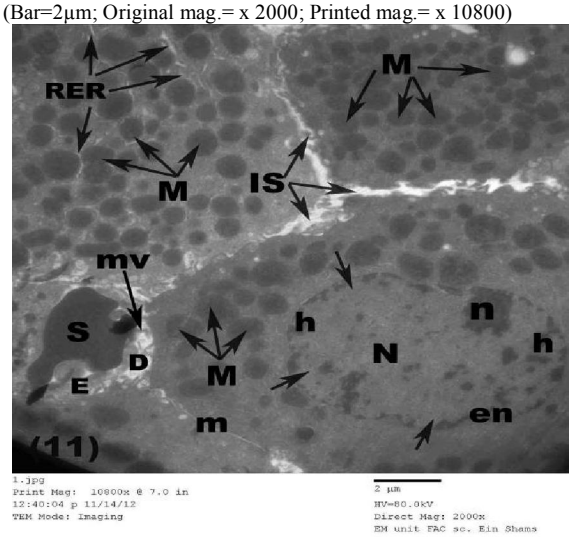

Figure (11): An electron micrograph of a cisplatin-treated rat's liver showing wide intercellular space (IS) between the neighboring hepatocytes. The nuclei (N) of hepatocytes exhibit electron-dense peripheral nucleoli (n), marginal condensed heterochromatin (h) around the nuclear pores (arrow). The cytoplasm contains many atrophic small sized, round-shaped, electron-dense mitochondriae (M) and few dilated rough endoplasmic reticulum cisternae (RER) in-between. Few short microvilli (mv) project from the sinusoidal surface of hepatocytes into the Disse space (D) deep to the flat endothelial cells (E) that line the congested blood sinusoids (S).
(Bar=2µm; Original mag.= x 2000; Printed mag.= x 10800)

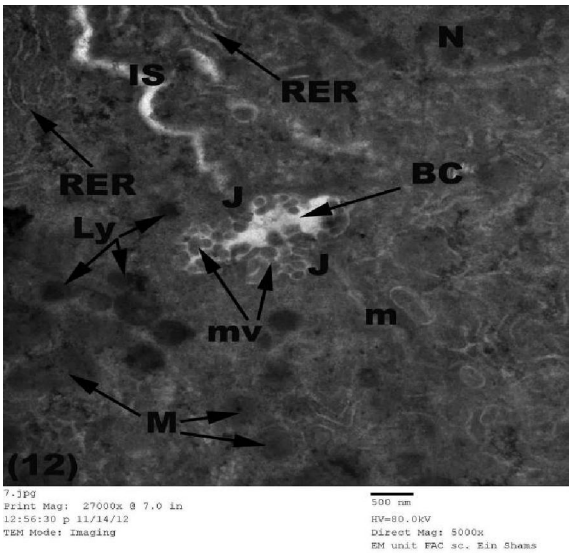

Figure (12): An electron micrograph of a cisplatin-treated rat's liver showing wide bile canaliculi (BC) exhibiting few short microvilli (mv) and tight junctions (J) at their ends between the cell membranes (m) of the neighboring hepatocytes. IS: wide intercellular space; M: mitochondria; RER: dilated cisternae of rough endoplasmic reticulum; M: mitochondria; Ly: lysosomes and N: nucleus.
(Bar = 500 nm; Original mag. = x 5000; Printed mag. = 27000)

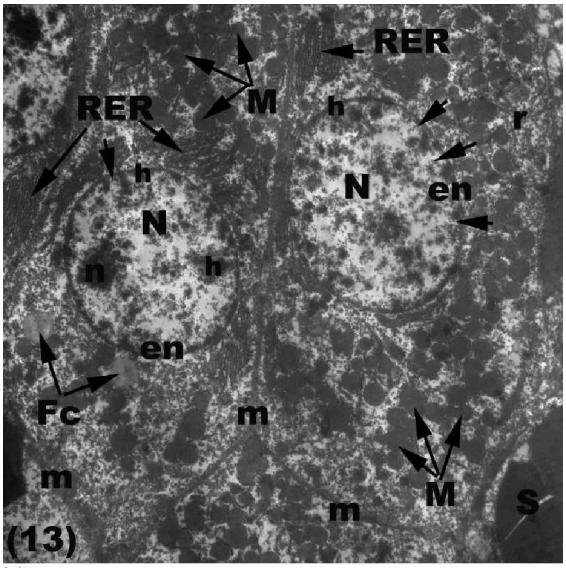

Figure (13): An electron micrograph of a combined aged garlic extract and cisplatin-treated rat's liver showing the fine granular cytoplasm of the polygonal hepatocytes containing numerous round-shaped mitochondria (M) with electron-dense matrix, an excessive amount of the rough endoplasmic reticulum cisternae (RER), free ribosomes (r) and a few fat globules (Fc). The oval-shaped nuclei (N) exhibit regular nuclear envelop (en), electron-dense peripheral nucleoli (n) and scattered masses of heterochromatin (h) around the nuclear pores (arrow). S: blood sinusoid.
(Bar=2µm; Original mag.= x 1000; Printed mag.= x 5400)

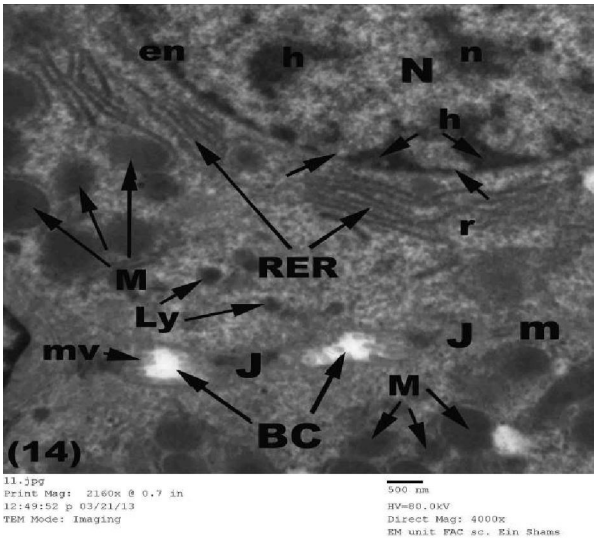

Figure (14): An electron micrograph of a combined aged garlic extract and cisplatin-treated rat's liver showing wide bile canaliculi (BC) between cell membranes (m) of the neighboring hepatocytes with tight junctions (J) at their ends. Short microvilli (mv) project from the limiting surface of hepatocytes within the biliary lumen. Many mitochondria (M),parallel rough endoplasmic reticulum cisternae (RER) and free ribosomes are seen within the pericanalicular cytoplasm. N: nucleus; n: nucleolus; en: nuclear envelop; h: heterochromatin; arrow: nuclear pores.
(Bar = 50 nm; Original mag. = 4000; Printed mag. = 21600)

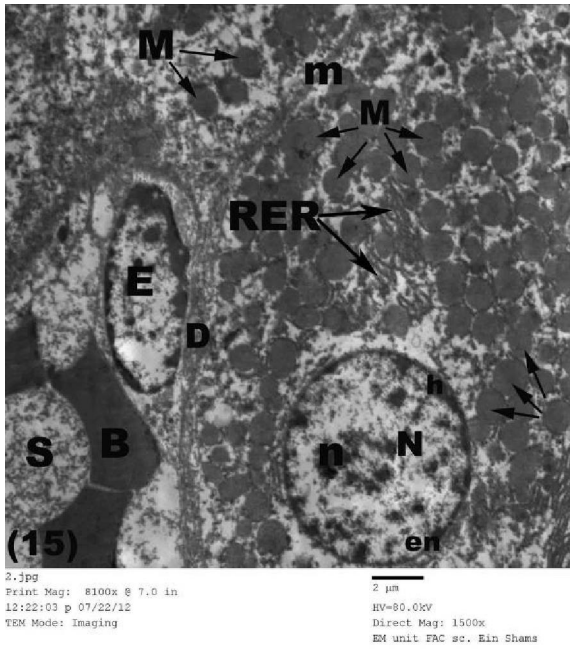

Figure (15): An electron micrograph of a combined aged garlic extract and cisplatin-treated rat's liver showing the flat endothelial cells (E) lining the blood sinusoids (S) with a narrow Disse space (D) between them and the sinusoidal surface of the hepatocytes. B: blood cells; M: mitochondria; N: nucleus; n: nucleolus; h: heterochromatin; en: nuclear envelop; RER: rough endoplasmic reticulum.
(Bar = 2µm; Original mag. = 1500; Printed mag. = 8100)

4.Discussion

CP is one of the most cytotoxic agents that is widely used to treat a variety of cancers but it is associated with toxic side effects on different body organs. Different natural products and dietary compounds have been recently investigated and evaluated as potential protective antioxidant agents against cisplatin-induced hepatotoxicity.[1,4,17] AGE, natural dietary substance, has been previously investigated to ameliorate the toxic side effects of different substances through its antioxidant, radical-scavengering and antiperoxidative activities.[7,10,18]

In the present study, a significant reduction in the final body weight and liver weight was demonstrated in CP -treated rats compared to control rats. In accordance with the results of present study, a significantly decreased in the animal's body weight was reported after single CP injection (6-7.5 mg/kg).[9,13,14] The reduction in the animal body weight is attributed in part to loss of appetite, excessive emesis, disturbance in food intake, food absorption and assimilation as a result of the toxic effect of CP on the functions of the gastrointestinal tract of the treated rats.[17,19]

The reduction in the final body weight, absolute and relative liver weights of CP -treated rats was significantly improved by the oral intake of AGE for two week before and one week after CP injection. These results may be due to the high antioxidant properties of AGE as it contains S- allyl cysteine, S-allyl mercaptocyteine, allicin, and selenium. These compounds have highly bioavailable and significant antioxidants that protect the tissues against any oxidative stress.[7] Moreover, AGE has anti-emetic and anti-anorexic effects that can improve the digestive, assimilation and metabolic functions of the rats.[4, 13]

AST and ALT are the most sensitive biomarker enzymes used in evaluation of the function and integrity of liver cells. These enzymes are present mainly in the cytoplasm of hepatocytes, thus presence of such enzymes within the circulation is a clear evidence on the damage of the cell membrane of hepatocytes.[20] In the present study, a significant elevation of serum levels of AST & ALT in CP-treated rats has been demonstrated compared to control animals. The results of our work were supported by Kart et al.[4]; Mansour et al.[1]; Arhoghro et al. [13]; Yildirim et al.[21] who stated an increase in the levels of liver biomarkers in CP-treated rats. In addition, alterations in the activity and serum level of liver enzymes may be considered a secondary event following CP-induced liver damage with subsequent leakage from hepatocytes.[1,13]

The total serum proteins measures both synthetic and execratory functions of the liver cells.

Thus, the reduction of the serum proteins in cisplatin-treated rats could be due to the direct impairment effect of cisplatin on these functions of liver cells.[22]

Oral administration of AGE prior to and after cisplatin significantly reduced its toxic effect on serum levels of AST & ALT enzymes compared to untreated rats. In agreement with the present study, administration of AGE caused a significant reduction in the serum levels of AST & ALT in rats treated with N- Nitrosodiethylamine[23]; cadmium[10]; lead[18] and doxorubicin[8]. The reduction of the liver enzymes in AGE pre-treated rats may be due to its antioxidant effect that reduces the free radical-induced oxidative damage in the liver, thereby stabilizing the membrane permeability and reducing the leakage of enzymes into the blood.[24]

Similar to the results of this study, reduction in serum levels of AST & ALT enzymes was reported with administration of other herbal plants such as aqueous extract of Scent leaf[13] ; Silymarin[9]; Pomegranate seed extract[21] and ginseng extract[17]in CP-induced hepatotoxicity.

In agree with our results, the biochemical findings in CP-treated rats were confirmed by the histopathological and ultrastructural changes in the liver[9, 14,19], where centrilobular necrotic changes, apoptotic nuclear changes, dilated congested central vein and blood sinusoids and wide portal tracts were observed. Venous and sinusoidal congestion within the hepatic parenchyma might be in part due to the direct irritant effect of cisplatin or secondary to the fibrotic changes in periportal areas affecting the intra-biliary system.[25]

In the current study, the oral intake of AGE significantly improved most of the histopathological findings of cisplatin hepatotoxicity. Similarly, an improvement of the histopathological changes in liver parenchyma in CP-treated rats was observed by various natural antioxidant agents such as silymarin, caffeic acid phenyl ester, aqueous extract of Scent leaf, Pomegranate seed extract and ginseng extract.[4, 9, 13, 17, 21] Due to its ability to reduce free radical-induced oxidative damage in the liver and to scavenge the hydroxyl and peroxyl radicals, AGE has been shown to improve the histopathological changes of the damaged liver cells.[23]

In consistent with the ultrastructural findings of the present study reduced cell size with wide intercellular space, vacuolated cytoplasm containing few dilated cisternae of rough endoplasmic reticulum, loss of mitochondria-RER association, small sized round-shaped mitochondria with condensed electron-dense matrix and fragmented cristae were observed in CP-treated liver tissues. Irregular outlined nuclei with marginal heterochromatin content, segregated nucleoli were seen in some hepatocytes. Also, dilated bile canaliculi with short microvilli, congested blood sinusoids with perisinusoidal accumulation of collagen fibers were noticed as well.[4, 9, 14,17,19,25]

Vacuolization and rarification of the of cell cytoplasm may be caused by the oxidative damage of cisplatin to the lipid components of the membranous organelles or due to dissolution of hepatic cords.[26] Moreover, the heterogeneity of the mitochondria might be a regulatory response of organelles to the energetic or nutritional state of the cell. In addition, authors added that, mitochondria might be one of the targets of potency in CP-mediated cancer chemotherapy and toxicity.[19]

The oxidative stress through the generation of reactive oxygen species (ROS) with subsequent lipid peroxidation and tissue damage was one mechanism of CP-induced hepatotoxicity.[9, 19] In addition, direct alteration of CP on the enzymatic part of the antioxidant defense system was reported as well.[4]

On the other hand, administration of AGE improved most of the ultrastructure changes induced by CP. In agree with the results of present study, Abdelmaguid et al.[9] who used silymarin and Nasr[14] who used misoprostol against CP-induced hepatotoxicity, similar findings were reported. Moreover, AGE or garlic powder were used to protect the liver against different other toxic agents including lead[18]; cadmium[10] and doxorubicin[8]. It seems that, AGE may has a protective effect against different agents induced-hepatotoxicity.

Conclusion:

Cisplatin administration induced reduction in body and liver weights, elevation of liver biomarkers and appearance of different histological and ultrastructural changes in rat's liver cells. However, the oral intake of AGE ameliorated most of the morphological, biochemical, histological and ultrastructural changes of CP-induced hepatotoxicity. Thus, AGE may be considered a useful dietary supplementary compound to patients treated with antineoplastic drugs including CP. This provides a cheap protective strategy in the management of acute hepatotoxicity or CP-induced liver damage. However further studies are needed to explore the exact mechanisms of cytoprotective effect of AGE as well as the effect of AGE on the antineoplastic effect of CP.

Conflict of Interest

The author declares that no conflict of interest exists.

Corresponding author:
Ashraf Youssef Nasr
Anatomy Department, Faculty of Medicine, Zagazig University, Zagazig, Egypt
E-mail: ashrafnaeem2013@gmail.com; ashrafnaeem2013@yahoo.com

References

1. Mansour HH, Hafez HF, Fahmy NM: Silymarin modulates Cisplatin 10 mg kg-[1] induced oxidative stress and hepatotoxicity in rats. *J Biochem Mol Biol 2006; 39: 656-661.*
2. Razo-Rodríguez AC, Chirino YI, Sánchez-González DJ, Martínez-Martínez CM; Cruz C, Pedraza-Chaverri J: Garlic powder ameliorates cisplatin-induced nephrotoxicity and oxidative stress. *J Med Food 2008; 11 (3): 583-586.*
3. Sastry J, Kellie SJ : Severe neurotoxicity, ototoxicity and nephrotoxicity following high-dose cisplatin and amifostine. *Pediatr. Hematol. Oncol 2005; 22: 441–445.*
4. Kart A, Yilmaz C, Musa K, Hasan O: Caffeic acid phenethylester (CAPE) ameliorates cisplatin-induced hepatotoxicity in rabbit. *Experimental and Toxicological Pathology 2010;* 62:45-52.
5. Yin X, Zhou J, Jie C, Xing D, Zhang Y: Anticancer activity and mechanism of *Scutellaria barbata* extract on human lung cancer cell line. *Life Sci 2004; 75: 2233-2244.*
6. Gill Cl, Boyed A, McDermott E, McConn M, Servili M, Selvaggini R, et al.: Potential anticancer effects of virgin olive oil phenols on colorectal carcinogenesis models in vitro. *Int. J. Cancer 2005; 117 (1): 1-7.*
7. Capasso A: Antioxidant Action and Therapeutic Efficacy of Allium sativum L. *Molecules 2013; Jan 4;18(1):690-700.*
8. Alkreathy H, Zoheir AD, Nessar A, Mark S, Soad SA, Osman AM: Aged garlic extract protects against doxorubicin-induced cardiotoxicity in rats. *Food and Chem Toxicol 2010; 48: 951-956.*
9. Abdelmeguid NE, Chmaisse HN, Abou Zeinab NS: Silymarin Ameliorates Cisplatin-Induced Hepatotoxicity in Rats: Histopathological and Ultrastructural Studies. *Pak J B Sci 2010; 13: 463-479.*
10. Padalko V, Kozlova E, Leonova I: Protective efficacy of garlic on cadmium induced oxidative stress in young and adult rats. *Oxid Antioxid Med Sci 2012; 1 (2): 101-109.*
11. Reitman S, Frankel AS: A colorimetric method for the determination of serum glutamic oxaloacetic and glutamic pyruvic transaminase. *Am J Clin Pathol 1957; 28:53–60.*
12. Schmidt M, Eisenburg J: Serum bilirubin determination in newborn infants. A new micro method for the determination of serum of plasma bilirubin in newborn infants. *Fortschr Med 1975; 93: 1461-1466.*
13. Arhoghro EM, Kpomah DE, Uwakwe AA: Curative Potential of Aqueous Extract of Lemon Grass (*Cymbopogon Citratus*) on Cisplatin Induced Hepatotoxicity in Albino Wistar Rats. *J Phys Pharm Adv 2012; 2 (2): 282-294.*
14. Nasr AY: Morphological, biochemical, histological, and ultrastructural protective effects of misoprostol on cisplatin induced-hepatotoxicity in adult male rats. *Saudi Med J 2013; 34 (12): 1237-1247.*
15. Bancroft JD, Gamble M: Theory and practice of histological techniques. 5[th]ed. Edinburgh. New York, London, Philadelphia Churchill Livingstone pub. 2002; PP. 125–138 172-175, 184-193, 593-620
16. Bozzola JJ ; Russell LD: *Electron microscopy. 2nd ed. Jones & Bartlett Publishers.1998.*
17. Abdel-Wahhab KG, Mahmoud MS, Mona EK and Mohamed AA: Protective effect of a natural extract against cisplatin induced hepatotoxicity. *Journal Applied Research 2013; 9 (4): 3129-3140.*
18. Sharma A, Sharma V, Kansal L: Amelioration of lead-induced hepatotoxicity by *Allium sativum* extracts in Swiss albino mice. *Libyan J Med 2010; 5: 4621- 4630.*
19. Pratibha RK, Dayanand AB: Cisplatin induced histological and ultrastructural alterations in liver tissue rat. *J Cytol Histol2011; 2: 128-132.*
20. Adaramoye OA, Osaimoje DO, Akinsanya AM, Nneji CM, Fafunso MA, Ademowo OG: Changes in antioxidant status and biochemical indices after acute administration of artemether, artemether-lumefantrine and halofantrine in rats. *J Compilation Basic Clin Pharmacol Toxicol 2008; 102: 412 - 418.*
21. Yildirim S, Ali Karadeniz, Akar Karakoc, Abdulkadir Yildirim, Yildiray Kalkan, Nejdet Simsek: Effects of royal jelly on liver paraoxonase activity in rats treated with cisplatin. *Turk J Med Sci 2012; 42 (3): 367-375.*
22. El-Sharaky AS, Newairy AA, Kamel MA, Eweda SM: Protective effect of ginger extract against bromobenzene-induced hepatotoxicity in male rats. *Food Chem Toxicol 2009; 47: 1584-1590.*
23. Shaarawy M Sabry, Amany AT, Saad ME, Zakaria YA, Abeer B, Maha SM, Emad K, Khalid M: Protective effects of garlic and silymarin on NDE-induced rats hepatotoxicity. *Int. J. Biol Sci 2009; 5: 549-557.*
24. Pradeep K, Mohan CVR, Gobianand K, Karthikeyan S: Silymarin modulates the oxidant-antioxidant imbalance during diethylnitrosamine induced oxidative stress in rats. *Eur J Pharmacol 2007; 560:110-116.*
25. Shona S, Abdel-Wakeel Essawy, Sherif M Zaki, Tarek I. Abdel-Galil: Effect of cisplatinum on the liver of the adult albino rat and the possible protective role of Vitamin E (Histological and Ultrastructural Study). *Anatomy & Physiology 2012; 2 (3): 1-5.*
26. El-Sayyad HI, Ismail MF, Shalaby FM, Abou-El-Magd RF, Gaur RL: Histopathological effects of cisplatin, doxorubicin and 5-flurouracil (5-FU) on the liver of male albino rats. *Int J Biol Sci 2006; 5: 466-473.*

7/19/2014