Ameliorative Effect Of Garlic Oil Extract After Hepatotoxicity aggravated By Subchronic Effect Ethephon In White Albino Rat

Original Article

Hosam-Eldin Hussein Osman[1], Ayman El-Meghawry El-Kenawy[2], Doaa Galal ELSahra[3] and Rasha R. Salem[4]

[1]Department of Anatomy, [2]Department of Pathology, Faculty of Medicine, Taif University, Saudi Arabia

[3]Modern Technology and Information University

[4]Department of Anatomy, faculty of Medicine, Alexandria University, Egypt

ABSTRACT

Introduction: Lately the artificial ripening and increase the shelf life of fruits and vegetables by chemicals as organophosphorus plant growth regulator ethephon. Medicinal plants are used due to their natural origin, their cost-effectiveness, and side effects. Garlic oil extract normally exists in nature containing many variable antioxidant effects.

Aim of the Work: Our study is directed to reveal the possible ameliorating properties of garlic oil extract in dealing with the toxic properties of ethephon on liver of adult albino rat.

Material and Method: 80 male of albino rats (200-250 gm) were be used and divided into four groups each was 20 rat; the first group was a control group and received normal distilled water orally for two weeks ; second group was received 100 mg garlic oil extract/kg body weight/day orally for 2 months, third group was received ethephon150 mg//kg b.wt /day orally for 2 months and fourth group was received ethephon150 mg/kg body weight/day for 2 months plus 100 mg garlic oil extract/kg b.wt. Blood and liver were be experimented and ready for laboratory hematological, chemical, histopathological and microscopic examinations respectively.

Results: The results revealed that red blood cell count and hemoglobin level values were decreased by ethephon intake, but leucocytosis, neutrophilia, monocytosis and lymphocytopenia were detected. Significant elevations were recorded in serum transaminases and total bilirubin levels, and significantly decreased the effect of antioxidant enzymes CAT, GSH and SOD. In contrast, MDA significantly increased when compared with the control group. Histopathological examination of the liver revealed that there were pyknosis of hepatocytes nuclei, fibrosis , cytoplasmic vacuolation and dilatation of central vein. There were also several electrons microscopic deteriorations occurred. Garlic oil extract somewhat alleviated the injury occurred in the liver.

Conclusion: Our results revealed the preventive effect of garlic oil extract in inhibition ethephon - induced major damage in the hepatocytes of albino rats.

Received: 29 May 2021, **Accepted:** 21 June 2021

Key Words: Ethephon, garlic, histopathology, liver, rat.

Corresponding Author: Rasha R.Salem, PhD, Department of Anatomy, Faculty of Medicine, Alexandria University, Egypt, **Tel.**: +20 10 2571 2480, **E-mail:** elreshy2@yahoo.com

ISSN: 1110-0559, Vol. 45, No. 3

INTRODUCTION

Recent agricultural methods for artificial ripening lead to use of many plant growth regulators (PGR). Excessive intake of the (PGR) would combine with multiple health dangers An example of PGRs is ethephon utilized on several of crops like fruits, several vegetable, cereals and oil seed crops artificial ripening acting by effect of ethylene, it affects frankly many physiological processes, like ripening, maturation, and stimulating the production of endogenousethylene[1]. Ethephon inhibit also plasma cholinesterase and cause hematological changes in albino rat[2]. Hepatotoxicity of ethephon on rat was reported by[3,1] reported that the mutations, deformations and congenital anomalies of ethephon on the pregnant mice with their fetuses[4]. Added that ethephon induced oxidative stress to gonad of albino rat. Toxicity of reproductive system, genotoxicity and major oxidative stress of ethephon were detected and recorded in mice[1].

Herbal and natural goods have been handled in traditional treatment to deal several health problems including cancers[5]. Herbal medicine activating the immune system, for example cell differentiation, and affecting apoptosis of malignant cells so make anticancer effect[6]. Garlic [Allium sativum L. (A. sativum)] is an example of herbal medicine in old times and also used as the flavoring agent and habitual medicine for healing. Garlic is considered to be a common food spice in old medicine which belongs to the Alliaceae family is to enhance physical and mental health, which is used all over the world as a food flavoring agent[7].

 DOI: 10.21608/ejh.2021.60275.1488

Recently different types of garlic like aged garlic extracts (capsules, drugs and fluids), garlic powder (tablets), garlic oil (capsule) is commercially accessible. AGE is a neutral smell garlic product produced by expanded withdrawal of new and clean garlic for not less than twenty months under ordinary room temperature[8].

Garlic oil extract o have varied biological activities, for example anticarcionogenic, decrease level of cholesterol, anticoagulant, decrease level of glucose, and numerous other biological actions[9]. Drinking of garlic juice led to better usage of glucose in glucose tolerance test done in experimental rabbits. Also produced a marked decrease in blood sugar amount in rabbits[10].

Researches carried out on garlic has major functional property in experimental animals, like hypotensive and vasodilative effects, decrease serum cholesterol, and inhibits oxidation,allergy ,inflammation and infection with immunomodulatory properties[11] So The present work objectives were done to estimate the probable defensive effect of garlic extract on some blood parameters, and on the activity of lipidperoxidation in hepatocytes of the rats that given Ethephon.Aged garlic extract also has major active antioxidative effect[12].

MATERIALS AND METHODS

Chemical substances

Ethrel, containing 48% ethephon (2-chloroethylphosphonic acid) which is the commercial form was bought from Chema Industries.

Serum albumin was evaluated by means of commercial kit and (TSB): total serum billirubin were got from Sigma Aldrich and Spectrum, in that order were purchased from Bio diagnose, Egypt.

Medications

A capsule of 10 mg/kg of concentrate pure garlic oil which equal to 1000 mg of fresh garlic bulb. produced by The Vitamin Shoppe Co. U.S.A. It was given to the animal as 100 mg/kg b.wt. as the method of[13] by an gastric tube 3 d per week for 2 months.

Animals

After approval of Ethics of Animal Experiments committee of Alexandria university, the recommendations in the Guide for Care and Use of experimental Animals approved by the. 80 male rats (200—250 gm), were got from Taief laboratory . Animals were kept in healthy and clean cages and obtained water and well-adjusted diet ad libitum and adjusted to laboratory conditions.

Experimental design

Adaptation of rats one week of, the animals were haphazardly grouped into four equal groups in split plastic animal house cages,

First group (group I) was served as control and received normal distilled water orally for 2 months .

Second Groups (group II) received garlic oil extract (100 mg/kg b.wt orally.) for 2 month[8].

Third Groups (group III) received ethephon150 mg/ kg/day International Programme on Chemical Safety 2002 for 2 months.

Fourth Groups (group VI) received ethephon150 mg/ kg/day for 2 months, next to successive intake of garlic (100 mg/kg b.wt orally.) for 2 months.

Blood samples and tissue specimens

At the end of 2nd month, the rats were given anesthesia by ether inhalation. Blood samples were withdrawn from puncture from each heart rat. One sample was collected on EDTA (heparinized tubes) in order to calculating of blood parameters while the second sample was clotted at 37 ◦C and centrifuged at 3000 rpm for 15 min. The serum (supernatant) was composed and kept at −20 ◦C for chemical tests .Tiny slices of liver of rat was cut, cleaned , washed with usual saline. Half of each liver was prepared for histological examination and the second half was prepared for ultra structure evaluation.

Hematological results

The samples of blood which were heparinized, tested for the count of red blood cells (RBCs), hemoglobin concentration (Hb%), white blood cells (WBCs) and the differential count of polymorphs and lymphocytes in relation to standard techniques by means of an Animal Blood Counter-ABCvet (Horiba ABX, France).

Biochemical studies

Liver biomarkers assessment

Calculation of Aspartate transaminase (AST) and alanine transaminase (ALT) enzymes done by means of saleable kits (Roche Diagnostics, GmbH, D-68298,Mannheim, Germany)[14].

Serum albumin was measured by means of marketable kit and total serum bilirubin (TSB) was calculated[15].

The liver samples were dissected and put in Petri dishes. After washing with physiological saline (0.9% NaCl), part of these samples was taken for histopathological investigations and the remaining part was kept at -80oC until used. The collected tissues were grinded with liquid nitrogen in a mortar. The grinded tissues (0.5 g) were then homogenized in 2mL 50mM phosphate buffer (pH 7.8) containing 1mM EDTA and 1% PVP. The homogenate was centrifuged under cooling at 15,000xg for 20 min, and the supernatant was stored at -80oC until the estimation of catalase (CAT), superoxide dismutase (SOD), all amount of glutathione (GSH) and malondialdehyde (MDA) content.

Histological results

Slices from each liver were fixed in 10% buffered formalin solution for 24 h, dehydrated, cleared and embedded in blocks of paraffin. Several sections (3–5 _m) were done by microtome. Sections were put in the oven

untill dewaxing. After that, the sections were subjected to hematoxylin & eosin for common histological aspects purpose and staining with Masson's trichrome was done in order to clarify connective tissue, estimation of mucopolysaccharides as PAS positive materials was done by the use of Periodic acid Schiff (PAS) stain[16].

Electron microscopy

Liver was incised into tiny pieces 1 mm and fixed in phosphate buffer solution (pH 7.2) for 3 h at 4°C, after which the tissues were distant and post fixed in buffered 2% OsO4 for an hour. Post fixed tissues were washed in the buffer and dehydration was done by means of a graded levels of ethanol, after that epon-araldite mix in labeled beam capsules for embedding was used . Ultrathin sections (50 nm thick) were obtained then uranyl acetate for 1/2 hour was applied with lead citrate for 20-30 min incised after their putting on naked copper-mesh grids[17].

Statistical analyses

Findings were calculated as mean ±SEM. evaluation of means was done by the Student's t-test (One way ANOVA) and the Mann–Whitney U test. After that the result value of ($p \leq 0.05$)were measured statistically significant. Estimation of the statistical findings was done with SPSS version 16.0 (SPSS Inc., Chicago, IL,USA).

RESULTS

Hematological findings

Data shown in (Table 1) revealed that rat in group III showed obvious decrease in RBC number, level of hemoglobin,total count of WBC and count of lymphocyte but neutrophils and monocytes were notably elevated. While rats in group IV showed appreciably rise in RBC count, hemoglobin level, total WBC count and lymphocyte but neutrophils and monocytes were considerably reduce (ameliorated the toxic effects of ethephon on hematologicalparameters).

Liver biomarkers

Data shown in (Table 2) showed that rat in group III revealed noticeable raise in serum AST, ALT and TSB measures and considerable reduce in serum albumin as regard to group I . But rats in group IV had noticeable reduction in AST, ALT and TSB levels and raise in serum albumin .relative to group III. No marked change was recorded in the previous serum AST, ALT and TSB levels and in serum albumin between garlic oil extract (group IV) and control group

Lipid peroxidation and antioxidants

Data shown in (Table 3) revealed that rat in group III showed considerable reduction in GSH, SOD, and level of CAT but considerably increased in MDA level as regarding the group I. Meanwhile rat in group IV revealed marked increase of SOD, CAT and GSH levels and considerably reduction in MDA level relative to group III. No marked change was recorded in the previous serum SOD, CAT and GSH and MDA levels among garlic oil extract (group IV) and control groups

Examination by light microscope

The Liver specimens of a control rat and garlic extract groups revealed a typical appearance of its cells of hexagonal lobules with normal central veins. The hepatocytes have large spherical nucleus with obvious nucleolus were set and running intensely around the central vein and the blood sinusoids and Kupffer cells were present lining. them (Figures 1,2).

The hepatocytes of rats that given ethephon revealed that the degenerative changes in the form of several vacuoles in the cytoplasm with shrinked nuclei. The sinusoids of the liver were enlarged with multiple Kupffer cells. In some liver specimens , ethephon showed necrotic changes in most of cells in the form of pycnotic nuclei of the cells with aggregated chromatin, absence of nucleolus moreover to that there were also portal fibrosis (Figures 3,4).

Garlic oil extract in combination with ethephon showed reduction of toxic effects of ethephon on the hepatocytes reduction in the sinusoidal dilation, decline in portal fibrosis. It is also noticed that the occurrence of minimal regions of inflammation in the liver sinusoids and few cells that had light cytoplasm as shown in, (Figures 5,6).

Histochemical analysis

The light microscopic comments showed that, the hepatic tissues of the control rat and garlic extract groups had (positive PAS)reaction in the cytoplasm of the cells (Figure 7)

The Liver tissues of the rats subjected to ethephon alone had a significant reduce in (PAS reaction) in the cytoplasm of the cells (Figure 8).

A minor intensification in concentration of PAS (positive reaction) in the cytoplasm of the cells was detected among liver tissues of the rats subjected to garlic oil extract parallel with ethephon for eight weeks (Figure 9).

Transmission electronmicroscopic findings

The hepatocytes of a control rat and garlic extract group showed healthy usual ultrastructure like normal euchromatic nucleus of the liver cells and thier nucleolus with Golgi apparatus, multiple rough endoplasmic reticulum plus numerous mitochondria (Figure10).

In group III which exposed to ethephon alone revealed damage of cell membrane, damage of the nuclear membrane and less obvious nucleolus (n), regression and damage of mitochondria , with raise in cytoplasmic fat droplets , with many vacuoles in the cytoplasm and decrease in rough endoplasmic retinacula (Figure11).

Exposure to garlic oil extract with ethephon, in experimental group IV revealed presence of sporadic areas of pathological lesions in the cells and in the sinusoids as shown in (Figure 12).

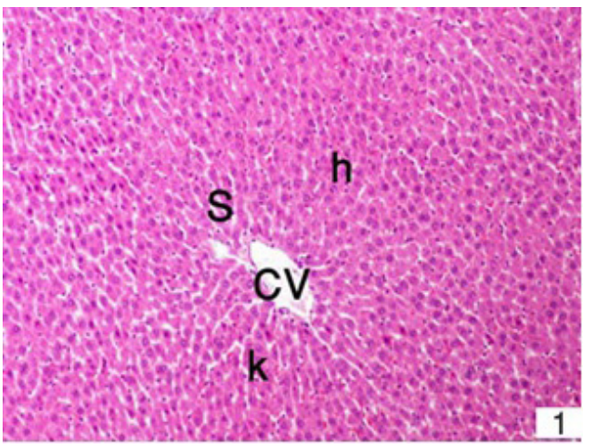

Fig. 1: Light photomicrography of liver of a control rat (group I) showing normal structure of hepatocytes (h) are hexagonal in shape, arranged into hepatic cords that run radiantly from the central vein (CV) and are parted by adjacent blood sinusoids (S) that have Kupffer cells (k).(H&E. x400.)

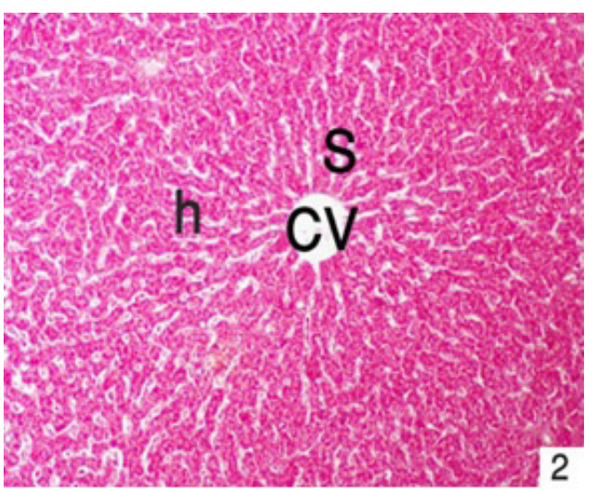

Fig. 2: Light photomicrography of liver of a control rat (group I) showing normal structure of hepatocytes organized into hepatic cords that run radiantly from central vein (CV) and are parted by blood sinusoids (S). (Mallory's x400.)

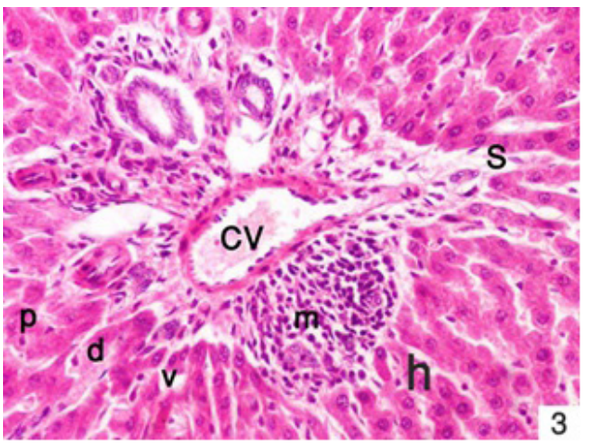

Fig. 3: Light photomicrograph of liver of rat next to eight weeks of taking ethephon (group III) showing that the shrunken hepatocyte (h) with vacuolated cytoplasm of hepatocyte (v); with condensed nuclear chromatin. Necrosis of some hepatocytes and the nuclei are contracted, pycnotic (p). Degenerated areas (d). Widening of blood sinusoids with gathering of mononuclear cells (m)in the nearest areas of sinusoids (S). The wall of sinusoid show many Kupffer cells (Kc).(H&E 400.)

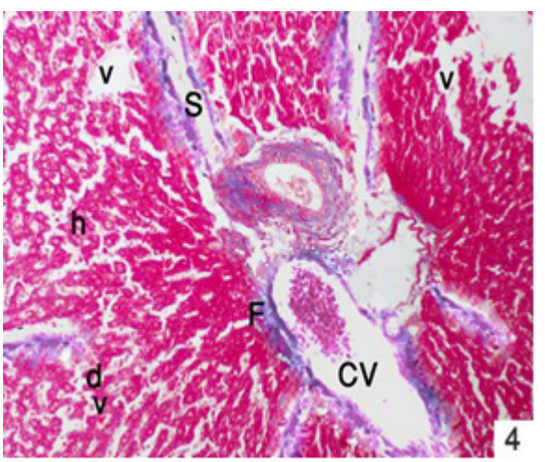

Fig. 4: Light photomicrograph of liver of rat next to eight weeks of taking ethephon (group III),showing degenerated area (d), vaculated cytoplasm (v) , degenerated hepatic cells (h), widening of central vein (CV) and sinusoidal spaces (S) with portal fibrosis (F).(Mallory's x400.)

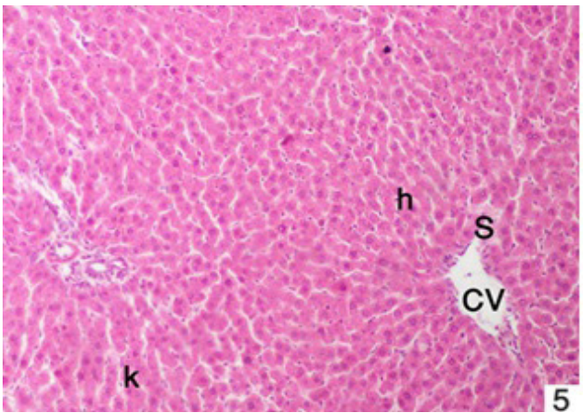

Fig. 5: Light photomicrograph of liver of rat next to eight weeks of taking garlic oil extract in combination with ethephon (group IV), showingthat the liver organization appears normal with nearly normal hepatocytes (h) ,decreased widening of blood sinusoid (S) containing Kupffer cells (k) , decrease in fragmentation of hepatocytes nuclei and decrease the mononuclear cells infiltration around the central vein. (H&E, x400.)

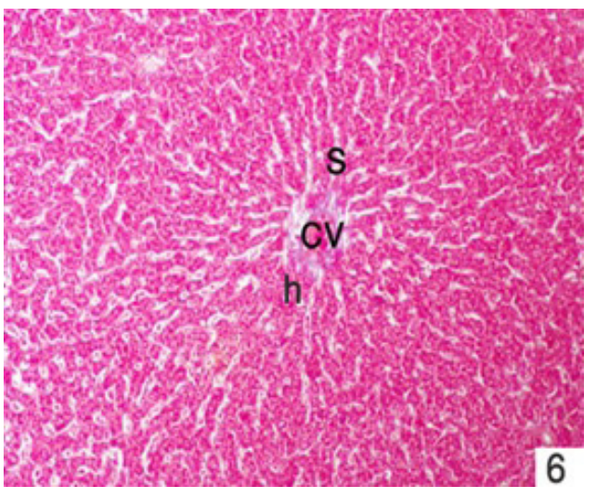

Fig. 6: Light photomicrograph of liver of rat next to eight weeks of taking garlic oil extract in combination with ethephon (group IV), showing marked decreased in degenerated hepatic cells (h), normal sized central vein (CV) and sinusoidal spaces (S) with marked absence portal fibrosis around the central vein. (Mallory's x400.)

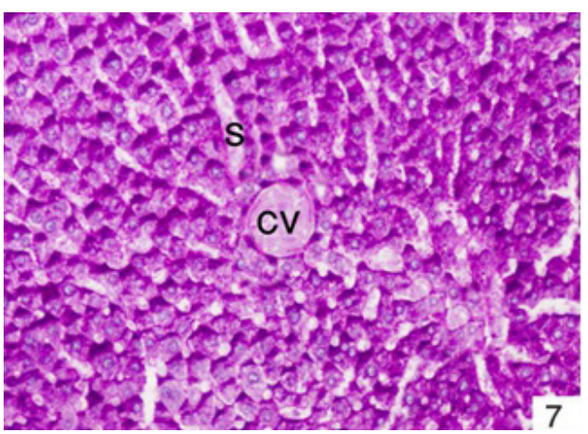

Fig. 7: Light photomicrograp of liver of a control rat (group I), showing a strong positive reaction of PAS in all its components particularly hepatocytes the central vein (CV) and the blood sinusoid (Periodic acid-Schiff's X 400.)

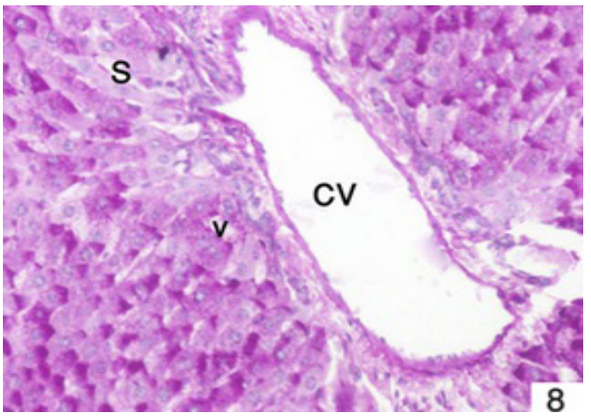

Fig. 8: Light photomicrography of liver of rat following eight weeks of application of ethephon (group III), showing a obvious decrease in PAS reaction in hepatic tissues principally hepatocytes and central vein (CV). N.B. blood sinusoid (S) and Vacuoles (V) .(Periodic acid-Schiff's X 400.)

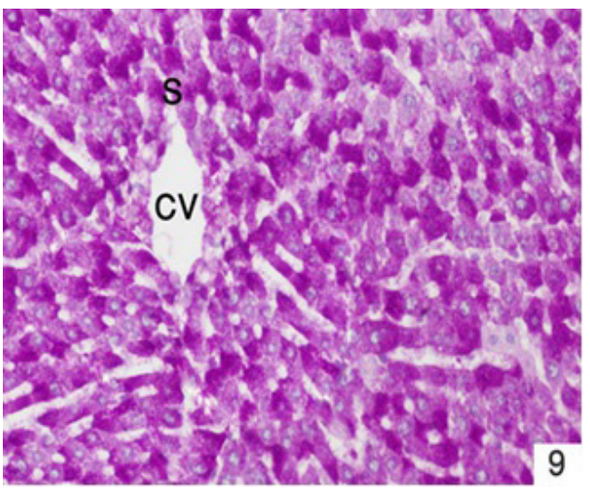

Fig. 9: Light photomicrography of liver of rat that subjected to garlic oil extract with ethephon for 8 weeks (group IV), showing marked PAS reactions particularly the hepatocytes. N.B; Central vein (CV). Blood sinusoid (S). (Periodic acid-Schiff's X 400.)

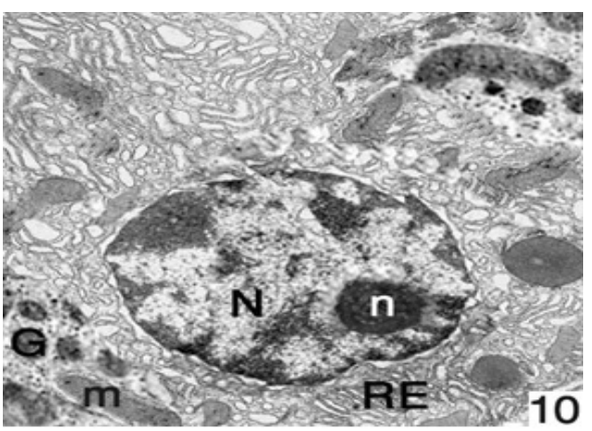

Fig. 10: Transmission electron microscopic Photomicrograph of a section of the liver of control rat (group I), showing, a typical euchromatic nucleus (N) of hepatocyte and obvious nucleolus with numerous mitochondria (M), normal Golgi apparatus (G) and Rough endoplasmic reticulum (RE). (TEM mag. =8000X.)

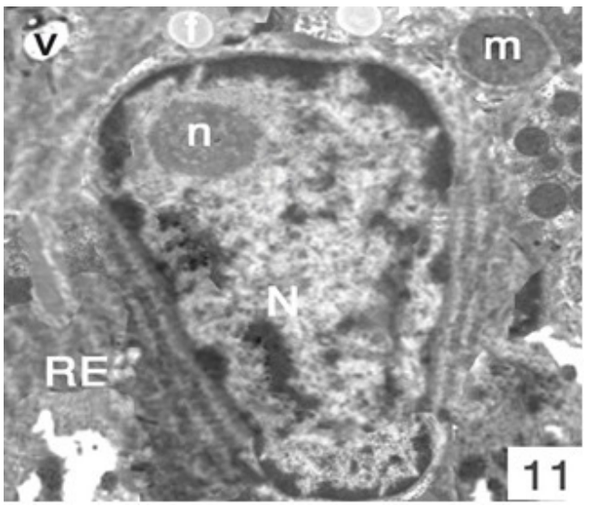

Fig. 11: Ultramicrograph of a section of liver of rat exposed to ethephon for 8 weeks (group III),, showing that destruction of cell membrane of liver cell with damage of the nuclear membrane (N) and less prominent nucleolus (n), regression and edematous mitochondria (m), cytoplasmic fat droplets (f), many cytoplasmic vacuoles (V) decrease in and edematous of rough endoplasmic retinacula. (RE) (TEM mag.12000X.)

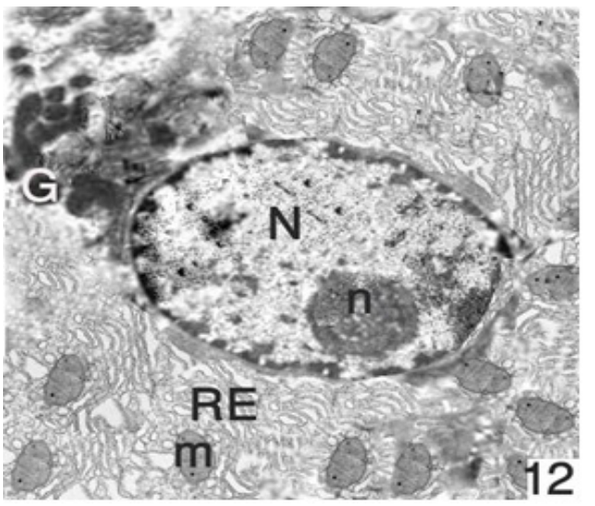

Fig. 12: Transmission electron microscopic Photomicrograph of a section of the liver of the rat that subjected to garlic oil extract with ethephon for 8 weeks (group IV), showing that the hepatocyte with nearly normal euchromatic nucleus (N) with prominent nucleolus (n) . many mitochondria (m) ,apparent Golgi apparatus (G) and rough endoplasmic reticulum (RE) were detected. (TEM mag. =8000X.)

Table 1: Effect of ethephon used alone or with garlic oil extract on Mean + SD of blood counts of rat of rats

Parameters	Group I(control)	Group II(Garlic)	GroupI II (Ethephon)	Group IV (Garlic + Ethephon)
RBCS (x 106/µL)	10.10±0.66	10.33±0.40	7.97 ± 0.82*	8.99±0.12**
Hb (g / dl)	13.34±0.06	13.78±0.02	10.97 ± 0.82*	12.17±0.12**
HCT(%)	51.84±0.46	52.04±0.27	44.47 ± 0.27*	48.07±0.42**
WBCS(x103/ ul)	7.01±0.46	6.89±0.26	9.52 ± 0.37*	7.64±0.17**
Lymphocytes (%)	39.25±0.51	39.75±0.41	29.92±0.81*	38.90±0.62**
Eosinophils (%)	0.05±0.01	0.05±0.01	0.02±0.01	0.50±0.02
Basophils (%)	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
Neutrophils (%)	56.25±0.51	56.79±0.31	60.62±0.81*	58.90±0.62**
Monocytes (%)	4.34±0.31	4.98±0.11	7.02±0.21*	5.10±0.32**

Number per group = 20 SD = standard deviation Group I (control)
Group II given ethephon (150 mg/kg/day).
Group III given ethephon (150 mg/kg/day for 8 weeks, after successive intake of garlic oil extract 100 mg/kg b.wt orally).
* = $p < 0.05$ (significant difference when compared with groupI)
** = $p < 0.05$ (significant difference when compared with group III)

Table 2: Effect of ethephon used alone or with garlic oil extract on Mean + SD of liver functions of rat

Parameters	Group I(control)	Group II(Garlic)	GroupI II (Ethephon)	Group IV (Garlic + Ethephon)
AST (U/ L) Aspartate amino transferase enzyme	100.74±0.66	99.67±0.66	160.77 ± 0.87*	120.67±0.12**
ALT (U/ L) Alanine amino transferase enzyme	76.14±0.76	75.74±0.34	100.27 ± 0.82*	80.17±0.32**
TSB(mg/ dl) total serum bilirubin;	0.94±0.06	0.95±0.01	1.97 ± 0.07*	1.01±0.02 **
Albumin(mg/ dl)	4.14±0.06	4.55±0.02	2.12 ± 0.07*	3.77±0.02 **

Number per group = 20 SD = standard deviation
Group I (control) given equal volume of distilled water/day.
Group II given ethephon 150 mg/kg/day.
Group III given ethephon 150 mg/kg/day for 8 weeks, after successive intakeof garlic oil extract (100 mg/kg b.wt orally).
* = $p < 0.05$ (significant difference in comparison with groupI)
** = $p < 0.05$ (significant difference in comparison with group III)

Table 3: The effect ofethephon used alone or with garlic oil extract on Mean + SD on the antioxidant enzymes in albino rats

Parameters	Group I(control)	Group II(Garlic)	GroupI II (Ethephon)	Group IV (Garlic + Ethephon)
SOD SuperOxide Dismutase	2.82±0.54	2.95±0.14	1.92 ± 0.76*	2.18±0.01**
CAT CATalase	2.78±0.16	2.88±0.12	1.07 ± 0.82*	2.78±0.02**
GSH GlutaStHione	14.94±0.95	15.14±0.92	7.92±0.18*	13.97 ± 0.89**
MDA MalonDiAldehyde	22±0.12	23±0.14	42±0.25*	24±0.92**

Number per group = 20 SD = standard deviation
Group I (control) received equal volume of distilled water/day.
Group II given ethephon 150 mg/kg/day.
Group III given ethephon 150 mg/kg/day for 8 weeks, after sequential intake of garlic oil extract (100 mg/kg b.wt orally).
* = $p < 0.05$ (significant difference in comparison with groupI)
** = $p < 0.05$ (significant difference in comparison with group III)

DISCUSSION

Ethephon is an example of pesticides. This study was done to discover and clarify its toxic and lethal effects on blood of rats, on its liver enzymes and histopathological alterations in liver of rats, Furthermore, the protective use of garlic oil extract against ethephon-induced toxic effects were studied[18]. and doxorubicin[19].

Hematopoietic system is considered as sensitive system to clarify the hazards results of poisons in animals and human beings[19]. In the presents study showed that ethephon led to marked side effect in blood parameters in rats and the pretreatment with garlic oil extract had fruitfully changed the blood conflict induced by ethephon these results were agreement with[20] who reported that garlic extract ameliorated the hematological disturbances caused by cisplatin with major success.

The present study revealed that rat given ethephon showed marked reduce of RBC count, Hb level and Hct value, these findings are in agreement with those mentioned by[1] who observed obvious reduce of Hb and Hct value in mice intoxicated with ethephon. In addition, this research showed leucocytosis, neutrophilia and lymphopenia. Parallel to our present results,[21,22] who found that that ethephon produced reduction in the number of peripheral lymphocytes in the blood of mice this may be due to toxic effect of ethephon on lymphoid organs.

Garlic-also induced an elevation in RBCs count might be due to it either to erythropoiesis activation or to the facility to declining membrane inflexibility intrinsic to the effect of cholesterol[23].

According to the liver function the AST and ALT seemed to be the enzymes used in estimation the role and reliability of hepatocytes. They are nearby the cytoplasm of liver cells[24]. In the present work, rats that were given ethephon revealed rise of levels of ALT, AST,TSB in the serum but low serum level of albumin relative to control group. The high levels of the serum enzymes in the liver, revealed pathological damage in the hepatocytes while the low level of serum albumin revealed the presence of destruction in the synthetic and the execratory functions of the hepatocytes. These results were agreed with the findings of this study in liver and results of[22]; also our results were in agreement with[25] who said that ethephon raises serum ALT and AST while decreases total protein in the serum of rat these results might be due to ethephon caused inflammatory and degenerative changes in the liver. The decreased albumin level indicates compromised liver excretory and synthetic functions[22,26,27].

Giving garlic extract orally before and after ethephon caused expressively reduction in its side effects on serum levels of AST & ALT enzymes as regard to untreated rats. Parallel to the findings of[20] who reported that treatment of AGE produced a considerable drop in the serum levels of AST & ALT in rats given cisplatin .The diminution of the liver enzymes in AGE treated rats due to its protective effect cause that decreases the free radical-induced oxidative injury in the liver. these resuts were in agreement with the results of[28] who found that the decrease in the values of liver enzymes in administration of garlic to rat because of its antioxidant achieve that lowers the free radical-induced oxidative injury in the liver, thereby supporting the membrane permeability and decreasing the escape of enzymes into the blood.

In addition to that, decrease in serum levels of AST & ALT enzymes was happened with intake of several herbal plants for example silymarin[4].

About antioxidants and lipid peroxidation,the present study showed that ethephon induced elevated MDA value, but reduction SOD, CAT and GSH levels in liver tissue of albino rat. Rise in MDA level increased the lipid peroxidation and raised ROS making added to change and decrease in membrane role and integrity[29].

The present work showed that there were prophylactic act of garlic extract fighting ethephon induced oxidative stress on hepatic tissues in rats, these results were in agreement with[20] who reported that intake with AGE induced marked reduce in hepatic MDA level with marked rise in hepatic CAT, SOD and GSH level in CP-treatedrats.

As regard to histological structure of liver of control group our results were in agreement with findings of[30].

About the histopathological changes ,in the present study the size of the nucleus of liver of rats treated with ethephon showed reduction in their size this suggests that these cells are probably hypoactive. Some of the nuclei of liver cells appeared pyknotic, these might be due to suggestive of degenerative changes. These findings were agreement with[3] who found that ethephon cause several histopathologicasl changes like vacuolations in the cytoplasm,pyknotic, heterochromatic nuclei and most of the cells appeared apoptotic and shrunken , in addition to that[31] also reported that the use of a gibberellic acid as plant growth regulator caused mild area of necrosis of cells of liver in experimental rats .The present study also showed that some of the degenerating cells became too shrunken and appeared degenerated .These findings are suggestive of hepatocellular degeneration and were consistent with the findings of[32] who mentioned hepatocellular necrosis after a high dose of the regulator of a plant growth .Also the present study showed that giving garlic extract prior to and after ethephon ameliorated most of the histopathological changes of ethephon induced liver toxicity. The same results were recorded by[20] who reported that established histopathological injuries in hepatocytes in CP-treated rats and their enrichment via AGE

Our results revealed that intake of garlic oil extract with ethephon change the bad toxic properties of ethephon by its antioxidant then antiperoxidative actions, these results were agreement with[8].

CONCLUSION

The present research showed that intake of ethephon led to hepatotoxic effect these were detected by change of antioxidant enzymes as CAT, GSH, and SOD, liver biomarkers, lipid peroxidation biomarker (MDA), and various histopathological aspects. Moreover, disturbance of blood parameters were detected in ethephon rats. But, the pre-treatment of garlic extract had a helpful role in ethephon induced these pathological changes through its antioxidant and effects. Thus, we concluded that garlic extract might be considered as a helpful and beneficial supplementary factor in diet to patients subjected to ethephon. This will help in protection of acute hepatotoxicity of ethephon.

CONFLICT OF INTERESTS

There are no conflicts of interest.

REFERENCES

1. Wolterink G, Inoue K, Zarn J, editors. Ethephon. The Joint FAO/WHO meeting on pesticide residues; 2015: Inc. FAO/WHO Geneva.
2. Dutta U, Deka M. Protective activity of Bambusa balcooa Roxb. against repeated exposure to Ethephon induced haematological alteration in Albino rat. Toxicology Letters. 2015;2(238):S84-S5.
3. Bhadoria P, Nagar M, Bahrioke V, Bhadoria A. Effect of ethephon on the liver in albino rats: a histomorphometric study. Biomedical journal. 2015;38(5).
4. Dutta U. Evaluation of ethephon induced oxidative stress to gonadal disorder and its amelioration by ethanolic extract of shoot of Bambusa balcooa roxb. in albino Rat. Toxicology Letters. 2015;2(238):S269-S70.
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 A549. Life sciences. 2004;75(18):2233-44.
6. Gill CI, Boyd A, McDermott E, McCann M, Servili M, Selvaggini R, *et al*. Potential anti-cancer effects of virgin olive oil phenolson colorectal carcinogenesis models in *vitro*. International Journal of Cancer. 2005;117(1):1-7.
7. Rajani Kanth V, Uma Maheswara Reddy P, Raju TN. Attenuation of streptozotocin-induced oxidative stress in hepatic and intestinal tissues of wistar rat by methanolic-garlic extract. Acta Diabetologica. 2008;45(4):243-51.
8. Capasso A. Antioxidant action and therapeutic efficacy of Allium sativum L. Molecules. 2013;18(1):690-700.
9. Agarwal KC. Therapeutic actions of garlic constituents. Medicinal research reviews. 1996;16(1):111-24.
10. Jain R, Vyas C. Garlic in alloxan-induced diabetic rabbits. The American journal of clinical nutrition. 1972;28(7):684-5.
11. Schafer G, H Kaschula C. The immunomodulation and anti-inflammatory effects of garlic organosulfur compounds in cancer chemoprevention. Anti-Cancer Agents in Medicinal Chemistry (Formerly Current Medicinal Chemistry-Anti-Cancer Agents). 2014;14(2):233-40.
12. Colín-González AL, Santana RA, Silva-Islas CA, Chánez-Cárdenas ME, Santamaría A, Maldonado PD. The antioxidant mechanisms underlying the aged garlic extract-and S-allylcysteine-induced protection. Oxidative medicine and cellular longevity. 2012;2012.
13. Riad N, Fares NH, Mostafa O, Mahmoud YI. The effect of garlic on some parasitological parameters and on hepatic tissue reactions in experimental schistosomiasis mansoni. J Appl Sci Res. 2007;3(10):949-60.
14. Reitman S, Frankel S. A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. American journal of clinical pathology. 1957;28(1):56-63.
15. Schmidt M, Eisenburg J. Serum bilirubin determination in newborn infants. A new micromethod for the determination of serum of plasma bilirubin in newborn infants. Fortschritte der Medizin. 1975;93(30):1461-6.

16. Bancroft J, Gamble M. Theory and practice of histological techniques 5th Edition, eds. Bancroft, JD and Gamble, M, Churchill Livingstone. 2002.

17. Watson ML. Staining of tissue sections for electron microscopy with heavy metals. The Journal of Biophysical and Biochemical Cytology. 1958;4(4):475.

18. Liju VB, Jeena K, Kuttan R. Acute and subchronic toxicity as well as mutagenic evaluation of essential oil from turmeric (Curcuma longa L). Food and Chemical Toxicology. 2013;53:52-61.

19. Alkreathy H, Damanhouri ZA, Ahmed N, Slevin M, Ali SS, Osman A-MM. Aged garlic extract protects against doxorubicin-induced cardiotoxicity in rats. Food and chemical toxicology. 2010;48(3):951-6.

20. Nasr AY. Protective effect of aged garlic extract against the oxidative stress induced by cisplatin on blood cells parameters and hepatic antioxidant enzymes in rats. Toxicology reports. 2014;1:682-91.

21. Wang S, Jin H, Tang Q, Fu J, Ren Z, Peng C, *et al*. The effect of ethephon on immune system in male offspring of mice. Environmental toxicology and pharmacology. 2017;49:119-23.

22. Abou-Zeid SM, Allam T, El-Bahrawy A, Mohamed A. Ameliorating effects of green tea on ethephon-induced immunotoxicity and oxidative stress in mice. Int J Pharm Sci Sci Res. 2018;4(1):1-9.

23. Hamlaoui-Gasmi S, Mokni M, Limam N, Limam F, Amri M, Aouani E, *et al*. Effect of garlic's mode of administration on erythrocytes and plasma parameters in Wistar rat. African Journal of Biotechnology. 2012;11(33):8259-63.

24. 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. Basic & clinical pharmacology & toxicology. 2008;102(4):412-8.

25. El Raouf A, Girgis S. Mutagenic, teratogenic and biochemical effects of ethephon on pregnant mice and their fetuses. Glob Vet. 2011;6:251-7.

26. Yazar S. The subchronic toxic effects of plant growth promoters in mice. Ankara Üniversitesi Veteriner Fakültesi Dergisi. 2008;55(1):17-21.

27. El-Okazy AM. The effects of combination of gibberellic acid-3 (GA3) and ethephon (2-chloroethyl phosphonic acid)(plant growth regulators) on some physiological parameters in mice. J Egypt Public Health Assoc. 2008;83(1-2):67-86.

28. Pradeep K, Mohan CVR, Gobianand K, Karthikeyan S. Silymarin modulates the oxidant–antioxidant imbalance during diethylnitrosamine induced oxidative stress in rats. European journal of pharmacology. 2007;560(2-3):110-6.

29. N. E. Lipid peroxidation: physiological levels and dual biological garlic on some parasitological parameters and on hepatic tissue reactions in experimental schistosomiasis mansoni. . J Appl Sci Res. 2009.

30. Gershbein LL, Elias H. Observations on the anatomy of the rat liver. The Anatomical Record. 1954;120(1):85-98.

31. Hussein WF, Farahat FY, Abass MA, Shehata AS. Hepatotoxic potential of Gibberellic Acid (GA3) in adult male albino rats. Life Sci J. 2011;8(3):373-83.

32. Altin DT OC, Yesilkaya E, Babül A, Bideci A, Cinaz P. Effect of the plant growth regulator. (2011).

الملخص العربى

التأثير التحسيني لمستخلص زيت الثوم بعد تفاقم السمية الكبدية بالتأثير شبه المزمن للإيثيفون في الجرذ الأبيض الذكر البالغ ألبينو

حسام الدين حسين عثمان[١]، أيمن المغاوري القناوي[٢]، دعاء جلال الصحر[٣]، ورشا سالم[٤]

[١]قسم التشريح، كلية الطب، جامعة الطائف، المملكة العربية السعودية

[٢]قسم الباثولوجيا، كلية الطب، جامعة الطائف، المملكة العربية السعودية

[٣]جامعة التكنولوجيا والمعلومات الحديثة

[٤]قسم التشريح، كلية الطب، جامعة الإسكندرية، مصر

المقدمه: تم مؤخرًا النضج الاصطناعي وزيادة العمر الافتراضي للفاكهة والخضروات بواسطة المواد الكيميائية مثل منظم نمو نبات الفوسفور العضوي الإيثيفون. تستخدم النباتات الطبية بسبب أصلها الطبيعي ، وفعاليتها من حيث التكلفة، وآثارها الجانبية. يوجد مستخلص زيت الثوم عادة في الطبيعة يحتوي على العديد من التأثيرات المضادة للأكسدة المتغيرة.

الهدف: أجريت دراستنا لإثبات الآثار الوقائية المحتملة لمستخلص زيت الثوم في إدارة الخصائص السامة لمنظم نمو نبات الفوسفور العضوي الإيثيفون على كبد الجرذان البيضاء البالغة.٨٠ ذكر من الجرذان البيضاء (٢٠٠-٢٥٠ جم) استخدمت وانقسمت إلى أربع مجموعات كل منها ٢٠ فأر.

المواد وطرق البحث: المجموعة الأولى هي مجموعة تحكم وتلقوا الماء المقطر العادي عن طريق الفم لمدة أسبوعين ؛ المجموعة الثانية تلقت ١٠٠ مجم من مستخلص زيت الثوم / كجم من وزن الجسم / يوم عن طريق الفم لمدة شهرين ، المجموعة الثالثة تم تناول الإيثيفون ١٥٠ مجم // كجم وزن / يوم عن طريق الفم لمدة شهرين والمجموعة الرابعة تم تناول الإيثيفون ١٥٠ مجم / كجم من وزن الجسم / لمدة شهرين بالإضافة إلى ١٠٠ مجم مستخلص زيت الثوم / كجم وزن حي. تم أخذ عينات من الدم والكبد وتحضيرهما لإجراء فحوصات الدم والكيمياء الحيوية والأنسجة المرضية وفحوصات البنية التحتية على التوالي.

النتائج: أظهرت النتائج أن عدد خلايا الدم الحمراء ومستوى الهيموجلوبين انخفض عن طريق تناول الإيثيفون، ولكن تم الكشف عن كثرة الكريات البيضاء، العدلات، كثرة الوحيدات، قلة اللمفاويات. تم تسجيل ارتفاعات كبيرة في الترانساميناسات في الدم ومستويات البيليروبين الكلية ، ويقلل بشكل كبير من تأثير إنزيمات مضادات الأكسدة CAT و GSH و SOD. في المقابل، زاد MDA بشكل ملحوظ عند مقارنته بمجموعة التحكم. كشف الفحص التشريحي المرضي للكبد عن وجود تغلظ في نوى خلايا الكبد ، وتليف ، وتفريغ سيتوبلازمي ، وتوسع في الوريد المركزي. كما حدثت العديد من التدهور الخلوي المجهري.

الإستنتاج: خفف مستخلص زيت الثوم إلى حد ما من الإصابة التي تحدث في الكبد. كشفت نتائجنا عن التأثير الوقائي لمستخلص زيت الثوم في تثبيط الإيثيفون الناجم عن أضرار جسيمة في خلايا الكبد في الجرذان البيضاء.