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## **A STUDY OF THE POSSIBLE PROTECTIVE ROLE OF ONION AND GARLIC VOLATILE OILS**

**Amro, N.E\*, M. Fathy Abdel-Hamid\*\* and  
Cherine M.K. \*\*\***

\* *National Centre for Research and Radiation Technology,  
Cairo, Egypt.*

\*\**Dept. of Agric. Biochem., Fac. of Agric. Ain Shams Univ.,  
Cairo, Egypt.*

\*\*\**Dept. of Pharmacology, Fac. of Medicine, Suez Canal  
Univ., Ismilia, Egypt.*

### **ABSTRACT**

This study has put stress on the capability of onion and garlic oils as chemopreventive agents in relieving and detoxifying the aflatoxin B<sub>1</sub> (AFB<sub>1</sub>) and carbon tetrachloride (CCl<sub>4</sub>).

Both AFB<sub>1</sub> and CCl<sub>4</sub> are known as carcinogenic agents. The hepato-carcinogenicity effect was studied by evaluation of glutathione-S-hydrogenase and glutathione-S-transferase activity in liver tissues of rats treated with AFB<sub>1</sub>- CCl<sub>4</sub>. This study was carried out on rats weighting 110-130g each.

The AFB<sub>1</sub> was used in a dose of 6 mg/kg b. wt., CCl<sub>4</sub> in a dose of 0.05 mg/kg b.wt. and onion and garlic oils in a dose of 600 mg/kg b. wt. Rats were treated for 16 weeks. They were divided into 6 groups randomly as follows:

Group I: received saline and served as a control group.

Group II: rats were treated with AFB<sub>1</sub> for 4 weeks, then, CCl<sub>4</sub> for 8 weeks, then, 4 weeks without any treatment.

Group III: rats were treated with the same regimen as in group II till the end of the 12<sup>th</sup> week, then, they received onion oil for another 4 weeks.

Group IV: rats were treated with the same regimen as in group II till the end of the 12<sup>th</sup> week, then, they received garlic onion oil for another 4 weeks.

Group V: rats were treated with onion oil in the first 4 weeks and then treated with AFB<sub>1</sub> for 4 weeks followed by treatment with CCl<sub>4</sub>

for 8 weeks.

Group VI: rats were treated with garlic oil in the first 4 weeks and then treated with AFB<sub>1</sub> for 4 weeks followed by treatment with CCl<sub>4</sub> for 8 weeks.

Treating rats with AFB<sub>1</sub>-CCl<sub>4</sub> exerted gradual increase in GSH level up to the twelfth week and then it tended to decrease by preventing further ASFB<sub>1</sub>-CCl<sub>4</sub> application up to the sixteenth week. The relative weights (% of body weight) of liver increased during the first 12 weeks in rats compared with the control group.

On the other hand, rats treated with onion and garlic oils before AFB<sub>1</sub>+CCl<sub>4</sub> application depressed the increase of the relative weight of liver organ. Data obtained clearly indicated that administration of both onion and garlic oils have relieved the liver damage induced by treating rats with AFB<sub>1</sub>+CCl<sub>4</sub>.

Oil samples diminished total bilirubin levels nearly to the normal level (control group), even when oil samples administrated before or after AFB<sub>1</sub>+CCl<sub>4</sub> treatment, with the exception of onion oil samples fed after AFB<sub>1</sub>+CCl<sub>4</sub> application, which exerted somewhat lower levels than control group.

A depressive effect of AFB<sub>1</sub>+CCl<sub>4</sub> was noticed on all levels of total protein, albumin, globulin, and A/G ratio compared with the control group. Either onion or garlic oils enhanced the accumulation of protein fraction, they effectively regulated A/G ratio after 16 weeks in rats treated with AFB<sub>1</sub>+CCl<sub>4</sub>.

In general, treating rats with either onion or garlic essential oils resulting in chemopreventive impacts, as decreased the elevation in both urea and creatinine concentrations throughout the experimental period (16 weeks).

**Key words:** Onion and garlic oils, AFB<sub>1</sub>, CCl<sub>4</sub>, GSH, GST, Rat, liver, Kidney.

## INTRODUCTION

Primary hepato cellular carcinoma is a major public health hazard in the developing countries of Africa and Asia. The etiology of this disease implicates both environmental (diet) and infectious (hepatitis B, C) factors (Montalto et al., 2002).

The AFB<sub>1</sub> is the commonest compound of the aflatoxins exerted by *Aspergillus flavus* (Bradburn et al., 1993). AFB<sub>1</sub> is activated mainly by cellular cytochrome P450 to form reactive

intermediate AFB<sub>1</sub>-8, 9 epoxide which can bind to DNA forming AFB<sub>1</sub>-DNA adducts. It is considered to be a critical step in the carcinogenicity of AFB<sub>1</sub> (Choy, 1993) and (Eaton and Gallagher, 1994). Uda, et al., (2006) stated that aged garlic extract inhibits development of preneoplastic lesions in rat's hepatocytes.

Several studies have showed that CCl<sub>4</sub> exposures (both short and long term) produce liver and kidney damage in humans and animals. The metabolism of CCl<sub>4</sub> has been investigated in rats, rabbits, dogs and human. Nearly half of the absorbed CCl<sub>4</sub> is excreted without any change, whilst the remainder is metabolized to trichloromethyl radical via cytochrome P450 enzyme system (Zangar et al., 2000). The free radical may bind directly to microsomal lipids and other cellular macromolecules contributing to the breakdown of membrane structure and disrupting cell energy processes.

The GSTs are a group of enzymes which reduced GSH and a wide variety of hydrophobic compounds as substrates. Functionally, they are known to catalyze the conjugation of electrophilic groups of hydrophobic xenobiotics and drugs to form GSH (Boyland and Chasseaud, 1969). GSTs protect cellular constituents of electrophiles and xenobiotics. Consequently, these enzymes help to detoxify certain extremely reactive substances by direct covalent binding of electrophilic agents to protein (Jacoby, 1978).

Organosulfur constituents of garlic and onion oils such as diallyl sulfide (DAS) and diallyl disulfide (DADS) enhanced the activity of either glutathione reductase and superoxide dismutase (Gudi and Singh, 1991).

Petal (2000) stated that the elevation of serum amino transferase is common clinical marker in liver injury, which can reflect increased apoptosis, as well as necrosis.

Apoptosis may play key role in many diseases was thought to be initiated by necrosis in the past (Rust and Gores, 2000).

Neil (2000) added that, in the liver, apoptosis generally is spotty, where, necrosis tend to be zonal.

Wang et al. (1996) gave fresh garlic homogenate (5g/kg) to mice before or immediately after acetaminophene (a leading analgesic and antipyretic drug used in USA) which reduced the levels of ALT and lactate dehydrogenase).

A common manifestation on nephritic damage in acute renal failure is characterized by decline in glomerular filtration rate (GFR),

which may have been induced by toxic chemicals including AFB<sub>1</sub> and CCl<sub>4</sub>) (Davis and Berndt, 1994).

This study was initiated to test the possibility of using anion and garlic volatile oils as hepatopreventing and / or hepatoprotecting agents against the harmful effect of AFB<sub>1</sub>- CCl<sub>4</sub> through investigating liver functions and kidney functions.

## MATERIALS AND METHODS

### 1) Chemicals:

AFB<sub>1</sub> (purchased from sigma co.

USA) was dissolved in dimethylsulfoxide and then completed to the required volume by sterile phosphate, buffered saline solution, dose 6 mg/kg b. wt (Butler and Neal, 1973).

CCl<sub>4</sub> (5 ml/45 ml corn oil) dose 0.05 ml / kg b.wt. (Qin et al., 1998).

Onion and garlic oils (extracted by steam distillation) were purchased from Kato Aromatic, Giza, Egypt. The stock solution was prepared by adding 7 ml of onion and garlic oils to 93 ml of corn oil (1 ml of this solution contain 120mg of onion and garlic oils). The used dose was 600 mg / kg b.wt.

### 2) Animals:

Male Wister rats, 5-6 weeks old, 110-130g b.wt., were purchased from private sector breeding laboratory in Giza, Egypt. Rats were adapted for ten days before use.

### 3) Study design:

Rats were treated for 16 weeks where animals were divided into 6 groups randomly as follows:

Group I: received saline and served as a control group.

Group II: rats were treated with AFB<sub>1</sub> for 4 weeks, then, CCl<sub>4</sub> for 8 weeks, then, 4 weeks without any treatment.

Group III: rats were treated with the same regimen as in group II till the end of the 12th week, then, they received onion oil for another 4 weeks.

Group IV: rats were treated with the same regimen as in group II till the end of the 12th week, then, they received garlic onion oil for another 4 weeks.

Group V: rats were treated with onion oil in the first 4 weeks and then treated with AFB<sub>1</sub> for 4 weeks followed by treatment with CCl<sub>4</sub> for 8 weeks.

Group No.	Treatment	Time of sampling in weeks				
		0	4	8	12	16
I	Control					
II	AFB <sub>1</sub> +CCl <sub>4</sub>	← AFB <sub>1</sub> → → CCl <sub>4</sub> → without				
III	AFB <sub>1</sub> +CCl <sub>4</sub> + Onion oil	← AFB <sub>1</sub> → → CCl <sub>4</sub> → O				
IV	AFB <sub>1</sub> +CCl <sub>4</sub> + garlic oil	← AFB <sub>1</sub> → → CCl <sub>4</sub> → G				
V	Onion oil + AFB <sub>1</sub> +CCl <sub>4</sub>	← O <sup>a</sup> → → AFB <sub>1</sub> → → CCl <sub>4</sub> →				
VI	Garlic oil + AFB <sub>1</sub> +CCl <sub>4</sub>	← G <sup>a</sup> → → AFB <sub>1</sub> → → CCl <sub>4</sub> →				

O = onion oil

G= Garlic oil

Group VI: rats were treated with garlic oil in the first 4 weeks and then treated with AFB<sub>1</sub> for 4 weeks followed by treatment with CCl<sub>4</sub>, for 8 weeks.

### Biochemical analysis:

GST was determined by the method of Habig et al. (1974)

GSH was measured colorimetrically according to the procedure of Mitchell et al (1973).

### Sampling of Blood and Tissues:

At 4 weeks intervals, the diets were removed from the cages at 8 am. The animals (3 from each group) were lightly anesthetized with ether, killed between 2 p.m. and 4 p.m. and the blood samples were collected from animals, through decapitation avoiding first drops. Suitable volumes of fresh blood were immediately taken after addition of EDT A as anticoagulant for hematological and immunological examinations. The other parts of blood samples were allowed to coagulate at room temperature then centrifuged at 4°C and the clear non-haemolysed sera were separated and stored at -20°C till used in biochemical analysis.

Animals were dissected as quickly as possible and the liver and kidney were excised, wiped with filter paper and weighted. Parts of these organs were fixed in 10 % neutral buffered formalin and stored in 70 % ethanol for histopathological examinations. The rest of liver parts were homogenized in icecold 100 mM phosphate buffer, pH 7.4.

Homogenates were centrifuged at 7000 r.p.m for 20 min at 4°C and the resulting supernatant were stored at -80°C.

### **I-Biochemical Assays.**

#### **A-Liver function tests (LFTs).**

##### **1- Transaminases.**

##### **Determination of alanine aminotransferase (AL T) activity.**

ALT (E.C.: 2.6.1.2) activity in serum was determined according to the method of Reitman and Frankel, (1957) using reagent kits purchased from BioMerieux Chemical Company (France).

##### **Determination of aspartate aminotransferase (AST) activity.**

AST (E.C.:2.6.1.1) activity in serum was determined according to the method of Reitman and Frankel, (1957) using reagents kits purchased from Randox Company (United Kingdom).

##### **2- Total bilirubin.**

Total bilirubin was determined by a coupling reaction with diazotized sulfanilic acid, in the presence of caffeine to give an azo dye. The same reaction was used to measure direct bilirubin, but in the absence of caffeine (Jendrassik and Grof, 1938; Gambino, 1956 and Tietz, 1983).

##### **3-Quantitation of serum proteins. Determination of serum total protein concentration.**

Serum total protein was determined by the biuret reagent as described by Gornall et al., (1949).

##### **Determination of serum albumin concentration.**

Serum albumin concentration was determined according to the method of Doumas et at, (1971) using reagent kits purchased from Spin react Company (Spain).

##### **B-kidney function tests. Determination of serum urea concentration.**

Serum urea was quantified with the urease-glutamic dehydrogenase reaction

##### **Determination of serum creatinine concentration.**

Creatinine was determined by its reaction with picric acid in alkaline medium, the colored complex was proportional to the concentration of creatinine in the sample, which was measured at 510 nm (Larsen, 1972 and Bartles et at, 1972).

## RESULTS AND DISCUSSION

The level of GSH and GST are inversely related to each other, i.e., an increase in GST activity may mean low levels of GSH in rat liver, and vice versa. Therefore, it is better to discuss the influence of feeding rats with either onion or garlic volatile oils on GSH (Fig. 1). Together with on GST (Fig. 2) in liver tissues of rats treated with AFB<sub>1</sub>- CCl<sub>4</sub> Results reveal the following statement:

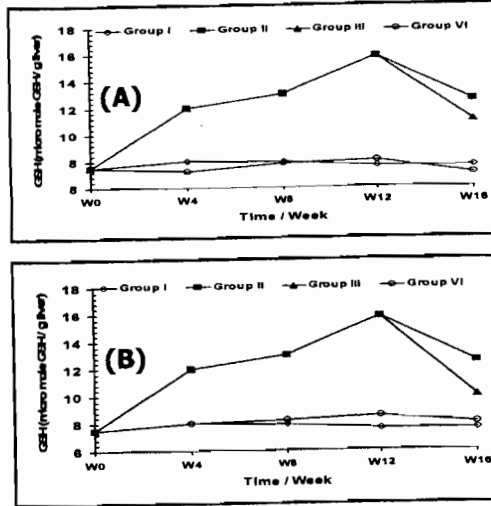


Fig (1): Effect of Onion oil (A) and Garlic oil (B) on Glutathione (GSH) level (micro mole of reduced GSH/g liver) in liver tissues of rats treated with AFB<sub>1</sub>- CCl<sub>4</sub>

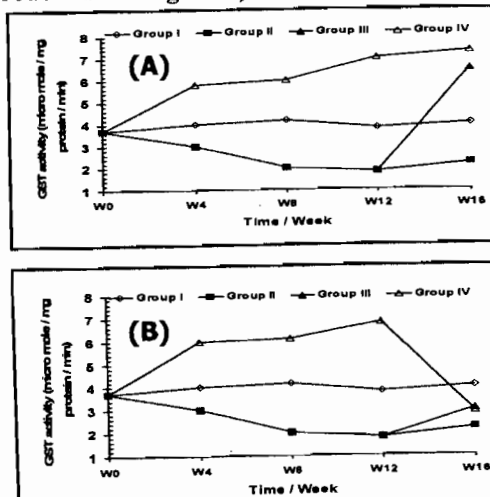


Fig (2): Effect of Onion oil (A) and Garlic oil (B) on Glutathione S-transferase (GST) activity (micro mole/mg protein/min) in liver tissues of rats treated with AFB<sub>1</sub>- CCl<sub>4</sub>

1) Treating rats with AFB<sub>1</sub>- CCl<sub>4</sub> exerted gradual increase in GSH up to the 12<sup>th</sup> week, and then it tended to decrease by preventing further AFB<sub>1</sub>- CCl<sub>4</sub> application up to the 16th week. On the other hand, GST behave the opposite trend shown in case of GSH.

2) In general, treating rats with all samples of onion and garlic oils have relieved the deleterious impact of AFB<sub>1</sub>-CCl<sub>4</sub> to variable extends, which depended on time of oil application, e.g., pretreating rats with onion or garlic oils were so effective in improving GSH levels to be close to the normal level during all time of sampling. On the other hand, post-treating with these oils exerted such decreases in GSH values, but to less extent, compared with pretreated rats. Post-treating rats with garlic oils seemed to be most effective than onion oils did.

3) Behavior of GST seemed to be somewhat complicated as some of oil addition was the most limiting factor in reducing GSH activity. Pre-treating rats with either onion or garlic oils exerted marked elevation in GST activity in comparison with control up to 12th week. On the other hand, after sixteen weeks, onion oil added before or after AFB<sub>1</sub>-CCl<sub>4</sub> exerted significant high activities compared with both control and rats treated only with AFB<sub>1</sub>- CCl<sub>4</sub>. However, the latter treatment showed the lowest GST activity during the course of the experiments. Application of garlic oils prior to AFB<sub>1</sub>- CCl<sub>4</sub> treatment showed also elevation in GSH activity as onion oil samples did during the first 12 weeks , but it differed from those of onion oils during the last four weeks, as marked decreases attained in GST activity at the end of the experiment compared with the normal level recorded in control group.

4) The observed increase in GST activity due to both onion and garlic oils application are good agreement with those reported by Wu et al. (2004), how observed significant increase in GST activity in rat liver and kidney due to DAS treatment. On the other hand, Sheen et al. (1996) found significant decrease in GST, glutathione reductase and GPx activities in cultured rat hepatocytes treated with 5mM/L DAS. However, it has been reported that DAS has induced GST- $\pi$  in mouse liver and fore stomach (Hu et al., 1996), GSH-  $\alpha$  and  $\mu$  In rat liver and glutathione reductase (Maurya and Sigh, 1991).

GSTs are a family of phase II detoxification enzymes that catalyze the conjugation of GSH to a wide variety of toxic endogenous and exogenous electrophilic compounds (Townsend and

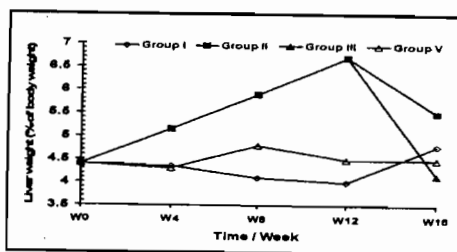


Tew, 2003). Anyway the introduction of detoxifying enzymes is rather slow process and in many cases low in magnitude (Sparnins et al., 1988).

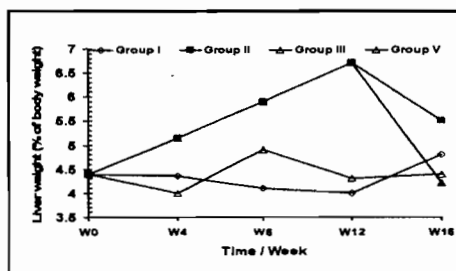
### Changes in the weight of some organs

Data clearly indicated the following aspects:

Treating rats with AFB<sub>1</sub>+ CCl<sub>4</sub> (Group II) for 12 weeks led to increase the relative weight of liver (Fig. 3 A,B) together with kidney and spleen compared with those of untreated rats (Group I, control). Anyhow the enlargement of these organs tended to decrease markedly after 16 weeks, but still higher than those of control. However, this is reasonable, since during the last 4 weeks rats were kept without AFB<sub>1</sub>+ CCl<sub>4</sub> treatment. On the other hand, testes weight showed the opposite trend and decreased in case of AFB<sub>1</sub>+ CCl<sub>4</sub> treated rats compared with control ones, especially during the first 12 weeks of the experiment.



**Fig (3A): Effect of Garlic oil on liver weight (% of body weight) of rats treated with AFB<sub>1</sub>- CCl<sub>4</sub>**



**Fig (3B): Effect of Onion oil on liver weight (% of body weight) of rats treated with AFB<sub>1</sub>- CCl<sub>4</sub>**

The harmful effect of (AFB<sub>1</sub>+ CCl<sub>4</sub>) on the relative weight of the above mentioned organs has been markedly mediated with applying onion or garlic essential oils. However, this may mean that both garlic and onion oils have such protective and remedial effect applied before or after (AFB<sub>1</sub>+ CCl<sub>4</sub>) treatment.

Hepatic and kidney damage due to CCl<sub>4</sub> ingestion or inhalation has been reported (U.S. EPA 1995). Also DHS, (1987) indicated that both subchronic and chronic exposure affected the same targets as acute exposure on nervous system and liver and kidney. Moreover liver / body weight ratios are dose dependent in mice received several CCl<sub>4</sub> doses ranged between 12 up 12.00 mg/kg for 90 days (Hayes et al., 1986).

Several compounds have been demonstrated to have such chemoprotective effect against AFB<sub>1</sub> and CCl<sub>4</sub> Abd El-Rahman (2005) was dealing with inositol hexa phosphate (IP<sub>6</sub>) which depressed the increase in hepatic weight, Yassien (2007) showed that to Bowman Birk protease inhibitor (BBI) depressed the increase of relative weight of liver, kidney and spleen, and it decreased the relative weight of testes compared with control animals.

### **Effect of onion and garlic essential oils on some biochemical parameters in rats treated with AFB<sub>1</sub>+ CCl<sub>4</sub>**

#### **(I) liver functions.**

It is well known that, liver cancer (primary hepatocellular carcinoma) and other hepatotoxicity factors leading to liver damage can be followed up successively by determination of :

A- Transaminases (AL T and AST).

B- Total bilirubin.

C- Total protein, albumin (A), globulin (G) and A/G ratio.

#### **A- Transaminases.**

Serum enzymes including AL T and AST are used in evaluating hepatic diseases. An increase in these enzyme activities reflects liver damage, either chronic or acute. Acute inflammatory hepato cellular disorders resulted in elevated transaminases levels (Kaplan, 1972 and Forstan et al., 1985).

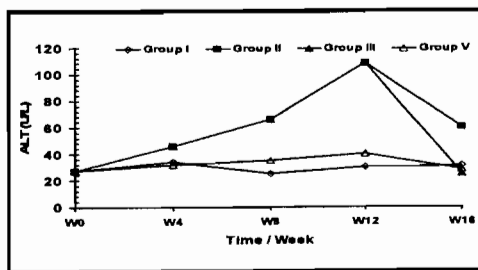
Changes in serum transaminases (AL T and AST) activity due to application of either onion or garlic essential oils before or after AFB<sub>1</sub>+ CCl<sub>4</sub> treatment are illustrated in Figs 4 A,B and 5 A,B which reveal the following main aspects:

1- Treating rats with AFB<sub>1</sub>+ CCl<sub>4</sub> for 12 weeks resulted in serious liver damage, as AL T activities increased significantly and reached maximum after 12 weeks (end of AFB<sub>1</sub>+ CCl<sub>4</sub> application). On the other hand its activity tended to decrease during the rest four weeks, i.e., after 12 weeks, as rats were kept without AFB<sub>1</sub>+ CCl<sub>4</sub> treatment.

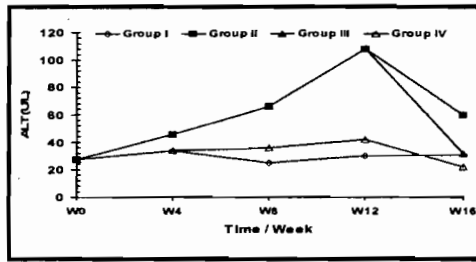
2- AST seemed to be more sensitive than AL T; as it exhibited dramatic increase during the first four weeks (AFB<sub>1</sub> treatment period), then it showed slight increase up to 12 weeks, but with stopping CCl<sub>4</sub> application, it tended to decrease up to 16 weeks (Fig. 5A,B).

3- Dealing with onion essential oil, data clearly indicated that administration of oil have releaved the liver damage induced by treating rats by AFB<sub>1</sub>+ CCl<sub>4</sub>, however this hold true whether onion essential oils were administrated before or after AFB<sub>1</sub>+CCl<sub>4</sub> treatments, as AL T activities were somewhat close to that of control animals.

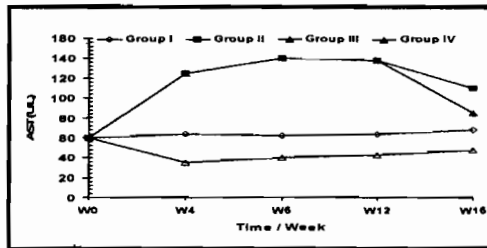
4- All onion and garlic essential oils resulted in nearly the same effective influence in relieving liver damage initiated and induced by treating with AFB<sub>1</sub>+ CCl<sub>4</sub>. Therefore it is so difficult to prefer such oil to other ones.



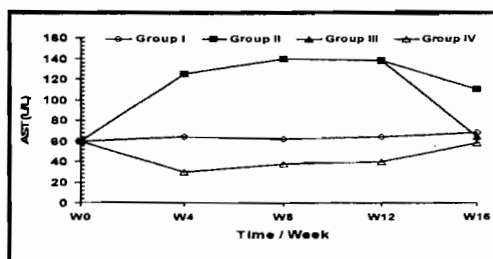
**Fig (4A):** Effect of onion oil on ALT (U/L) of rats treated with AFB<sub>1</sub>- CCl<sub>4</sub>



**Fig (4B):** Effect of Garlic oil on ALT (U/L) of rats treated with AFB<sub>1</sub>- CCl<sub>4</sub>



**Fig (5A):** Effect of Onion oil on AST (U/L) of rats treated with AFB<sub>1</sub>- CCl<sub>4</sub>



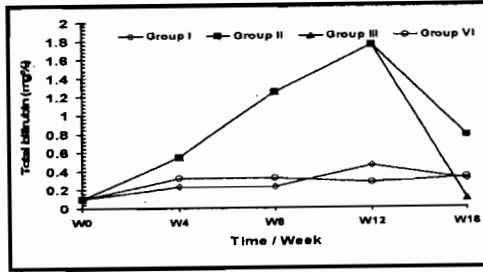
**Fig (5B): Effect of Garlic oil on AST (U/L) of rats treated with AFB<sub>1</sub>- CCl<sub>4</sub>**

Depending upon the above mentioned findings we can conclude that all samples of onion and garlic essential oils, effectively relieved liver damage induced by AFB<sub>1</sub>+CCl<sub>4</sub>, whether they were administered before or after AFB<sub>1</sub>+CCl<sub>4</sub> treatment; as these oils effectively mediated the elevation of serum transaminases (AL T and AST).

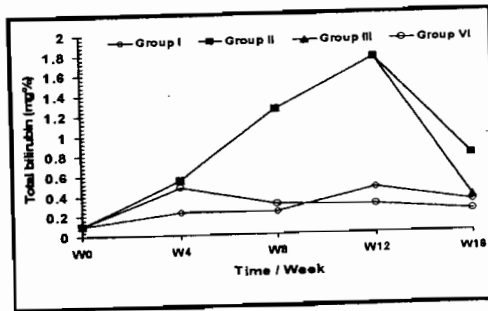
It has been reported that CCl<sub>4</sub> treatment alone elevated AST activity and caused massive cell death. Kupffer cells, as well as, hepatocytes have inducible P450 2E1, and thus they are capable of metabolizing CCl<sub>4</sub> and ethanol (Edwards et al., 1993). Moreover it has been suggested that kupffer cell activation is a crucial step in the hepatocyte injury induced by CCl<sub>4</sub> or ethanol.

### **B- Total bilirubin.**

It is well known that accumulation of total bilirubin in blood is also a second marker of liver damage and/or metabolic disturbance in liver. If the liver is unable to form bilirubin glucuronides, which is secreted into bile, or if there is excessive destruction of red cell, bilirubin may accumulate in blood plasma. Thus it is so interesting to follow up the response of total bilirubin to AFB<sub>1</sub>+ CCl<sub>4</sub> treatment and to what extent onion or garlic essential oils exert their hepatic protective effect against these carcinogens (Fig 6 A, B).



**Fig (6A): Effect of Onion oil on total bilirubin (mg %) in serum of rats treated with AFB<sub>1</sub>- CCl<sub>4</sub>**



**Fig (6B): Effect of Garlic oil on total bilirubin (mg %) in serum of rats treated with AFB<sub>1</sub>- CCl<sub>4</sub>**

Data clearly indicated that total bilirubin increased progressively up to 12 weeks, and then it significantly decreased up to the end of the experiment (16 weeks). However this decreased is reasonable since rats were kept out CCl<sub>4</sub> treatment during the remainder four weeks of the experiment.

Regarding the effect of all onion and garlic essential oil samples investigated in the present study, data clearly reveal such hepatic protective effect, as oil samples diminished total bilirubin levels

nearly to the normal levels (Control group) even when oil samples administrated before or after AFB<sub>1</sub>+CCl<sub>4</sub> treatment, with the exception of onion oil samples fed after AFB<sub>1</sub>+CCl<sub>4</sub> application, which exerted somewhat lower levels than control group.

In this connection Cheesborough (1992) suggested that the rise in serum levels of ALT, AST and total bilirubin in rats treated with AFB<sub>1</sub>+CCl<sub>4</sub> compared with control may be due to liver cell damage, or metabolic disturbance in liver involving defective conjugation and / or excretion of bilirubin. The bilirubin route of elimination is perhaps the most important contributing source for the excretion of animal metabolites. Since the liver encounters nutrients, environmental toxicants and waste products, within this framework, it extracts the environmental toxicants and waste products to prevent their circulation to other parts of body.

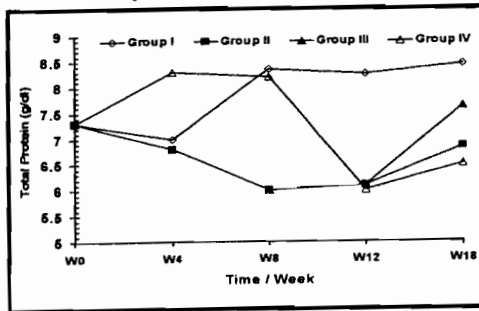
### **C- Total protein, albumin (A), globulin (G) and A/G ratio.**

The behaviour of total protein, albumin (A), globulin (G) and A/G ratio in rats treated with onion and garlic essential oils are illustrated in Figs 7-9. The main effect which has captured our attention is the depressive effect of AFB<sub>1</sub>+CCl<sub>4</sub> on all levels of total protein and their fractions compared with normal rats. This depressive influence reached significance with continuous treating rats with these carcinogens up to the twelfth week, and then they tended to increase by stopping AFB<sub>1</sub>+CCl<sub>4</sub> application during the last four week, i.e., 16 weeks.

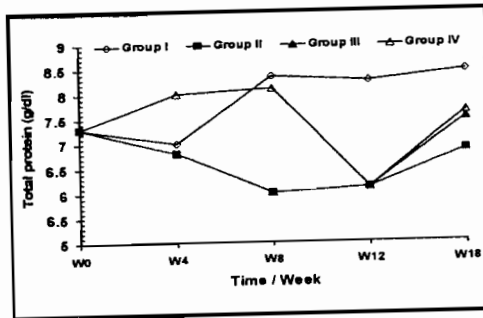
The other main aspect which one must put stress is the remedial effect of all onion and garlic oils, as they enhanced the accumulation of all protein fractions to variable extents which depended upon; type of protein fraction, sort and time of oil samples given. However this may be explained briefly in the following statements:

#### **1- Total protein.**

Rats fed with onion or garlic oils during the first four weeks, i.e., before AFB<sub>1</sub>+CCl<sub>4</sub> application, exhibited significant increases compared with control, then slight differences were achieved after 8 weeks, but after 12 weeks, total protein decreased significantly in case of all onion and garlic oils compared with normal rats (control). After 16 weeks, total protein content increased significantly in case of all treatments, but it did not reach that of control (Fig. 7 A,B).



**Fig (7A):** Effect of Onion oil on protein (g/dL) of rats treated with AFB<sub>1</sub>- CCl<sub>4</sub>

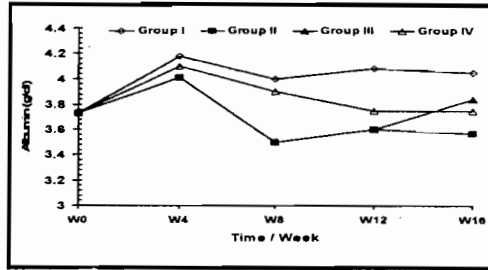


**Fig (7B):** Effect of Garlic oil on protein (g/dL) of rats treated with AFB<sub>1</sub>- CCl<sub>4</sub>

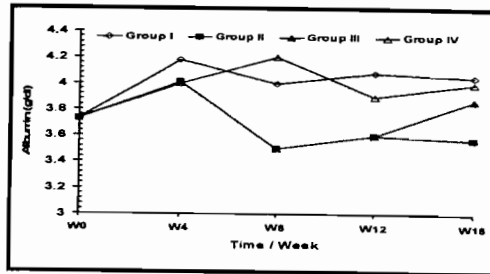
### 2-Albumin.

Beginning of the experiment with feeding rats with either onion or garlic oils resulted in slight differences up to 8 weeks, then clear decreases were attained up to 16 weeks. Regarding the effectiveness of garlic oils, they could be arranged in descending order as follows: garlic oil, before AFB<sub>1</sub>+ CCl<sub>4</sub> application followed by garlic oil after AFB<sub>1</sub>+ CCl<sub>4</sub> treatment (Fig. 8 A,B).





**Fig (8A): Effect of Onion oil on albumin (g/dL) of rats treated with AFB<sub>1</sub>- CCl<sub>4</sub>**

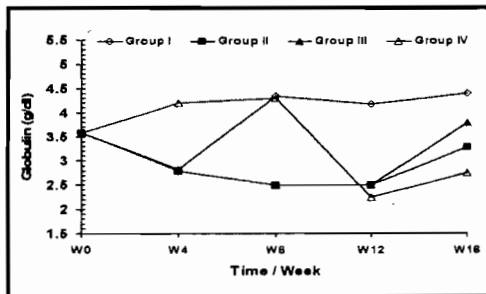


**Fig (8B): Effect of Garlic oil on albumin (g/dL) of rats treated with AFB<sub>1</sub>- CCl<sub>4</sub>**

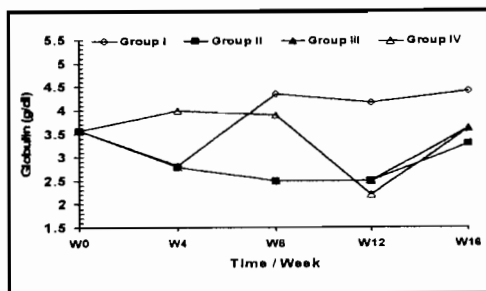
### 3- Globulin.

Treating rats with onion oil before AFB<sub>1</sub>+CCl<sub>4</sub> or after AFB<sub>1</sub>+CCl<sub>4</sub> relieved significantly the depressive effect of these carcinogens after 16 weeks, as globulin content tended to approach its normal level, but it is still beyond normal level.

Other treatments enhanced its accumulation after 16 weeks, but they did not reach the normal level in control group. During the first four weeks, globulin levels were nearly unaffected due to AFB<sub>1</sub> application; consequently this may mean that globulin responded significantly only with the beginning of CCl<sub>4</sub> administration (Fig. 9 A,B).



**Fig (9A):** Effect of Onion oil on globulin (g/dL) of rats treated with AFB<sub>1</sub>-CCl<sub>4</sub>



**Fig (9B):** Effect of Garlic oil on globulin (g/dL) of rats treated with AFB<sub>1</sub>-CCl<sub>4</sub>

#### 4- A/G ratio:

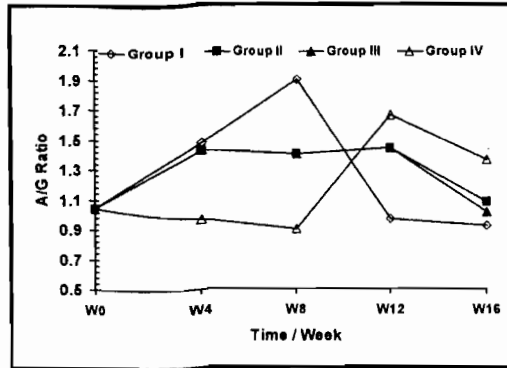
Regarding the A/G ratio (Fig. 10 A,B) data revealed the following main statements:

(a) A/G ratio in control rats increased significantly up to the eighth week then changed slightly up to the end of the experiment (16 weeks).

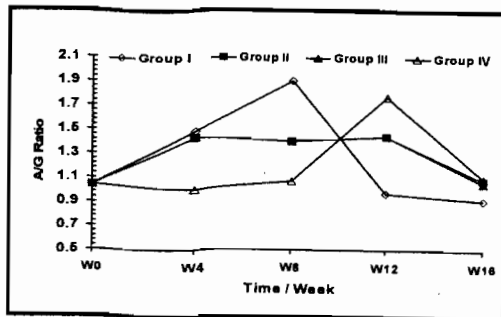
(b) Application of all onion oils effectively regulated A/G ratio after 16 weeks, excepted onion oil before AFB<sub>1</sub>+ CCl<sub>4</sub> the other treatments slightly affected A/G ratio.

(c) Garlic oils showed less efficiency for regulating A/G ratio compared with onion oils, as all samples showed such high values compared with control.

(d) Supplementation of garlic oil, before or after AFB<sub>1</sub>+ CCl<sub>4</sub> was useless treatments, as they did not improve A/G ratio resulted by application of these carcinogenic agents, i.e., AFB<sub>1</sub>+ CCl<sub>4</sub>



**Fig (10A):** Effect of Onion oil on A/G ratio of rats treated with AFB<sub>1</sub>-CCl<sub>4</sub>



**Fig (10B):** Effect of Garlic oil on A/G ratio of rats treated with AFB<sub>1</sub>-CCl<sub>4</sub>

## (II) Effect on kidney function; urea and creatinine content.

Changes in serum urea and creatinine have been used as important indices for evaluating the impact of chemicals on kidney functions. Increasing of urea and creatinine concentration in blood suggest the inability of kidney to excrete these waste products, and

consequently further suggest a decrease in glomerular filtration rate (GFR).

Data illustrated in Fig. (11 and 12 A,B) clearly revealed that both urea and creatinine increased steadily in rats treated with only AFB<sub>1</sub>+CCl<sub>4</sub> for 12 weeks, then their concentrations tend to decrease during the last four weeks of the experiment as no further treatment of AFB<sub>1</sub>+CCl<sub>4</sub> were carried out.

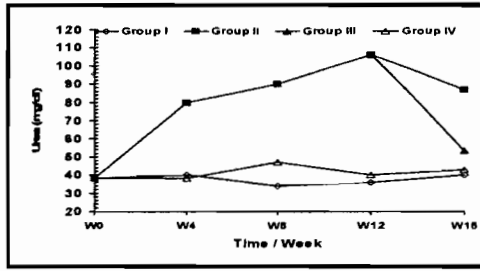
In general; treating rats with either onion or garlic essential oils resulted in such chemopreventive impact, as decreased the elevation in both urea and creatinine concentration throughout the experimental period (16 weeks).

Otherwise, the remedial influence varied due to; time of oils administration (before or after AFB<sub>1</sub>+CCl<sub>4</sub> application) sort of oil administrated; and finally due to the response of urea and creatinine, however our results could be summarized as follows:

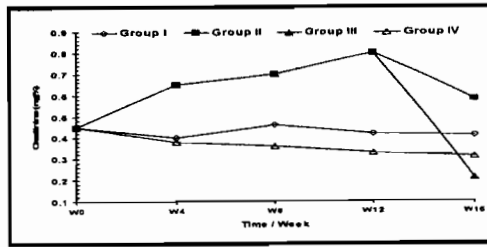
Time of oil administration, data proved that addition of all oils investigation before AFB<sub>1</sub>+CCl<sub>4</sub> application showed the highest efficiency in regulating both urea and creatinin concentration, which nearly approached that of normal level in control rats. On the other hand; post treating rats with all oils after AFB<sub>1</sub>+CCl<sub>4</sub> mediated significantly the elevation in urea but its values are still far from its normal value.

Sort of oil administrated; garlic oils seemed to be more effective in diminishing urea concentration in comparison with samples of onion oils, especially when oils administration was carried out before AFB<sub>1</sub>+CCl<sub>4</sub> application.

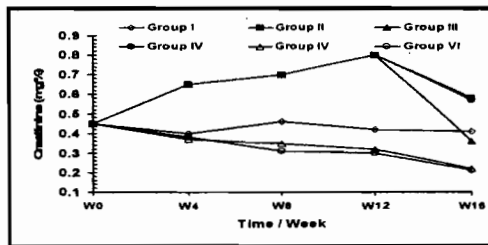
Regarding creatinine, data show marked decreases in creatinine concentration compared with control, whether oils were added before or after AFB<sub>1</sub>+CCl<sub>4</sub> treatment, which was similar to that of Group II (AFB<sub>1</sub>+CCl<sub>4</sub>).



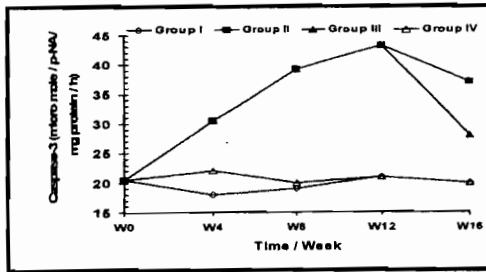
**Fig (11A):** Effect of Onion oil on urea of rats treated with AFB<sub>1</sub>-CCl<sub>4</sub>



**Fig (11B):** Effect of Garlic oil on urea of rats treated with AFB<sub>1</sub>-CCl<sub>4</sub>



**Fig (I2A):** Effect of Onion oil on creatinine of rats treated with AFB<sub>1</sub>-CCl<sub>4</sub>



**Fig (12B): Effect of Garlic oil on creatinine of rats treated with AFB<sub>1</sub>- CCl<sub>4</sub>**

At close, depending upon the above mentioned findings, one concludes the following statements:

1- Garlic oils effectively regulated urea concentration compared with onion oils investigated in the present study, especially when administrated to rats before AFB<sub>1</sub>+ CCl<sub>4</sub> application.

2- Urea responded (significantly) to the added oils compared with creatinine, as urea values were nearly close to normal level in control rats, while creatinine showed the lowest values compared with control.

3- Administration of either onion or garlic oils before AFB<sub>1</sub>+ CCl<sub>4</sub> exerted more chemopreventive effect compared with addition after AFB<sub>1</sub>+ CCl<sub>4</sub>.

## REFERENCES

- Abd El-Rahman, A.A. (2005). Effect of some plant products on the immune response of albino rats treated with aflatoxin. M.Sc. thesis. Faculty of Agriculture, Minia university.
- Bartles, H.; Bohmer, M. and Heirli, C. (1972). Clin. Chem. Acta. 37: 193.
- Boylard, E. and L.F. Chasseaud, (1969). The role of glutathione and glutathione S-transferases in mercapturic acid biosynthesis. Adv. Enzymol., 32: 173-219
- Bradburn N., G. Blunden, R.D. Coker and K. Jewers (1993). Aflatoxin contamination of maize. Trop. Sci., 33:418-428.
- Butler, W.H. and G.E. Neal (1973).The effect of aflatoxin B1 on the hepatic structure and RNA synthesis in rats fed a diet marginally

- deficient in choline. *Cancer Research*, 33: 2878-2885.
- Cheesborough, M. (1992). *Medical Laboratory Manual for tropical countries*. Vol I pp.472-505. Butter worth-Heinemann Ltd., Hakkey court, Jordan Hill.
- Choy, W.N. (1993). a review of the dose response induction of DNA adducts by AFBI and its implications to quantitative cancer risk assessment. *Mutate. Res.*, 296: 181-198.
- Davis, M.E. and Berndt, W.D. (1994). Renal methods for toxicology. In Hayes A.W. (eds). *Principles and Methods of toxicology* 3rd Ed. New York Raven, pp 871-894.
- DHS (1987). Part B. Health effects of carbon tetrachloride. Report to the Air Resources Board on Carbon Tetrachloride Submitted to the Scientific Review Panel for Review. California Department of Health Services. Currently the Air Toxicology and Epidemiology Section, Office of Environmental Health Hazard Assessment, California Environmental Protection Agency.
- Doumas, B.T.; Watson, W.A. and Biggs, H.G. (1971). Determination of serum albumin. *J. Clin. Chem. Acta.* 31: 8789.
- Eaton, D.L. and E.P. Gallagher (1994). Mechanisms of aflatoxin carcinogenesis. *Annu. Rev. Pharmacol. Toxicol.* 34, 135-172.
- Edwards, M.J.; Kauffman, F.F. and Thurman, R.J. (1993). The involvement of kupffer cells in CCl<sub>4</sub> toxicity. *Toxicol. App. Pharmacol.* 119: 276-279.
- Fortsan, W.e.; Tedesco, F.J. and Starens, F.e. (1985). Marked elevation of serum transaminase activity associated with extra hepatic biliary tract disease. *J. clin. Gastroenterol.* 7: 502-515.
- Gambino, S.R. (1956). In standard methods of clinical chemistry. S. Meites, (Ed), New York, Academic press. 5: 55-64.
- Gornall, A.G.; Bardawill, C.J. and David, M.M. (1949). Determination of serum protein by means of the biuret reagent. *J. Bioi. Chem.* 177: 751-766.
- Gudi, V.A. and S.V. Singh (1991). Effect of DAS, a naturally occurring anti-carcinogen on GSH dependent detoxification enzymes of female CD-1 mouse tissues. *Biochem. Pharmacol.*, 42: 1261-1265.
- Habig, W. H. Pabst, M. J., and Jacoby, W. B. (1974): Glutathione s-transferase. The first step in mercapturic and formation. *J. Bioi. Chem.*, 249: 7130-7139.
- Hayes J, Condie L, Borzelleca J (1986). Acute, 14-day repeated

- dosing, and 90-day subchronic toxicity studies of carbon tetrachloride in CD-1 mice. *Fund. Appl. Toxicol.* 7:454-63.
- Hu, X., P.J. Benson, S.K. Srivastava, L.M. Mack, H. Xia, V. Gupta, H.A. Zaren and S.V. Singh (1996). Glutathione S transferases of female A1J mouse liver and forestomach and their differential induction by anticarcinogenic organosulfides from garlic. *Arch. Biochem. Biophys.*, 336(2): 199-214.
- Jacoby, W.B. (1978). The glutathione S-transferases: A group of multifunctional detoxification proteins. *Adv. Enzymol. Relat. Areas Mol. Biol.*, 46: 383-414.
- Jendrassik, L. and Grof, P. (1938). *Biochem.* 81: 7297. Kaplan, M. (1972). Alkaline phosphatase. *Gastroenterology.* 62: 452-465.
- Larsen, K. (1972). *Clin. Chem. Acta.* 41: 209. Maurya, A.K. and S.V. Singh (1991).
- Differential induction of GST isoenzymes of mice stomach by DAS, a naturally occurring anticarcinogen. *Cancer Lett.*, 57(2): 121-129.
- Mitchell, J.R.; Jollow, D.J.; Patter, W.Z.; Davis, D.C.; Gillette, J.R. and Brodie, B.B. (1973). Acetaminophen - induced hepatic necrosis: Role of drug metabolism. *J. Pharmacol. Exper.* 187: 185-194.
- Montalto, G., M. Cevello, L. Giannitrapani, F. Dantona, A. Terranova, and L.A.M. Castagnetta (2002). Epidemiology, Risk Factors, and Natural History of Hepatocellular Carcinoma. *Ann. NY. Acad. Sci.*, 963: 13-20.
- Neil, W. (2000). Mechanisms of liver cell injury (Liver Diseases: and beyond). *J. Hepatol.* 32: 39-47.
- Petal, T. (2000). Apoptosis in hepatic pathophysiology. *Clinics in liver disease.* 4: 295-317.
- Qin, G.; Y. Ning, J. Su, H. Shinozuka, and P.D. Lotlikar (1998). Enhancement of aflatoxin B<sub>1</sub>-induced enzyme altered hepatic foci in rats by treatment with carbon tetrachloride. *Exper. Molec. Medicine.*, 30: 186-191.
- Reitman, S. and Frankel, S. (1957). Colourimetric of serum transaminases. *Am. J. Clin. Pathol.* 68: 28-56.
- Rust, C. and Gores, G.J. (2000). Apoptosis and liver disease. *physiology in medicine.* 108: 567-574.
- Sheen, L.Y., C.K. Lii, S.F. Sheu, R.H.C. Meng, and S.J. Tsai (1996). Effect of the active principle of garlic-diallyl sulfide-on cell viability, detoxification capability and the antioxidation system of



- primary rat hepatocytes. *Food Chem. Toxicol.*, 34: 971-978.
- Sparnins V.L., G. Barany, and L.W. Wattenberg (1988). Effects of organosulfur compounds from garlic and onions on benzo[a]pyreneinduced neoplasia and glutathione Stransferase activity in the mouse. *Carcinogenesis*, 9: 131-134.
- Tietz, N.M. (1983). *Textbook of clinical chemistry*. W.B. saunders Co. Try. 64: 1033-1043
- Townsend, D.M. and K.D. Tew (2003). Caspases: enemies within. *Science*, 28: 1312-1316.
- U.S. EPA (1995). Technical fact sheet. Part of Drinking Water and Health, U.S.Environmental Protection Agency. EPA 811-F-95-004-7, PB 95140232.
- Uda, N.; N. Kadhimoto, I. Sumioka, E. Kyo, S. Sumi and S. Fukushima (2006). Aged garlic extract inhibits development of putative preneoplastic lesions in rat hepatocarcinogenesis. *J. Nutr.*, 136: 855-860.
- Wang, E.J.; Li, Y.; Lin, M.; Chen, L.; Stein, A.P.; Reuhl, K.R. and Yang, C.S. (1996). Protective effects of garlic and related organosulfur compounds on acetaminopheninduced hepatotoxicity in mice; *Toxicol. Appl. Pharmacol.* 136: 146154.
- Wu, C.c., J.G. Chung, S.J. Tsai and L.V. Sheen (2004). Differential effects of allyl sulfides from garlic essential oil on cell cycle regulation in human liver tumor cells. *Food and Chemical Toxicology*, 42: 1937-1947.
- Yassien, Eman, E. (2007). Effect of plant protease inhibitor on rats treated with a cancer inducer. M.Sc. Thesis. Faculty of Agriculture, Minia University.
- Zangar R.c., J.M. Benson, V.L. Burnett, and D.L. Springer (2000). Cytochrome P450 2E1 is the primary enzyme responsible for low-dose carbon tetrachloride metabolism in human liver microsome. *Chern. Bioi. Interact.*, 125: 233-243.

## دراسة للتأثيرات الواقية للزيت التيار للبصل والثوم

عمرو نجاح الشحات\*، محمد فتحى عبد الحميد\*\*، شيرين مورييس خليل\*\*\*  
 \*المركز القومى لبحوث وتكنولوجيا الأشعاع، هيئة الطاقة الذرية، مصر.  
 \*\*قسم الكيمياء الحيوية، كلية الزراعة، جامعة عين شمس، القاهرة، مصر.  
 \*\*\* قسم الفارماكولوجى، كلية الطب، جامعة قناة السويس، الأسماعيلية.

تتضمن هذه الدراسة امكانية استخدام زيت البصل والثوم كمواد مانعة للأصابة أو تزيل سمية الأفلاتوكسين B1 ورابع كلوريد الكربون المسببان لسرطان الكبد. وقد تمت دراسة سلوك كل من الجلوتاثيون وأنزيمات الجلوتاثيون- أس الناقل فى كبد الفئران التى تعرضت ل AFB<sub>1</sub>-CCL<sub>4</sub>. تم معاملة ذكور الفئران لمدة 16 أسبوع وكانت أوزانها تتراوح بين 110-130 جرام لكل فأر ثم استخدم الأفلاتوكسين بجرعة 6 مجم/ كجم ورابع كلوريد الكربون بجرعة 0.05 مجم/ كجم وكل من زيتى البصل والثوم بجرعة 600 ملليجرام/ كجم فأر. تم تقسيم الفئران المعاملة الى 6 مجموعات كما يلى:  
 المجموعة الأولى: هى المجموعة الضابطة وقد أعطيت 1 مل من محلول كلوريد الصوديوم بالحقن البروتونى.

المجموعة الثانية: تم معالجة الفئران لمدة الـ 4 أسابيع الأولى من التجربة بالأفلاتوكسين ثم المعاملة برابع كلوريد الكربون لمدة أربع أسابيع ثم أربع أسابيع بدون أى معاملة.  
 المجموعة الثالثة: تم معاملة الفئران بنفس الطريقة فى المجموعة الثانية حتى الأسبوع الثانى عشر ثم المعاملة لمدة أربع أسابيع أخرى بزيت البصل.  
 المجموعة الرابعة: تم معاملة الفئران بنفس الطريقة فى المجموعة الثانية حتى الأسبوع الثانى عشر ثم المعاملة لمدة أربع أسابيع أخرى بزيت الثوم.  
 المجموعة الخامسة: خلال الأربع أسابيع الأولى تم معاملة الفئران بزيت البصل ثم المعاملة بالأفلاتوكسين لمدة أربع أسابيع ثم المعاملة برابع كلوريد الكربون لمدة ثمانية أسابيع.  
 المجموعة السادسة: خلال الأربع أسابيع الأولى تم معاملة الفئران بزيت الثوم ثم المعاملة بالأفلاتوكسين لمدة أربع أسابيع ثم المعاملة برابع كلوريد الكربون لمدة ثمانية أسابيع. وقد أدت المعاملة بها الى زيادة متدرجة فى الجلوتاثيون حتى الأسبوع الثانى عشر ثم بدأت فى التراجع حتى الأسبوع السادس عشر بينما أدت المعاملة بزيت البصل والثوم للفئران التى سبق أن تعرضت للإصابة من خلال تناولها AFB<sub>1</sub>-CCL<sub>4</sub> الى تقليل الأثر الضار لتلك المواد المسرطنة.  
 وقد زادت الأوزان النسبية للكبد (%من وزن الجسم) خلال الـ 12 أسبوع الأولى فى الفئران بمقارنتها بالكنترول. ومن ناحية أخرى فإن الفئران المعاملة بزيت البصل أو الثوم AFB<sub>1</sub>+CCL<sub>4</sub> أدت الى انخفاض الزيادة فى الوزن النسبى للكبد.

وقد أوضحت النتائج المتحصل عليها ان إضافة أى من زيت البصل أو الثوم قد أدى الى انخفاض تلف الكبد فى الفئران المعاملة بـ AFB<sub>1</sub>+CCL<sub>4</sub>. كما أدت عينات الزيت الى انخفاض مستوى البليروبين الكلى حتى يصل تقريبا الى مستوى الكنترول وذلك سواء أضيف الزيت قبل أو بعد معاملة الفئران بـ AFB<sub>1</sub>+CCL<sub>4</sub>، أما فى تغذية الفئران على زيت البصل بعد معاملة الفئران بـ AFB<sub>1</sub>+CCL<sub>4</sub> فإن مستوى البليروبين انخفض حتى أقل من الكنترول.

لوحظ انخفاض فى كل من البروتين الكلى والألبومين والجلوبولين ونسبة الـ A/G فى الفئران المعاملة بالمواد المسرطنة وذلك بمقارنتها بالكنترول، لكن بإضافة زيت الثوم أو البصل فقد أدى الى تزايد البروتين وتراكمه وأثر على نسبة A/G بعد 16 أسبوع وذلك بعد معاملة الفئران بالمواد المسرطنة. وبصفة عامة معاملة الفئران بزيت البصل أو الثوم له تأثير واقى حيث أدى الى تقليل مستوى كل من اليوريا والكرياتينين خلال التجربة (16 أسبوع).