



EFFECT OF ONION AND GARLIC VOLATILE OILS ON SOME IMMUNOLOGICAL PARAMETERS IN RATS TREATED WITH AFLATOXIN B1-CARBON TETRACHLORIDE

Journal

Fathi, M. Abdel-Hamid¹; Cherine Maurice, K.²
and Amro, N.E.³

*J. Biol. Chem.
Environ. Sci.*, 2008,
Vol. 3(2): 463-472
www.acepsag.org

1. Department of Biochemistry, Faculty of Agriculture, Ain Shams University, Cairo, Egypt
2. Department of pharmacology, Faculty of Medicine, Suez Canal University
3. National Center for Research and Radiation Technology, Cairo, Egypt

ABSTRACT

The effect of non or γ irradiated onion and garlic volatile oils on male albino rats suffered from $\text{AFB}_1 + \text{CCl}_4$; could be summarized as follows:

1- All immunological parameters i.e. chemotactic movement, MIF and Ig responded negatively in rats suffered from these carcinogens; as the former parameters increased, whilst MIF values were less than normal level. On the other hand Ig increased significantly specially during the period of AFB_1 application (first four weeks) than CCl_4 did.

2- Pre or post treating with most onion and garlic oils, had limited effect on relieving the deleterious impact of $\text{AFB}_1 + \text{CCl}_4$ as chemotactic movement and Ig still higher than their normal levels, on the other hand MIF still less than these of control rats after the experiment course for 16 weeks.

3- As general trend; pre-treating rats with either onion or garlic oils resulted in strong chemotactic movement compared with control or post-treatments i.e. both onion and garlic volatile oils may exert such chemoprotective effect than curative action.

Key words: Garlic oil, Onion oil, Aflatoxin B1, Carbon tetrachloride, immunological parameters

INTRODUCTION

The importance of garlic and onion goes back for thousand of years, not only as food and spices but also, as remedy for many diseases. The antioxidants activity observed in garlic and onion oils can be due to their rich sulfur-containing constituents. Oils of garlic and onion contain sulfoxides, thiols and sulfides which can trap electrons from other systems, and in turn preventing superoxides formation and scavenging many free radicals including hydroxyl radicals, since free radicals have been related to several age related diseases (Weisburger, 2001 and Kumaraguruparan *et al.*, 2005). The extract of onion is used as anti-asthmatic, anti-inflammatory, anti-allergenic and anti-rhombic agents due to inhibition of cyclooxygenase and lipooxygenase.

DNA adducts are believed to be an initial step in carcinogenesis. In rat mammary gland, garlic powder decreased the occurrence of DMBA-DNA adducts *in vivo* and the amounts of total and individual adducts correlated positively with mammary tumor incidence. Garlic powder, garlic water extract, a deodorized garlic powder, a garlic powder with a high sulfur content, and SAC were also, effective against mammary DMBA-DNA binding (Amagase and Milner, 1993). However, DNA adducts induced by incubation of human bladder tumor cells with 2 AF were inhibited by DAS and DADS (Chung *et al.*, 2004).

Plant derived foods contain thousands of chemically dissimilar photochemicals, many of which have been investigated in studies *in vitro* and *in vivo* to determine their effects on cancer risk and their related mechanisms of action (Huang *et al.*, 1994; Ho *et al.*, 1994, and American Institution for Cancer Research, 1996). In one study, e.g., diallyl sulphide seemed to suppress cell division in human colon tumors cells by interfering with the cell cycle (Knowles and Milner, 2000).

The present work aims to study the effect of either non or γ irradiated onion and garlic oils on some immunological parameters in rats suffered from AFB₁ + CCl₄ treatments.

MATERIALS AND METHODS

1) Chemicals

AFB₁ (purchased from Sigma Co, U.S.A. lot No. 30801) was dissolved in dimethylsulfoxide and then completed to the required

Volume by sterile phosphate buffered saline solution, the investigated dose was 6 mg/ kg rat body weight (Butler and Neal, 1973).

Carbon tetrachloride (5 ml/45 ml corn oil) dose 0.05 ml/ kg B.W (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 120 mg of onion oil or garlic oil). The used dose was 600 mg/ k.g body weight.

2) Animals

Male albino rats (*Rattus norvegicus*) 5-6 weeks old (110-130 g body weight) were purchased from a private sector breeding laboratory in Giza, Cairo, Egypt. Rats were adapted for 10 days before use.

3) Study design

In order to assess the effects of onion and garlic essential oils as a chemoprotective agent in albino rats treated with AFB₁ as initiated and CCl₄ as promoted cancer agent. Two onions or garlic oil samples "unirradiated and the other were subjected to gamma irradiation with 7 KGy".

Eighty-seven of male white albino rats were divided into ten different groups as follows:

Group No.	Treatment	No of Rats	Time of sampling in weeks				
			0	4	8	12	16
I	Control	15	3	3	3	3	3
II	AFB ₁ -CCl ₄	12	← AFB ₁ →	← CCl ₄ →	without		
III A ₁	AFB ₁ -CCl ₄ - unirrd-onion oil	3	← AFB ₁ →	← CCl ₄ →	UIO ^a		
IV A ₂	AFB ₁ -CCl ₄ - irrd-onion oil	3	← AFB ₁ →	← CCl ₄ →	IO ^b		
III B ₁	AFB ₁ -CCl ₄ - unirrd-garlic oil	3	← AFB ₁ →	← CCl ₄ →	UIG ^c		
IV B ₂	AFB ₁ -CCl ₄ - irrd-garlic oil	3	← AFB ₁ →	← CCl ₄ →	IG ^d		
V A	unirrd-onion oil + AFB ₁ + CCl ₄	12	← UIO ^a →	← AFB ₁ →	← CCl ₄ →		
VIA ₂	irrd-onion oil + AFB ₁ + CCl ₄	12	← IO ^b →	← AFB ₁ →	← CCl ₄ →		
VB ₁	unirrd-garlic oil + AFB ₁ + CCl ₄	12	← UIG ^c →	← AFB ₁ →	← CCl ₄ →		
VIB ₂	irrd-garlic oil + AFB ₁ + CCl ₄	12	← IG ^d →	← AFB ₁ →	← CCl ₄ →		

(a) UIO: Unirradiated onion

(b) IO: Irradiated onion.

(c) UIG: Unirradiated garlic.

(d) IG: Irradiated garlic

4) Biological analysis

1- Chemokinesis and Chemotaxis.

Two assays systems were used to study the chemotactic and chemokinetic responses of the rat leucocytes.

A- The micropore filter assay (chemokinesis assay).

In an attempt to more precisely quantify the chemotactic response, a modified Boyden chamber assay was used (Boyden, 1962) in which cells migrate from one compartment, through micropore filter, towards a compartment containing chemoattractants. The method employed here is after (Neuman and Sorg, 1980; Gearing and Rimmer, 1985; Howell, 1987; Abu El-Maged, 1991 and El-Feki, 1994). The chemokinetic effect obtained was expressed by a chemokinetic index calculated as:

$$\text{Chemokinetic index} = \frac{\text{Mean number of cells per h.p field with chemokinetic stimulus}}{\text{Mean number of cells per h.p field with control stimulus}}$$

B- The agarose gel assay (chemotaxis).

The method used in this investigation was described by Nelson *et al.* (1975) and modified by (Gearing and Rimmer, 1985; Abu El-Maged, 1991 and El-Feki, 1994).

2- Cell-mediated immune response.

Macrophage Migration Inhibition Factor (MIF).

MIF was carried out as described by David *et al.*, (1964). The results are expressed as migration inhibition index (MII) as follows:

$$\text{MIF} = \frac{\text{Mean area of migration with antigen}}{\text{Mean area of migration without antigen}}$$

3- Humoral immune response.

Turbidity test for estimation of total immunoglobulins level (Total Ig).

Zinc sulphate turbidity had been applied for estimation of total immunoglobulins level (Total Ig) according to Mc Ewan *et al.*, (1970) and Pfeiffer *et al.*, (1977).

4- Statistical analysis.

The data presented in this work as means \pm standard error (SE). Differences between groups were determined using one way ANOVA. The level of significance was accepted with $P < 0.05$ (Steel and Torrie, 1960). SPSS computer program were used for statistical analysis.

RESULTS AND DISCUSSION

1- Chemotaxis and cell migration:

Data obtained in table 1 (a and b) could be briefly discussed in the following statements:

a) Control rats showed no chemotactic movement during the duration of experimental course for 16 weeks.

b) As general trend, rats suffered from AFB₁ + CCl₄ treatment showed such increase which reached maximum (strong chemotactic movement) after 12 weeks, then tended to be moderate during the last four weeks; however, this is reasonable since rats were kept out any further CCl₄ application.

c) Pre-treatment with all oils and only post treating with irradiated garlic oil caused very strong chemotactic movement after the sixteenth week of experiment.

d) Moderate chemotactic movement was observed in case of post treating with non or irradiated oils

Table (1a): Effect of onion oil on chemotactic movement of rats treated with AFB₁ + CCl₄.

Groups Weeks	Group I Control	Group II AFB ₁ + CCl ₄	Group III AFB ₁ + CCl ₄ O1	Group IV AFB ₁ + CCl ₄ O2	Group V O1.....AFB ₁ + CCl ₄	Group VI O2.....AFB ₁ + CCl ₄
Week 0	=	=	=	=	=	=
Week 4	=	++	++	---	---	---
Week 8	=	---	---	+++	+++	---
Week 12	=	---	---	+++	+++	+++
Week 16	=	++	++	---	+++	+++

= No chemotactic movement

++ Weak chemotactic movement

+++ Moderate chemotactic movement

---- Very strong chemotactic movement

Table (1b): Effect of garlic oil on chemotactic movement of rats treated with AFB₁ + CCl₄.

Groups	Group I Control	Group II AFB ₁ + CCl ₄	Group III AFB ₁ + CCl ₄ O ₁	Group IV AFB ₁ + CCl ₄ O ₂	Group V O ₁AFB ₁ + CCl ₄	Group VI O ₂AFB ₁ + CCl ₄
Week 0	=	=	=	±	=	=
Week 4	=	++	++	--	+++	+++
Week 8	=	+++	+++	+++	+++	++
Week 12	=	+++	+++	+++	+++	---
Week 16	=	++	---	---	+++	---

= No chemotactic movement.

- Weak chemotactic movement.

-- Moderate chemotactic movement.

+++ Very strong chemotactic movement.

2- Macrophage migration inhibition factor (MIF):

Application of all onion and garlic oils did not succeed in controlling MIF index, as all values, were significantly less than normal level in control group (figs. 1 and 2). Oils pretreatments seemed to be somewhat effective as pushed MIF index values towards normal level more than post treatments, and this hold true for all oils investigated. However, this may mean that MIF showed significant negative response (i.e, very sensitive) to AFB₁ + CCl₄ and their deleterious effect were not mediated or at least mediated by pre or post treating with either onion or garlic oils.

3- Total immunoglobulin (Ig):

Data illustrated in Figs. (3 and 4) showed that, total immunoglobulin (Ig) responded negatively to AFB₁ + CCl₄ treatment compared with control, as its values were significantly higher than normal level especially during the first four weeks (period of AFB₁ application) then its values varied slightly up to the sixteenth week. Anywise this may mean the responsibility of AFB₁ for increasing Ig rather than CCl₄ did during its application. i.e, Ig was more sensitive to AFB₁ than CCl₄. Any attempt to relieve this deleterious effect through administration of all onion and garlic oils, had limited influence, as its values were still significantly higher than normal level except for pretreating with irradiated garlic oil.

It should be concluded that, plant derived foods contain thousands of chemically dissimilar phytochemicals, many of which have been investigated in studies *in vitro* and *in vivo* to determine their effects on cancer risk and their related mechanisms of action. (Haung *et al.*, 1994; Ho *et al.*, 1994; American Institute for Cancer Research, 1996). Diallyl sulphide (found in allium vegetables such as garlic and onion) seemed to suppress cell division in human colon tumor cells by interfering with the cell cycle; cells remained in the inactive G phase instead of moving to the M phase, where mitosis occurs (Knowles and Milner, 2000).

REFERENCES

- Abu El-Maged, A.A. (1991). Encapsulation chemotactic and chemokinetic activities of the haemocytes of different stages of *Periplaneta americana*. Bull. Fac. Sci. Assuit. Univ. 20: 109-122.
- Amagase, H. and Milner, J.A. (1993). Impact of various sources of garlic and their constituents on 7,12-dimethylbenz[a]anthracene binding to mammary cell DNA, Carcinogenesis. 14: 1627-1631.
- American Institute for Cancer Research (1996). ed. Dietary phytochemicals in cancer prevention and treatment. New York: Plenum Press.
- Boyden, S.V. (1962). The chemotactic effect of mixtures of antibody and antigen on polymorphonuclear leucocytes. J. Exp. Med. 115:453-466.

- Butler, W.H. and Neal, G.E. (1973). The effect of aflatoxin B₁ on the hepatic structure and RNA synthesis in rats fed a diet marginally deficient in choline. *Cancer. Res.* 33: 2878-2885.
- Chung, J.G.; Yeh, C.K.; Chung, K.C.; Lin, S.S. and Lee, J.H. (2004). Inhibition of N-acetyltransferase activity and gene expression in human colon cancer cell lines by diallyl sulphide, *Food Chem. Toxicol.* 42: 195-202.
- David, J.R.; Al-Askari, S.; Lawrence, H.S. and Thomas, L. (1964). Delayed hypersensitivity *in vitro* I the specificity of inhibition of cell migration by antigen. *J. Immunol.* 93: 264-273.
- El-Feki, M.A. (1994). The role of chemoattractants in phagocytosis of catfish. *Bull. Fac. Sci. Assuit. Univ.* 23: 185-200.
- Gearing, A. and Rimmer, J.J. (1985). Amphibian lymphokines: I: Leucocyte chemotactic factors produced by amphibian challenge *in vitro* develop. *Comp. Immunol.* 9: 281-290.
- Ho, C.T.; Osawa, T.; Huang, M.T.; Rosen, R.T. (1994). eds. *Food phytochemicals for cancer prevention II—teas, spices, herbs.* Washington, DC: American Chemical Society.
- Howell, C.J. (1987). A chemokinetic factor in the carp *Cyprinus carpio*. *Develop. Comp. Immunol.* 11: 139-146.
- Huang, M.T.; Asawa, T.; Ho, C.T.; Rosen, R.T. (1994). eds. *Food phytochemicals for cancer prevention I—fruits and vegetables.* Washington, DC: American Chemical Society.
- Knowles, L.M. and Milner, J.A. (2000). Diallyl sulfide inhibits p34 cdc2 kinase activity through changes in complex formation and phosphorylation. *Carcinogenesis* 21: 1129-1134
- Kumaraguruparan, R.; Chandra Mohan K.V.; Abraham, S.K. and Nagini, S. (2005). Attenuation of N-methyl-N nitrosoguanidine induced genotoxicity and oxidative stress by tomato and garlic combination, *Life Sci.*, 76: 2247-2255.
- McEwan, A.D.; Fisher, E.W.; Selman, I.B. and Penhale, W.J. (1970). A turbidity test for the estimation of immunoglobulin levels in neonatal calf serum. *Clin. Chim. Acta.* 27: 155-163.
- Nelson, R.D.; Quie, P.G. and Simons, R.L. (1975). Chemotaxis under agarose. A new and simple method for measuring chemotaxia and spontaneous migration of human PMN leucocytes and monocytes. *J. Immunol.* 115: 1650-1656.

- Neuman, C. and Sorg, C. (1980). Sequential expression of functions during macrophage differentiation in murine bone marrow culture. *Eur. J. Immunol.* 10: 834-840.
- Pfeiffer, N.E.; Mc Guirn, T.C.; Bendel, R.B. and Weikel, J.M. (1977). Quantitation of bovine immunoglobulins: comparison of single radial immunodiffusion, zinc sulphate turbidity, serum electrophoresis and refractometer methods. *Am. J. Vet. Res.* 38: 693-698.
- Qin, G.; Ning, Y.; Su, J.; Shinozuka, H. and Lotlikar, P.D. (1998). Enhancement of aflatoxin B₁ induced enzyme altered hepatic foci in rats by treatment with carbon tetrachloride. *Exper. Molic. Medicine.* 30: 186-191.
- Steel, R.G.D. and Torrie, J.H. (1960). Principles and procedures of statistics, pp 99-160. McGraw-Hill, New York, NY.
- Weisburger, J.H. (2001). Antimutagenesis and anticarcinogenesis, from the past to the future, *Mutat. Res.* 480: 23-35.

تأثير الزيوت الطيارة للثوم والبصل على بعض القياسات المناعية في

الفئران المعاملة بالأفلاتوكسين B₁ ورابع كلوريد الكربون

محمد فتحي عبد الحميد¹ - شيرين مورييس خليل² - عمرو نجاح الشحات³

¹ قسم الكيمياء الحيوية - كلية الزراعة - جامعة عين شمس - شبرا الخيمة - القاهرة - مصر

² قسم الفارماكولوجي - كلية الطب - جامعة قناة السويس

³ المركز القومي لبحوث وتكنولوجيا الإشعاع - هيئة الطاقة الذرية - القاهرة - مصر

تم دراسة تأثير كل من الزيوت الطيارة للبصل والثوم (المشععة بأشعة جاما أو الغير مشععة) فيس
الفئران المعاملة بواسطة أفلاتوكسين B₁ + رابع كلوريد الكربون CCl₄ كعوامل مسرطنة وقد أسكن لتأخرين
النتائج فيما يلي :

1- جميع القياسات المناعية تحت الدراسة chemotactic movement , MIF وكذلك Ig تأثرت سلبيا
بالمقارنة بنظيراتها في الفئران الغير معاملة بواسطة AFB₁ + CCl₄ حيث حدث زيادة في العاملين
الأول والثالث بينما حدث انخفاض معنوي في MIF بالمقارنة بالمستوى الطبيعي في فئران مجموعة
المقارنة.

2- لوحظ تأثير محدود لزيوت البصل والثوم في تعديل مستويات MIF وكذلك Ig حيث ظلت بعيدة
لحد ما عن مستواها الطبيعي في فئران مجموعة المقارنة وذلك بعد 16 أسبوع من التجربة .

3- المعاملة بزيوت كل من البصل والثوم قبل تعريض الفئران لكل من CCl₄ + AFB₁ كان
لها تأثير وقائي حيث أدت الى زيادة في chemotactic movement .