Effects of clove oil on Apoptosis in digestive system of rats treated with Uranium

Widad J.H.Ali Al-Mashhadany , Kawkab S. Al-Kasiy. and Salim R. Al- Obaidi

Abstract

Uranium from the environment enters the human body by ingestion with food and drink, and by inhalation of air borne U containing dust particles or aerosols.

The digestive tract is the entry for radionuclide's following the ingestion of contaminated food and small intestine is the main area of U absorption through out the gastrointestinal tract.

The study demonstrate the apoptosis in digestive organs (stomach, small intestine, large intestine and liver), and found clove oil reduce apoptotic numbers where Uranium elevated the number of apoptotic cells. The results showed an increament significant ($P < 0.05$) in apoptosis cells in animals treated with 75 mg /kg UN (22.8%) in liver , and showed a reduction significant ($P < 0.05$) in apoptosis cells in animals treated with 500mg /kg clove oil (2.04 %) in small intestine compare with control.

الخلاصة

يؤدي التعرض لليورانيوم الى تجمع كميات كبيرة منه في معظم الاعضاء الحيوية للجسم ولقد تم افتراض نظرية الضرر الذي تسببه الجذور الحرة كسبب وراء تلف الانسجة نتيجة التعرض لليورانيوم حيث تكون حالة فرط الاكسدة هي الميكانيكية المتوقعة لمثل هذا التاثير . تم تصميم هذه الدراسة لتقييم التاثير الوقائي لجرعتين مختلفتين من زيت القرنف (500 ملغم/كغم و750 ملغم /كغم) من وزن الجسم في الجرذان البيض المعاملة بـ (75 ملغم /كغم) من وزن الجسم من نترات اليورانيوم التي تدعم تسمم اعضاء الجهاز الهظمي ودراسة عدد الخلاية التي تعاني الموت المبرمج في المقاطع النسيجية في (المعدة والامعاء الدقيقة و الامعاء الغليضة والكبد) للجرذان .

وأظهرت الدراسة انخفاض معنوي ($P< 0.05$) في اوزان الحيوانات المعاملة بـ (75 ملغم /كغم) نترات اليورانيوم.كما اظهرت ارتفاع معنوي في ذويالخلايا في الحيوانات المعاملة بـ (75 ملغم/كغم) نترات اليورانيوم . و أظهرت الدراسة انخفاض معنوي ($P< 0.05$) في معدلات ذوي الخلايا في الحيوانات المعاملة بـ (500ملغم/كم زيت القرنفل) بنسبة (2.04) مقارنة مع مجموعة السيطرة.

Introduction

Uranium from the environment enters the human body by ingestion with food and drink, and by inhalation of air borne U containing dust particles or aerosols (1).

The digestive tract is the entry for radionuclide's following the ingestion of contaminated food and small intestine is the main area of U absorption through out the gastrointestinal tract (2 ; 3).

Apoptosis is a highly regulated physiologic cell death process that is critical for development, host defense, and the prevention of malignancies throughout the body (4: 5 ; 6).

There are two major mechanism regulating apoptosis: (1) in mitochondria – regulated pathway. And (2) the death receptor path way induced by death signaling legends, such as tumor necrosis factor – α or fas-L and subsequent caspase 8 activation (4; 7) .

Oxidant-induced DNA damage which occurs after U exposure .

Apoptosis occurs when internal monitors recognize damage or malfunction and initiate signaling cascade that eventually activate the cell (8).

One of the important function of apoptosis is the elimination of preneoplastic cell (9).

The first modern usage of the term apoptosis comes from a 1972 edition of British Journal of cancer (10).

John kerr used the word (which in ancient Greek means "to prune" or the failing of leaves) to describe an unusual form of cell death, he encountered while studying a cute liver injury in rat models during the early 1960s.

Kerr first called the phenomenon " shrinkage necrosis " because the dying cells some how convert themselves into small round masses of cytoplasm, often containing tiny specks of condensed nuclear chromatin. These masses have their organelles intact and are eventually phagocytized and eaten by nearby cells, leaving no trace of the inflammation that accompanies classic necrotic cell death. The cells simply fold up and die as shown in (Fig.1) Seemingly of their own accord (11).

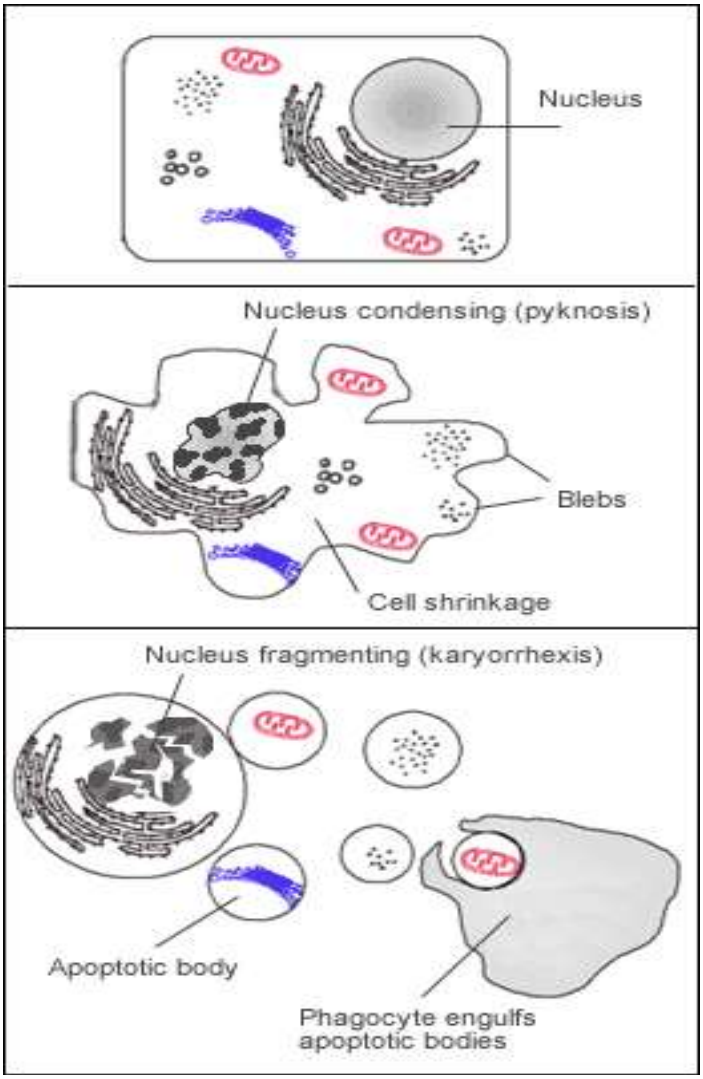


Fig. (1): Programmed cell death (11).

Today, apoptosis–sometimes called programmed cell death, is one of hottest areas of study in cellular biology. Why? Although there are countless ways to kill healthy functioning cells, in apoptosis many of most universal pathologies act through common pathways to do so. This rays the hope that by modulating apoptosis and the common pathways that lead to it, a wide variety of diseases–such as auto– immune disorders, cancer, neurodegenerative diseases and more–may be treated (12).

Eventually, the cell ingests its own DNA along with its structural proteins and phagocytizes itself (13).

Apoptosis, or programmed cell death is normal component of the developmental and health of multicellular organisms.Cells die in response to variety stimuli and during apoptosis they do so in controlled , regulated fashion (14)This makes apoptosis distinct from another form of cell death called necrosis , in which uncontrolled cell death leads to lysis of cells ,inflammatory responses and potentially , to serious health problems (15) .

Apoptosis by contrast , is a process in which cells play an active role in their own death which is why apoptosis is often referred to as cell suicide (16) .

There are a number of mechanisms through which apoptosis can be induced in cells. The sensitivity of cells to any of these stimuli can the expression of pro- and anti – apoptotic proteins, the severity of the stimuli and the stage of the cell cycly (17).

Some of the major stimuli that can induce apoptosis are:

In some cases the apoptotic stimuli comprise extrinsic signals such as the binding of death inducing legends to cell surface receptors called death receptors (12).

These legends can either be soluble factors or can be expressed on the surface of cells such as cytotoxic T- lymphocytes (18).

Characteristic morphology of apoptosis

A cell undergoing apoptosis shows a characteristic morphology that can be observed with microscope:

1. Cell shrinkage and rounding due to break down of the proteinaceous cytoskeleton by caspase.
2. The cytoplasm appears dense, and the organelles appear tightly packed.
3. Chromatin undergoes condensation into compact patches against the nuclear envelopes in a process known as pyknosis, ahall mark of apoptosis (19).
4. The nuclear envelope becomes discontinuous and the DNA inside it is fragment in a process refered to as karyorrhexis. The nucleus breaks into several discrete chromatin bodies or nucleosomal units due to the degradation of DNA (20).
5. The cell membrane shows irregular buels known as blebs.
6. The cell breaks apart into several vesicles called apoptotic bodies, which are then phagocytosed (20).

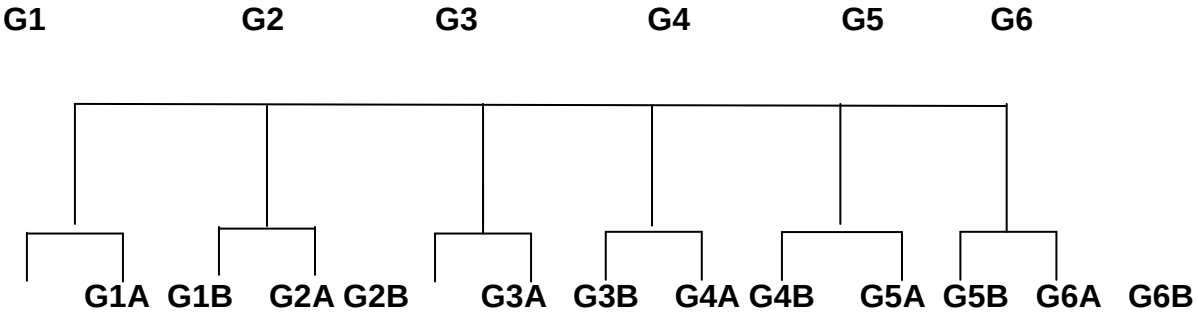
Why should a cell commit Suicide?

There are two different reasons.

1. Programmed cell death is as needed for proper development as mitosis is, as in the formation of the fingers, and by apoptosis, of the tissue between them (21).
2. Programmed cell death needed to destroy cells that represent a threat to the integrity of the organism (10).

Materials and methods

120 male rats divided into six groups as follow.



Group 1: Includes 20 animals administrated orally with ,two ml of, physiological saline PBS (Phosphate Buffer Saline) and used as negative control.

Group 2: Included 20 animals administrated orally with two ml of 75 mg/kg body weight UN and served as positive control

Group 3: Included 20 animals administrated orally with two ml of 500mg /kg body weight clove oil.

Group 4: Included 20 animals administrated orally with two ml of 750 mg /kg body weight clove oil

Group 5: Included 20 animals administrated orally with two ml of 500 mg / kg body weight clove oil + 75m g UN /kg body weight.

Group 6: Included 20 animals administrated orally with two ml of 750 mg / kg body weight clove oil + 75m g UN /kg body weight.

1. The body weight of each animal were measured each week.
2. Animals treated with clove oil or UN were treated between one day and another.
3. After one month we divided each group into two subgroups (A* and B*).
4. Before sacrificing the animals their body weight were measured and the blood samples were obtained by heart puncture to use in other biochemical studies.
5. The animals were sacrificed by anesthetized with ether (twenty four hour after the last treatment).
6. Digestive system was obtained for the assessment of tissue sections.
7. The remain animals of groups B* were left for another month and used the same treatment of groups A* after two months of the experiment this treatment added as long term study.

Ag – Nor Staining:

Sections:

Four µm paraffin section were stained with silver nitrate and according to the (22) modification as follows:

Statistical Analysis:

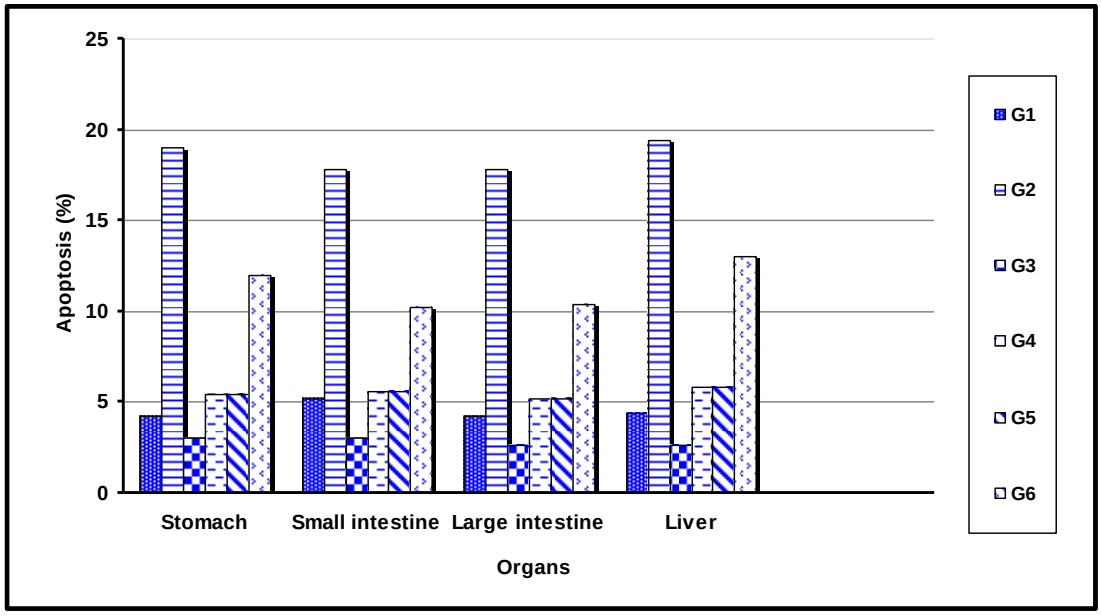
The data of this study were complied into the computerized data file and the frequency, distribution and statistical description (mean, rang, and SD) were derived using SPSS statically software.

We used statistical analysis of variance (ANOVA) test and least significantly difference (LSD) test by probability of less than 0.05 ($P<0.05$) according to (Duncan et al ,1983).

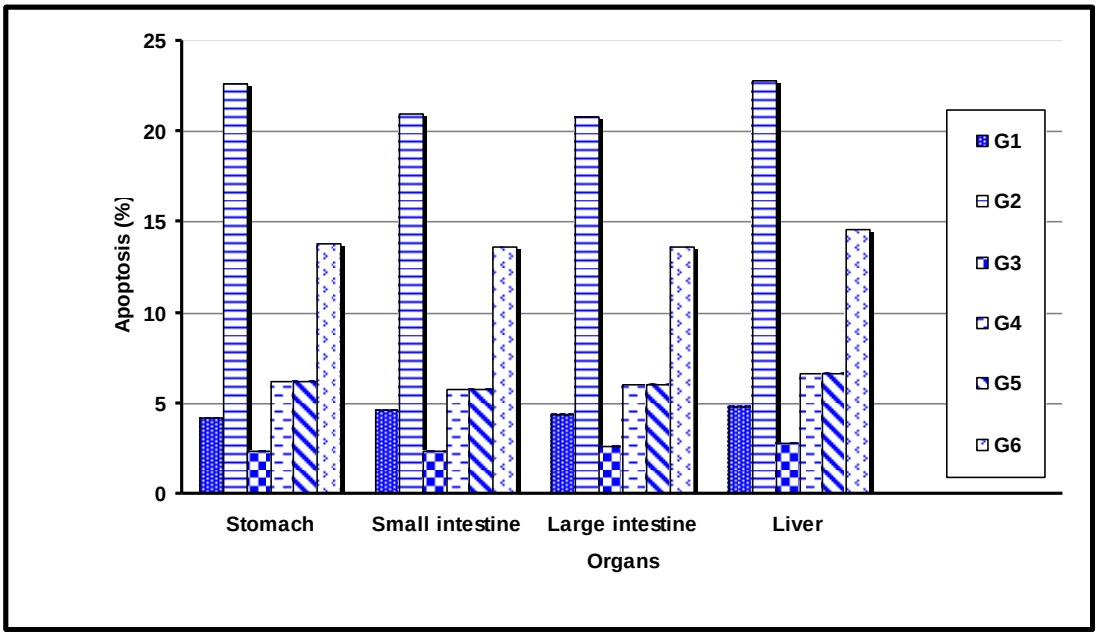
Results&Discussion

Effects of clove oil used of 500 mg / kg 0r 750 mg/kg on apoptosis in stomach , small intestine , large intestine and liver of male rats treated with 75 mg /kg UN for one month and for two months:

The data in (table 4-4 Appendix), showed significant increment (P<(0.05) in animals group G2 (75mg /kg UN)in apoptotic index in all organs (Stomach , S.int.,L.int and Liver) after one month administration, so more significant increment after two months of administration. However ,groups treated with clove oil aloneG3(500mg /kg clove oil) and G4 (750mg /kg clove oil) and in group G5 (500mg /kg clove oil +75mg/kgUN) showed significant reduction (P<(0.05) decrease in apoptotic index after one month and after two months of administration compare with G2 (75mg /kg UN) show in (Fig .4-10 and Fig.4-11).



(Fig . 2) Effects of clove oil used of 500 mg / kg or 750 mg/kg apoptosis in digestive system of male Rats treated with 75 mg /kg UN for one month. .(G1:Administrated with saline , G2: 75 mg /kg UN, G3:500 mg / kg clove oil,G4:750 mg/kg clove oil,G5: 500 mg / kg clove oil+75 mg /kg UN,G6: 750 mg/kg clove oil+75 mg /kg UN).



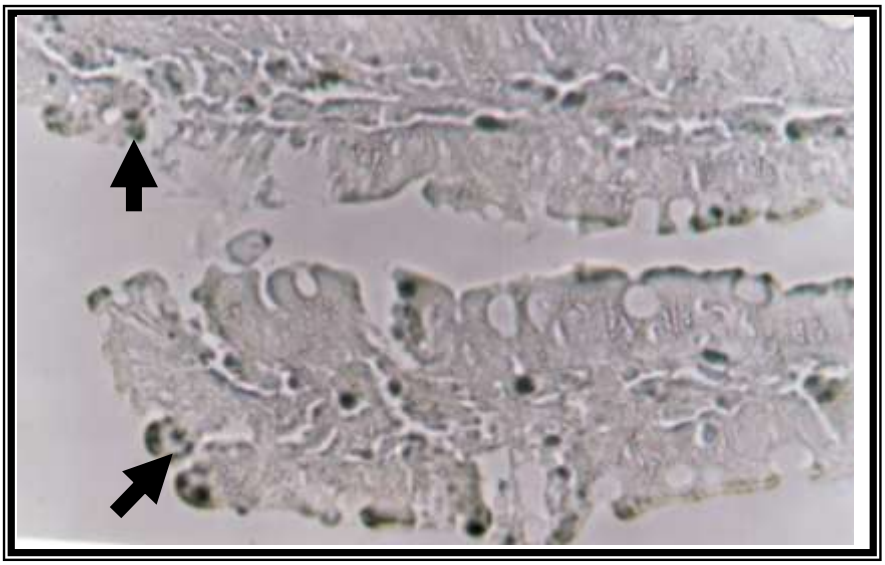
(Fig 3) Effects of clove oil used of 500 mg / kg or 750 mg/kg apoptosis in digestive system of male rats treated with 75 mg /kg UN for two months.(G1:Administrated with saline , G2: 75 mg /kg UN, G3:500 mg / kg clove oil,G4:750 mg/kg clove oil,G5: 500 mg / kg clove oil+75 mg /kg UN,G6: 750 mg/kg clove oil+75 mg /kg UN).

Apoptotic Examination

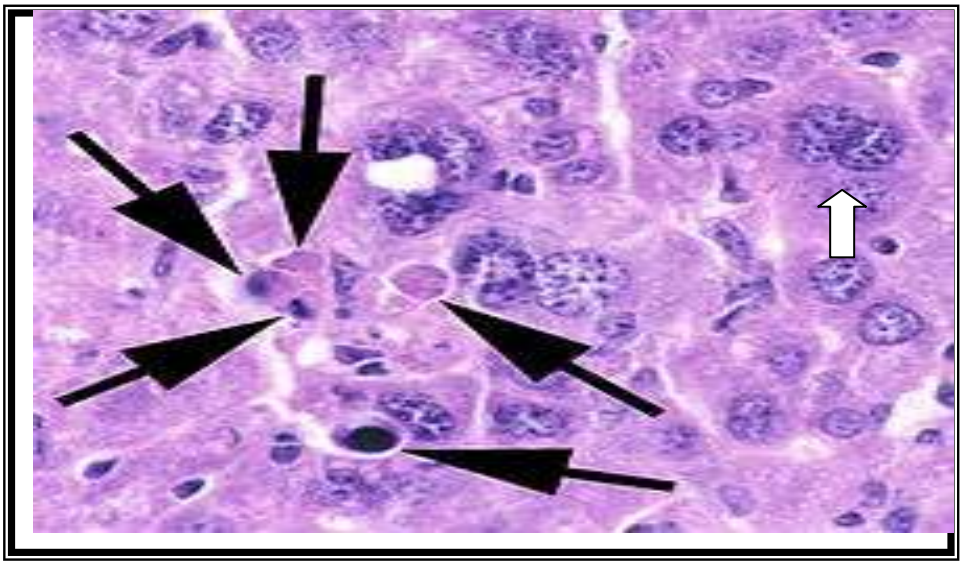
All stained sections that stained with silver nitrate AgNOR stain this study showed adequate staining , but variation in number of AgNOR stained nuclei was seen among the different administrated groups ,as shown in Fig. (4) .

The tissue sections prepared from small intestine of rats treated with saline only as control group G1 showed apoptotic cell with crescent shape normal epithelial mucosa as shown in Fig(4).

The tissue sections prepared from liver of rats treated with UN G2 (75 mg /kg UN) showing high number of apoptotic cells Fig(7), The tissue sections prepared from small intestine of rats treated with clove oil lone showing low number of apoptotic cells as illustrated in Fig(5) Fig6),and Fig7).



(Fig. 4): Section of small intestine of rat treated with saline for one month in G1A* Showing isolated apoptotic surface epithelial mucosa ( ). : 200 x (AgNOR stain) .



(Fig 6): Section of liver of rat treated with (75mg /kg UN)for one month in G2A* Showing isolated apoptotic of hepatic cells severe apoptotic cells ( ) ,mitotic division ( ) : 400 x (AgNOR stain) .

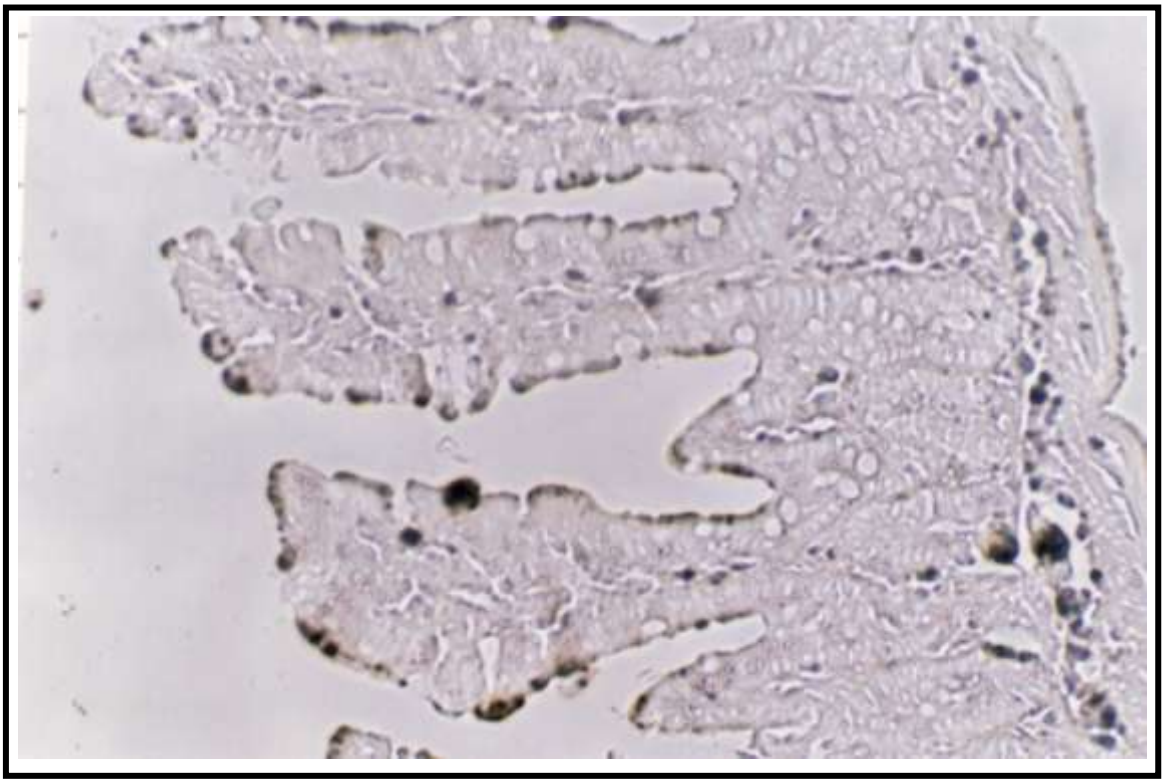
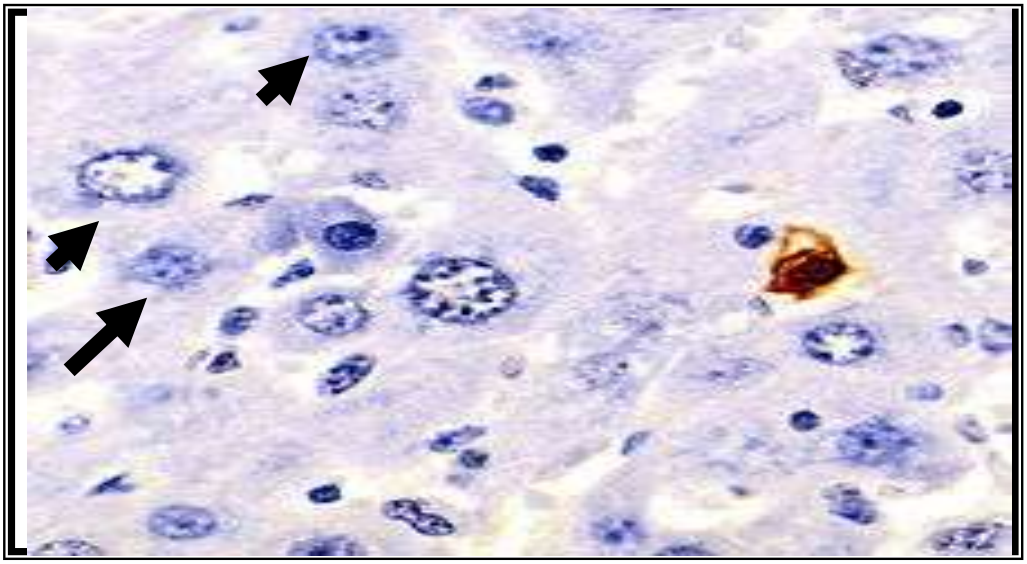
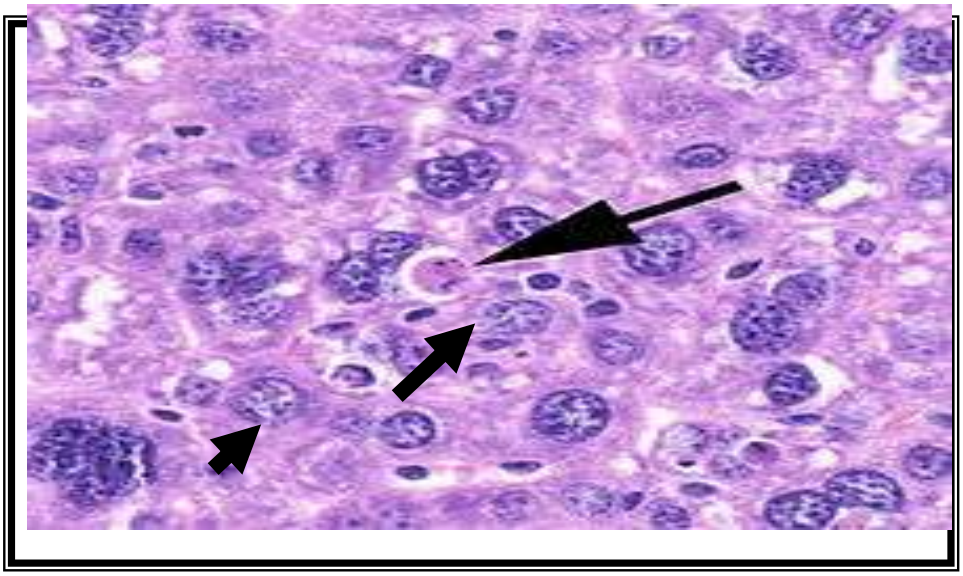


Fig. (40): Showing liver tissue in rat treated with 750 mg / Kg clove oil + 75mg / Kg UN two months G5B. Magnification: 200 X (AgNOR stain).



(Fig 7): Section of liver of rat treated with 500 mg /kg clove oil)for one month in G3A* Showing isolated apoptotic of hepatic cells ( ) Magnification : 400 x (AgNOR stain) .



(Fig. 8): Section of liver of rat treated with 750 mg / kg clove oil for two months G6B*Showing rat liver tissue apoptotic of hepatic cells ( ) Magnification: 200 x (AgNOR stain).

The death of higher cells according to genetically predetermined program probably has the biological aim of preventing cell compound from being released in a completely uncontrolled fashion which could lead to local inflammatory reaction , for example (24) . This avoided by the cells disintegrating into small pieces which are enclosed by the still intact cell membrane (so called apoptotic particles). The presence of cell membrane not only prevents the cell contents being released but is also a condition for effective phagocytosis of these particles by macrophages (25).

Apoptotic death may be initiated or modulated by the presence of some exogenous factors, such as drugs, radiation and also diet (26).

In the present study we reported that clove oil reduced apoptosis, however the role of the spice, clove, in regulating the balance between cell proliferation and apoptosis via modulating the expression of proliferation and apoptosis regulating genes in tumor cells, is not yet revealed.

It is clearly conceivable that increased or uncontrolled proliferation and an impaired apoptosis play a decisive role in the accumulation of malignant cells and eventually in the genesis of multistage carcinogenesis (27) .

Table (1):- Effects of clove oil used of 500 mg/kg or 750 mg/kg on apoptosis digestive system (stomach , small intestine , large intestine and liver) , of male rats treated with 75 mg/kg UN for one month and for two months

	Treated groups	n	G1 Saline μ±SD	G2 75 mg/kg UN μ	G3 500mg/kg Clove oil μ±SD	G4 750mg/kg Clove oil μ±SD	G5 500mg/kg Clove oil+ 75 mg UN μ±SD	G6 750 mg/kg Clove oil+ 75 mg /kgUN μ±SD
Stomach	A*	10	A,a 4.2±0.83	A,d 19.0±1.0	A,b 3.00±0.70	A,a 5.4±0.54	A,a 5.4±0.54	A,c 12.0±1.58
	B*	10	A,a 4.2±0.44	B,f 22.6±1.94	A b 2.4±0.54	A,d 6.2±0.44	A,d 6.2±0.44	A,e 13.8±1.30
Small intestine	A*	10	A,a 5.20±0.44	A,d 17.8±1.48	A,b 3.00±0.70	A,a 5.6±0.54	A,a 5.6±0.54	A,c 10.2±1.3
	B*	10	A,a 4.6±0.89	B,d 21.0±1.58	A,b 2.4±0.54	A,a 5.8±0.44	A,a 5.8±0.44	B,c 13.6±1.14
Large intestine	A*	10	A,a 4.2±0.44	A,d 17.8±0.44	A,b 2.6±0.54	A,a 5.2±0.83	A,a 5.2±0.83	A,c 10.4±1.14
	B*	10	A,a 4.4±0.54	B,f 20.8±2.16	A,b 2.6±0.89	A,d 6.0±1.22	A,d 6.0±1.22	B,e 13.6±1.14
Liver	A*	10	A,a 4.4±0.54	A,f 19.4±0.89	A,b 2.6±0.54	A,d 5.8±0.44	A,d 5.8±0.44	A,e 13.0±0.70
	B*	10	A,a 4.8±0.83	B,e 22.8±0.83	A,b 2.8±0.83	A,c 6.6±0.54	A,c 6.6±0.54	B,d 14.6±0.54

- Different small letter (a,b,c,d,e,f,) represent significant differences(P≤0.05) between means of the same column . Different capital letter (A,B,C,D,E<F,) represent significant differences(P≤0.05) between means of the same rows. A* for one month of administration, B* for two months of administration.

Referances

1-Taylar,D.M. and Taylar,S.K.(1997). Environmental Uranium and human health .Rev.Environ. Health .12: 147 -157.

2=Dublineau , I. ; Grison, S. ; Baudelin , C. ; Dudoignan , N. ; Souidi , M. ; Marquette , C. ; Paquel , F. and Aigueperse , J. (2005) . Absorption of Uranium through the entire gastrointestinal tract of the rat .Int . J. Radical . Biol. 81 (6) : 473 -482 .

3-Guegaen , Y. : Souid , M. : and Hadge, H. C. (1973). A history of Uranium poisoning (1824-1942). By H. C. Hodge, in Hand book of Experimental pharmacology.New series XXX VL, Uranium, Plutanium, Transplutonic Element, H. C. Hodge, eds springer- verlag, New york, p. (5-69).

4-Uncu, H. and Tuzuner, A. (2003). Epithelial Leiomyosarcoma of the gastrocolic ligament. Acta. Chir. Belg, 103 (1): 105- 107.

5-Forkas, R.H. and Gross kreutz, C.I. (2001). Apoptosis, neuropotection, and retinal ganglion cell death an overview for ophthalmol clin. J. 41 (1): 111- 130.

6-Burt,S.A. and Reinders, R.D. (2003).Antibacterial activity of selected plant essential oils against Es cherichia coli. Appl. Microbial. 36 (3); 57-61.

7-Steinmetz, k. A. and Potter, J. D. (1996).Vegetables, fruit, and cancer prevention are view. J. Am. Diet. Assoc. 96: 1027- 1039.

8-Kokileva, L. (1994). Multi- step chromatin degradation in apoptosis. Int. Arch. Allergy Immunal, 105: 339- 343.

9-Lowe, S. W.; Ruley, H. E.; Jacks, T. and Housmun, D. E. (1993). P53- Dependent. Apoptosis modulate the cytotoxicity of anti cancer agents. Cell. 34: 957- 967.

10-Kerr, J.F.R.; Wyllie, AH. and currie, AR. (1972). Apoptosis: a basic biological phenomenon with wide- ranging implication in tissue kinetics. Br. J. concer. 26 (4): 239- 257.

11-Givgliano D. (2000). Dietary antioxidants for cardiovascular prevention. Nat. Metab. Cardiovas. 10: 38- 44.

12-Todd, D.; Yang, G. and Brown, R. w. (1996).Apoptosis in renal cell carcinoma, Detection by in situ end – labeling of fragmented DNA and correlation with other prognostic factors. J. Hum. Pathol. 27 (10): 1012- 1017.

13-Rudzinski, M.; Wong, TP. And Saragrovi, H.U. (2004). Changes in retinal expression of neurotrophins and newotrophin receptors induced by ocular hypertension. J. neurobiol. 58 (3): 341- 354.

14-Shim, C.; Zhang, W. and Rhee, ch. (1998) profiling of differentially expressed genes in human primary cervical cancer by complementary DNA expression orray. J. clin. Cancer. Res. 4 (21): 3045- 3050.

15-Nicoletti, I.; Migliorat, G. and Pagliacci, M.C. (1991). A rapid and simple method for measuring thymocyte apoptosis by propidium iodide staining and flow cytometry [J]. J. Immunal methods, 139 (2): 271- 279.

16-Zuzarte- Luis. V. and Hurle, J.M. (2002)." programmed cell death in the developing limb " int. J. Dev. Biol. 46 (7): 871- 878.

17-Forkas, R.H. and Gross kreutz, C.I. (2001). Apoptosis, neuropotection, and retinal ganglion cell death an overview for ophthalmol clin. J. 41 (1): 111- 130.

18-Thompson ,C.D. (1995). Apoptosis in the pathogenesis and treatment of disease. J. Science. 267(5203): 1456- 1462

19-Santon, A. and Susin, A. (2000). Two Distionct pathways leading to Nuclear Apoptosis. Journal of Experimental medicine. 192 (4): 116-122.

20-Nicoletti, I.; Migliorat, G. and Pagliacci, M.C. (1991). A rapid and simple method for measuring thymocyte apoptosis by propidium iodide staining and flow cytometry [J]. J. Immunal methods, 139 (2): 271- 279

21-Lodish, H. (2004). Molecular cell Biology New york; W. H. Freedmen and company, pp. 4366- 4373.

22-Cronin ,K. ; Leftus ,B. and Dervan , P. A. (1989) . Are AgNORs useful in distinguishing follicular hyperplasia from follicular lymphoma ? J. Clin . Pathol . 42 : 1267 -1268 .

23-Duncan , R. C. ; Knapp , R. G. and Miller , M. C. (1983). Introductory Biostatistics of Health Sciences. A Wileg Medical Publication , John Wiley and Sons , London .pp: 161 -179 .

24-Jemal, A.; Murray, T.; ward, E.; Samuels, A.; Tiwari, R.C., Ghafoor, A.; Feuer, E. J. and Thun, M. J. (2005). CANCER STATISIES. Ca. Cancer. J. Clin. 55: 10- 30.

25-Hall , AH . and Rumack , BH . (2004) . Information System Micromedex . Inc . Englewood , Volum 122:32 -33 .

26-Hawkins, R. A.; Sangster, k. and Arends, M. J. (1998). Apoptosis death of pancreatic cancer cells induced with double bond number and involves an oxidative mechanism. J. pathol. 185: 61- 70.

carcinogenesis. Asian **27-Banerjee, S. and Das, S**. (2005). Anticarcinogenic of clove on skin Pacific . J. Cancer. Pred. 6: 304- 308.