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MOLECULAR DETECTION OF ANTIOXIDANT ACTIVITIES OF CINNAMON FLAVINOID EXTRACT AGAINST DIBUTYLNITROSAMINE CARCINOGENICITY

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ABSTRACT

Still remain conscious to improve the treatments of cancer by searching about new compound, which might be effective at the same time less harmful for human health. The present study aims to identification of the individual phenolic compounds and flavonoids of cinnamon extract (CFE) and its effects as chemopreventive agents on the carcinogenicity induced by dibutylnitrosamine (DBNA) in albino rats. The liver microsomal lipid peroxidation product such as malondialdehyde (MDA). reduced glutathione (GSH) and superoxide dismutase (SOD) as antioxidant enzymes and DNA damage (Comet assay) inside every cell were measured. The result of CFE analysis (HPLC) included. By using rat liver homogenate, the administration of 150 mg or 300 mg CFE/kg. bw/day in the presence and absence of (DBNA) for 4, 8 and 12 weeks showed increase then decrease in MDA concentration in the different treated groups. Flavinoids decreased the level of lipid peroxidation through the liver testes as, GSH and SOD, and ameliorate the damaged nuclei in the blood Lymphocytes. In conclusion. DBNA could dramatically change the biological activities in liver and CFE play a significant role in protecting pathogenesis of these changes.

Key words: Antioxidant- Cinnamon Flavinoids- Carcinogenicity- Oxidases. DNA damage.

INTRODUCTION

Nitrosamines are produced from nitrite and amines; N- nitroso compounds are one of the important groups of carcinogens frequently present in human environment and food chain **[Oshawa *et al.*, (2003)].** The presence of nitroso compounds and their precursors in human environment together with the possibility of their endogenous formation

in human body have led to suggestions of their potential involvement in human cancers **[Kaplan et *al.*, (1997)]. Uwagawa et al., (1991)** suggested that treatment rats with N. N`- dibutyl-nitrosamine (0.05% in drinking water) for 20 weeks were enhanced the incidences of preneoplastic and neoplastic lesions in liver, esophagus, forestomach and urinary bladder.

A free radical is any species (atom, group of atoms or molecule) that contain one or more unpaired electrons **[Gutteridge, (1995)]**, this situation is unstable making such species highly reactive and short lived. If two radical meet they can combine their unpaired electrons, join to form covalent bonds and both radical are lost, when they react with non radicals they either donate their unpaired electron to or take an electron from the other non radical, the result of these reactions is a chain reaction where one radical be gets another **[Mercuri *et al.*, (2000)]**. Nitroso compounds have been suggested to cause oxidative stress and cellular injury due to involvement of free radicals **[Aiub *et al.*, (2003)]**. There is considerable support to the concept that oxygen free radicals and related lipid peroxides also play a key role in the pathogenesis of normal senescence and of age-related chronic degenerative disease, including cancer and atherosclerosis **[Maxwell (2000)]**.

Antioxidant is a substance that delays or inhibits oxidative damage to a target molecule. It works in concert to provide protection against radical reaction by scavenging reactive oxygen species preventing the formation of reactive oxygen species **[Halliwell (1996)]**. Chelation or binding transition metal ions and repairing damage of the target organs **[Sies (1993)]**. Glutathione peroxidase (glutathione H_2O_2 oxidoreductase) is a widely distributed enzyme functioning in the prevention of the deleterious effects of peroxides generated in the course of normal metabolism **[Weiss et al., (1980)]**.

Flavonoids have been shown in a number of studies to be potent antioxidants, capable of scavenging free radicals including the superoxid radicals (O^{-}), hydroxyl radical (OH), hydrogen peroxide (H_2O_2) and lipid peroxide radicals have been implicated in number of disease including cataracts, macular degeneration **[Gerster (1989)]**, cardiovascular disease **[Hertog *et al.*, (1993)]**, diabetes **[Kahler *et al.*, (1993)**, gastrointestinal inflammatory disease **[Smirnov (1994)]**, liver disease **[Miguez *et al.*, (1994)]**, periodontal disease and other inflammatory process **[Bobyrev *et al.* (1994)]** and sthma **[Greene (1995)]**, Cancer **[Ginter (1995)]**.

Sudheer *et al.*, (2008) examined the effect of ferulic acid, naturally occurring phenolic compound on lipid peroxidation and endogenous antioxidant status on the activities of lactate dehydrogenase and alkaline phosphatase in plasma, DNA single strand breaks in peripheral blood of nicotine treated rats. The rats showed a significant decrease in the activities of circulating lactate dehydrogenase, alkaline phosphatase, the level of lipid peroxidative markers, DNA strand breaks (Comet parameters), micronuclei frequency (in the whole blood) and increase in the expression of cyclooxygenase-2 and Nf-kappaB and significant increase in antioxidant status.

MATERIALS AND METHODS

The male albino rats *Ratus norvigicus* weigtting 100 ± 5 gm were kept in the laboratory under constant conditions of temperature (24 ± 2 ° C) for at least one week before and through the experimental work, being maintained on a standard diet and water were available *ad-libitum*. The experiment rats were divided into six groups. The first group: were fed on the basal diet and normal water and left in normal condition for 12 weeks were served as control group. The rats of the second groups: were fed on the control diet and drink tap water containing 20 ml/L dibutylamine (DBN) and 5g/L sodium nitrite daily for 12 weeks. Third group: were fed the basal diet supplied with 150 mg/kg body weight of powdered cinnamon flavonoid extract (CFE). The fourth group: were fed the basal diet supplied with 300 mg/kg body weight of powdered (CFE). The fifth groups: were fed the basal diet supplied with 150 mg/kg body weight of (CFE) and drink tap water containing 20 ml/L dibutylamine (DBN) and 5g/L sodium nitrite. The sixth group: were fed the basal diet supplied with 300 mg/kg (CFE) and drink tap water containing 20 ml/L dibutylamine (DBN) and 5g/L sodium nitrite. The diet and drink (nitrosamine precursors **[Hashimoto *et al.*, (1976)]** prepared daily just before the feeding time, CFE dose were calculated according to the human recommended dose.

Five rats taken every 4 weeks from the experimental groups were sacrificed then blood was collected and left to clot and centrifuged at 3000 rpm for 10 min. The supernatant serum was aspirated and stored at –20°C until used in biochemical studies. Samples form liver were excised.

Part of liver was homogenized and used for biochemical assays of MDA, SOD and GSH according to **Lindsay (1968), Giannopolitis and Ries (1977)** and **Yashkochi and Masters (1979)** respectively.

Identification of individual phenolic compounds in prepared cinnamon extract was performed according to **Sauvesty *et al.*, (1992).**

The studying of DNA damages by single cell gel electrophoresis was performed according to **Sing *et al.*, (1988).**

Statistical analysis: The result was computed using SPSS software program version 10.

RESULTS

I: Identification of phenolic comp. *cinnamon* ethanolic extract:

Data illustrated in Figure (1) showed the phenolic compounds chromatogram resulted from HPLC system. The bark of *Cinnamomum verum* contains Pyrogalic acid (0.49%), Protocatechanic (0.57%), Catechin (1.05%), polyhydroxy benzoic (0.28%), *P.* Coumaric (2.88%), phenol (0.66%), *O.* Coumaric (1.54%), salicylic (0.26%), coumarin (4.5%), quercetin (0.016%) and cinnamic acid (8.035%) as the major components.

II- The antioxidant activity of cinnamon flavonoid extracts (CFE) in liver homogenate:

1- Malondialdehyde (MDA) concentration:

Table (1) showed the changes on malondialdehyde (MDA) concentration during different period of feeding diet contains 150 or 300 mg CFE/kg b.w./day in the presence and absence of nitrosamine precursors (DBA+NO2). In control group (G1), MDA concentration at 8 weeks reached to the highest value 3.85 nmol/mg tissue protein. Rats treated with nitrosamine (G2) showed significant increase in MDA concentration during all sampling periods by the ratio of 441.26, 613.25 and 1248.16% for 4, 8 and 12 weeks as a percent of control, respectively, in comparison with control group (G1). Feeding with CFE 150 mg/kg b. w./day showed decrease at 4 weeks then slight increase at 8 weeks and decrease at the last weeks by the ratio 0.05, 0.17 and 0.21% as percent of control, respectively. Increasing CFE to 300 mg/kg b. w./day showed non significant decreasing during all sampling periods by the ratio of 7.65, 22.60 and 31.29% for 4, 8 and 12 weeks. In G3 & G4 the MDA level

returned into the normal range with variations. Feeding 150 mg/kg b. w./day in the presence and absence of nitrosamine (G5) showed significant increasing at 4 weeks then started to decrease at 8 weeks and reached to the lowest value at 12 weeks by the ratio 289.07, 168.57 and 142.33 . Feeding rats with 300 mg CFE mg/kg b. w./day in presence of nitrosamine (G6) showed significant increase at 4 weeks then started to decrease at 8 weeks and reached to the lowest value at 12 weeks by the ratio 207.10, 205.45 and 178.53% as a percent of control, respectively, in comparison with control group. On the other hand the MDA level in G5 & G6 decreased significantly in comparison with nitrosamine group during all the period of treatments.

2- Superoxide dismutase activities:

Table (2) showed the changes in SOD activities during different periods of feeding diet containing 150 or 300 mg CFE/kg b.w./day in the presence and absence of nitrosamine. In control group (G1) SOD activity reached to the highest value 5.60 umol/mg tissue proteins at 8 weeks. Rats maintained on nitrosamine precursors (G2) showed continuous decreasing in enzyme activity by the ratio 55.07, 71.07 and 85.58% for 4,8 and 12 weeks as a percent of control, respectively, these decreasing are significant during all the experiment periods in comparison with control group. Feeding 150 mg CFE/kg b.w./day (G3) showed decreasing at 4 weeks and slight decreasing in 8 and 12 weeks by the ratio –2.66, -1.36 and –0.86%. Addition of 300 mg CFE/kg b.w./day (G4) showed increase at 4 weeks then decrease at 8 weeks and reached to the highest value at 12 weeks by the ratio 17.03, 13.93 and 21.17, these increase are significant during all the periods of treatments. Feeding 150 mg/kg b.w./day in presence of nitrosamine showed continuous decreasing at 4 and 8 weeks and reached to the highest value at 12 weeks by the ratio of –51.09, -57.14 and –47.81 % . On the other hand the SOD activity in G5 and G6 increased significantly in nitrosamine group during all the experiment periods.

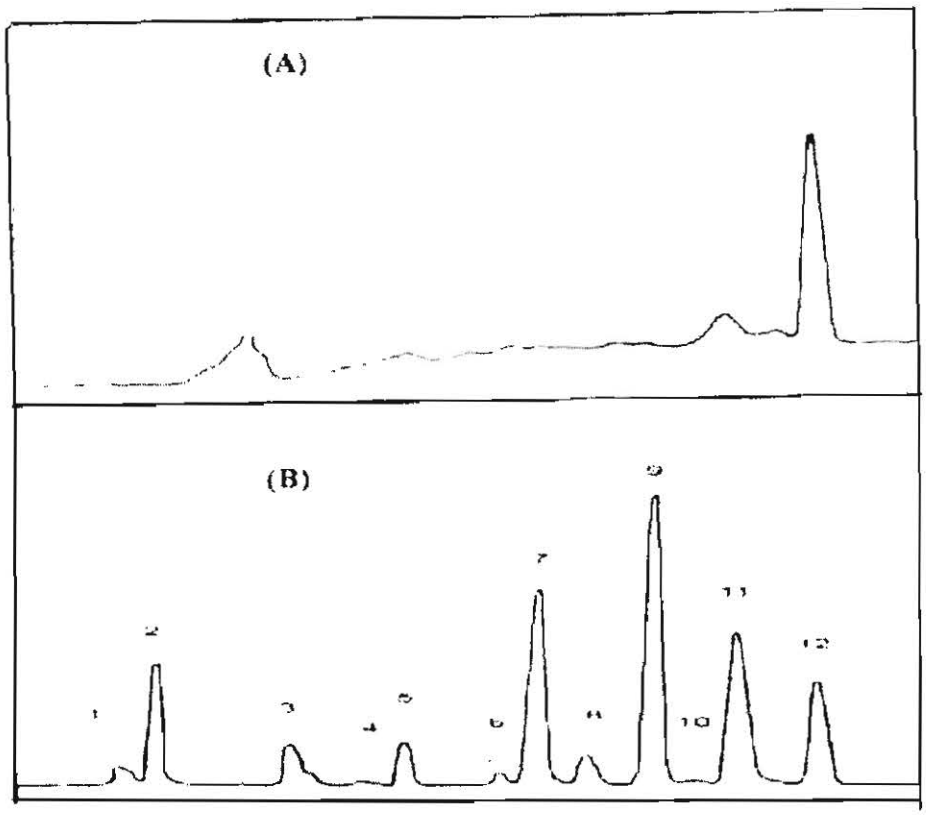
3- Reduced glutathione (GSH) content:

Table (3) showed the changes in GSH activities during different periods of feeding diet containing 150 or 300 mg CFE/kg b.w./day in the presence and absence of nitrosamine. In control group (G1), GSH content reached to the highest value 9.04 umol/mg tissue protein at 4 weeks. Rats maintained on nitrosamine precursors (G2) showed significant decreasing

during all sampling periods by the ratio 40.71, 48.24 and 62,05% for 4, 8 and 12 weeks as a percent of control, respectively in comparison with control group. Feeding rats with 150 mg CFE/kg b.w./day (G3) showed gradual increase at 4 weeks then started to increase significantly at 8 and 12 weeks. by the ratio +11.70, +71.52 and +106.4% respectively compared to control. The significant increasing were obtained after 8 and 12 weeks. An increasing CFE to 300 mg/kg b.w./day (G4) showed continuous increasing during all sampling periods by the ratio 20.6, 78.2 and 93.0% for 4,8 and 12 weeks as a percent of control, respectively, the significant increase was obtained during all the experiment periods. Feeding 150 mg CFE/kg b.w./day in the presence and absence of nitrosamine (G5) induced continuous decreasing at 4 and 8 weeks then reached to the highest value at 12 weeks by the ratio of –28.54, -35.92 and –21.5% as a percent of control, respectively. Increasing CFE to 300 mg/kg b.w./day (G6) showed continuous decreasing by the ratio of 31.42, 34.04 and 18.2% for 4, 8 and 12 weeks. In G5 & G6 the decreasing are significant after 4 and 8 weeks, then returned to the nearly normal at the end of the experiment. On the other hand the GSH content in G5& G6 were increased significantly in comparison with nitrosamine group (G2) at the end of experiment (12 weeks).

III- DNA damage:

Table (4) and Figure (2 a, b, c) represents the data of DNA migration in agarose gel after treatment with dibutylnitrosamine (DBNA) and the protective role of cinnamon flavonoid extract. Lymphocytes cells treated with DBNA showed greater migration as compared to control group. That means DBNA induced a greater number of cells with additional DNA-lesions (migration of DNA fragments) and with a higher proportion of DNA damage. According to the time of treatments damaged and strong damaged cells could be observed much more frequently as compared to the controls. Feeding CFE by the concentration of 150 mg /kg b.w./day and 300 mg /kg b.w./day showed a reduction in DNA damage cells, reduced cytotoxic activity by dibutylnitrosamine. CFE alone also induced a protection to the blood lymphocytes cells.



HPLC chromatograms of Cinnamon flavonoid ethanolic extract (A) and standard of phenolic compounds (B)*

Retention time for phenolic compound was performed on a Hewlett-packed HPLC (Model 110), using reversed-phase column (250 x 4.6mm) with 5µm particle size. Injection by Rehodyne injection value (Model 7125), as follow: solvent (a) 0.5% acetic acid : 99.5% D.W and solvent (b) 0.5% acetic acid : 99.5 acetonitrile,. Flow rate (1.5 ml/min) and Detector, UV at 254 nm. Phenolic compounds of sample were identified by comparing their relative retention times unit those of standards mixture chromatogram identification of peaks:

1- Pyrogallic	2- Gallic	3- Drotocatechuic
4- Catechin	5- P.OH benzoic acid	5- P.OH benzoic acid
6- P. Coumaric	8- *o*. Cumaric	9- Salicylic
10- Coumarin	11- Quecertin	12- Cinnamic acid

Fig. (1): Identification of phenolic compound in *cinnamon* ethanolic extract.

Table (1): The effect of feeding cinnamon flavonoid extract (CFE) on malondialdehyde (MDA) concentration (nmol / mg tissue protein) in liver tissue of rats drinking dibutylnitrosamine (DBNA) .

Period of treatment (weeks)	Control (G1)	DBNA (G2)		CFE feeding				DBNA drinking +CFE feeding			
				150 mg/kg of CFE (G3)		300 mg/kg of CFE (G4)		150mg/kg of CFE (G5)		300mg/kg of CFE (G6)	
	nmol / mg tissue protein	nmol / mg tissue protein	% of Control	nmol / mg tissue protein	% of Control	nmol / mg tissue protein	% of Control	nmol / mg tissue protein	% of Control	Nmol/ mg tissue protein	% of Control
4	3.66 ± 0.74^{c}	19.81 ± 5.65^{a}	-441.26	3.61 ± 0.62^{c}	-0.05	3.38 ± 0.53^{c}	-7.65	14.24 ± 1.20^{b}	+289.7	11.24 ± 1.17^{b}	+207.10
8	3.85 ± 0.87^{c}	27.46 ± 6.95^{a}	-613.25	4.02 ± 0.55^{c}	+0.17	2.98 ± 0.56^{c}	-22.60	10.34 ± 0.91^{b}	+168.57	11.76 ± 1.43^{b}	+205.45
12	3.26 ± 0.78^{c}	43.95 ± 5.90^{a}	-1248.13	3.05 ± 1.34^{c}	-0.21	2.24 ± 0.50^{c}	-31.29	7.90 ± 1.66^{b}	+142.33	9.08 ± 1.47^{b}	+176.53

- Numbers are expressed as mean ± S.D. of five rats in comparison to control.
- The mean different is significant at $p < 0.05$.
- Different letter (a,b,c) mean significant variations in comparison to control.

Table (2): The effect of feeding cinnamon flavonoid extract (CFE) on superoxide dismutase (SOD) activity (µmol / mg tissue protein) in liver tissue of rats drinking dibutylnitrosamine (DBNA) .

Period of treatment (weeks)	Control (G1)	DBNA (G2)		CFE feeding				DBNA drinking +CFE feeding			
				150 mg/kg of CFE (G3)		300 mg/kg of CFE (G4)		150mg/kg of CFE (G5)		300mg/kg of CFE (G6)	
	µmol / mg tissue protein	µmol / mg tissue protein	% of Control	µmol / mg tissue protein	% of Control	µmol / mg tissue protein	% of Control	µmol / mg tissue protein	% of Control	µmol / mg tissue protein	% of Control
4	5.52±0.14^{d}	2.48±0.40^{a}	-55.07	2.86±0.11^{c}	-2.66	6.46±0.27^{e}	+17.03	3.44±0.03^{b}	-37.68	2.70±0.18^{ac}	-51.09
8	5.60±0.35^{d}	1.62±0.31^{a}	-71.07	4.24±0.27^{e}	-1.36	6.38±0.14^{f}	+13.93	3.46±0.08^{b}	-38.21	2.40±0.32^{c}	-57.14
12	5.48±0.10^{c}	0.79±0.10^{a}	-85.58	4.62±0.44^{d}	-0.86	6.64±0.15^{e}	+21.17	2.44±0.55^{b}	-55.47	2.86±0.39^{b}	-47.81

- Numbers are expressed as mean ± S.D. of five rats comparison to control.
  - The mean different is significant at $p < 0.05$.
  - Different letter (a,b,c) mean significant variations comparison to control.

Table (3): The effect of feeding cinnamon flavonoid extract (CFE) on reduced glutathione concentration (µmol / mg tissue protein) in liver tissue of rats drinking dibutylnitrosamine (DBNA) .

Period of treatment (weeks)	Control (G1)	DBNA (G2)		CFE feeding				DBNA drinking +CFE feeding			
				150 mg/kg of CFE (G3)		300 mg/kg of CFE (G4)		150mg/kg of CFE (G5)		300mg/kg of CFE (G6)	
	µmol / mg tissue protein	µmol / mg tissue protein	% of Control	µmol / mg tissue protein	% of Control	µmol / mg tissue protein	% of Control	µmol / mg tissue protein	% of Control	µmol / mg tissue protein	% of Control
4	9.04±1.67^{a}	5.36±0.16^{b}	-40.71	10.10±1.32^{ac}	-11.7	10.90±2.02^{c}	+20.6	6.46±0.39^{b}	-28.5	6.20±0.35^{b}	-31.4
8	8.52±0.13^{a}	4.41±0.21^{b}	-48.24	14.62±2.99^{c}	+71.6	15.18±1.65^{c}	+78.2	5.46±0.19^{b}	-35.9	5.62±0.25^{b}	-34.0
12	8.96±0.86^{a}	3.40±0.37^{b}	-62.05	18.49±2.79^{c}	+106.4	17.29±3.17^{c}	+93.0	7.03±0.54^{a}	-21.5	7.33±0.65^{a}	-18.2

- Numbers are expressed as mean ± S.D. of five rats comparison to control.
- The mean different is significant at $p < 0.05$.
- Different letter (a,b,c) mean significant variations comparison to control.

Table (4): The effect of feeding cinnamon flavonoid extract (CFE) on DNA damage blood lymphocytes of rats drinking dibutylnitrosamine (DBNA) .

Period of treatment (weeks)	Control (G1)		DBNA (G2)		CFE feeding				DBNA drinking +CFE feeding			
					150 mg/kg of CFE (G3)		300 mg/kg of CFE (G4)		150mg/kg of CFE (G5)		300mg/kg of CFE (G6)	
	Mean±SD	%	Mean±SD	%	Mean ±SD	%	Mean ±SD	%	Mean ±SD	%	Mean ±SD	%
4	1.0 ± 0.7	2	6.2 ±0.84	+12a	1.4 ± 0.55	+2	0.8 ± 0.84	0	5 ± 0.71	+10a	4 ± 0.83	+8
8	1.0 ± 0.71	2	9 ± 0.72	+18b	0.6 ± 0.89	0	0.4 ± 0.55	0	7.2 ± 0.84	+14a	6.6 ± 0.83	+12a
12	2.0 ± 0.71	4	1.6 ± 1.14	+22b	0.4 ± 0.55	+2	0.4 ± 0.54	+2	8.4 ± 1.14	+16b	5.6 ±	+10a

- Numbers of damaged cells are expressed as mean ± S.D. of five rats.
- % of the change than the control.
- The mean different is significant at $p < 0.05$.
- Different letter (a,b,c) mean significant variations comparison to control.

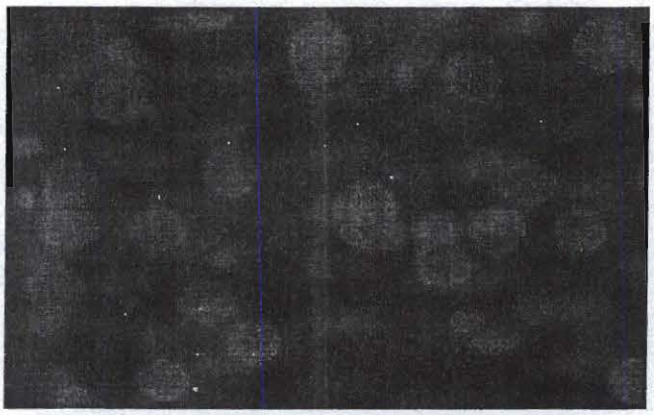


Fig. (2a): Fluorescent microscopic image showing Normal lymphocytes.

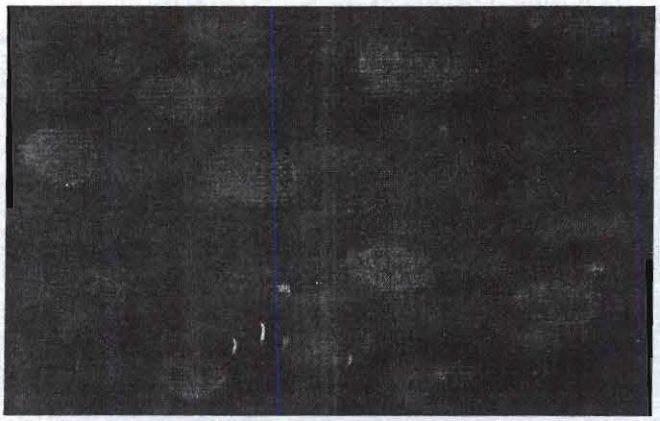


Fig. (2b): Fluorescent microscopic image showing damaged cells.

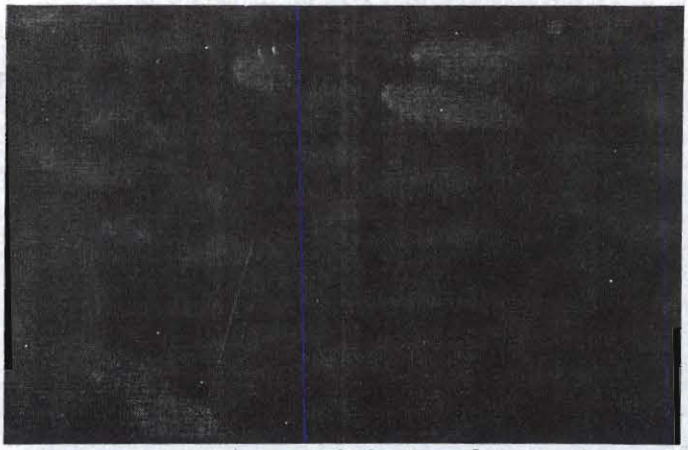


Fig. (2c): Fluorescent microscopic image of strong damages cells.

DISSCUSION

The obtained data of Cinnamon flavonoid extract was confirmed by **Newall et al. (1996)** who found that the cinnamon contains polyphenols (4-10%) tannins, gum, mucilage, restin, starch, sugars and traces of coumarin. also may be synergistic with vitamin and trace minerals. On the other hand, , the total phenolics and flavonoids were measured in Cinnamon by [**Nair *et al.*, (1998)**] which indicated that total phenolics and flavonoids content in cinnamon more than 100 mg/ 100gm, flavonoids content were measured as the sum of quercetin, kaemferol, luteolin and pelargonidin.

Lipid peroxidation (LPO) initiated by free radicals. It considered to be deleterious for cell membranes and has been implicated in a number of pathological situations. Flavonoids decreased the level of LPO in liver [**Tirkey *et al.*, (2005)**]. When fatty acid is peroxidized, it is broken down into aldhydes, which excreted. Aldehydes such as thiobarbituric acid reacting substance (TBARS) have been widely accepted as a general marker of free radical production. The most commonly measured TBARS is on malonaldehyde [**Sjodin *et al.*, (1990)**]. Our study showed that in DBNA treated rats, **MDA** was increased through all the periods. which is matched with **Ramakrishnan *et al.* (2006)** whose found that n-nitrosodiethylamine administration induced increase the relative liver weight, the levels of lipid peroxides, glutathione (GSH) and superoxide dismutase (SOD). The toxic action produced by nitrosamine might be attributed to generation of oxidative stress due to generation of reactive oxygen species and alter the antioxidant defense system in the cells and tissues [**Mittal *et al.*, (2006)**].

GSH content in the present study matches with the result of **Dhuley (1999).** The enzyme activity markedly restored when rat fed diet alone with cinnamon antioxidant. Moreover, antioxidative compounds such as polyphenol, flavonoids vitamin E and C have a number of biological activities, as immune stimulation, inhibition of nitrosamine formation, an alteration of metabolic activations of carcinogens and inhibition of genetic changes.

SOD and catalase are the major enzymes, which calalyze and help in elimination of ROS derived from redox process of xenobiotics in the liver tissue [**Poli (1993)**]. Such as shown in the present data SOD decreased in DBNA treated group all over the experiment period, which in agreement with [**Ramakrishnan *et al.* (2006)**]. Supplemented diet

with 300 mg/kg CFE increased SOD activity, to added to several studies which reported that CFE exert antioxidant protection through their ability to activate the antioxidant enzymes (SOD&GSH-PX) and also decreased MDA, nitric oxide (NO) and prostaglandin **[Dhuely (1999) and Wu *et al.*, (2006)]**.

DNA damage in the blood lymphocytes elevated in DBNA treated group, then considerable degree of repair with addition of CFE to the diet, which is in going with **Anderson *et al.* (1998)** who found that the flavonoid, silymarin, myricetin, quercitin, kaempferol, rutin and kaempferol-3-rutinoside have been examined in combination with the food mutagens, reduced antigenotoxic effect since DNA damage was reduced in the Comet assay in lymphocytes, this would suggest that the effects occur in somatic and germ cells. Also, the protective effect of ellagic acid, a natural polyphenolic compound, against nicotine toxicity was elucidated by analyzing the lipid peroxidative index viz., superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), reduced glutathione (GSH), Vitamins A, E and C. DNA damage and repair (Comet assay) and micronucleus assay. There was a significant increase in the level of lipid peroxidative idex, severity in DNA damage and micronuclei number in nicotine-treated rats, which was positively modulated by EA treatment **[Sudheer *et al.*, (2007)]**. In conclusion, Cinammon flavonoid extract ameliorate the biological changes induced by carcinogens.

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التحديد الجزيئي للنشاط المضاد للأكسدة لمستخلص مسحوق القرفه ضد السرطانات المحدثة بالنيتروزوأمينات

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مازال الإهتمام الواضح بمعالجة الأورام السرطانية بإستخدام مواد جديدة تكون فعالة وفي نفس الوقت أقل ضرراً علي صحة الإنسان. صممت هذه الدراسة لمعرفة المكونات الفينولية لمسحوق القرفة وإستخدامه كواقي غذائي ضد الأورام السرطانية المحدثة بالداي بيوتيل نيتروزأمين في الجرذان البيضاء. تم دراسة الإنزيمات الناتجة من الأيض الكبدي الدقيق للدهون مثل المالون داي ألدهيد والجلوتاثيون المختزل والسوبرأكسيد دسميوتيز كمضادات لأكسدة، ودراسة تكسير الحمض النووي داخل كرات الدم الليمفاوية وإصلاحه. سجلت في البحث نتائج تحليل مسحوق القرفة بجهاز الفصل الكهربي السائل عالي الكفاءة. بإضافة مستخلص مسحوق القرفة إلي غذاء الجرذان بنسبة ١٥٠مجم / كجم و ٣٠٠ مجم/ كجم من وزن الجسم في وجود أو غياب المادة المسرطنة لمدة٤-٨-١٢ أسبوع أدي إلي إنخفاض المالون داي ألدهيد والسوبر أكسيد والجلوتاثيون المختزل، وحافظ على أنوية كرات الدم من التحطم. والخلاصة أن النيتروزوأمينات تغير بشدة العوامل البيولوجية ويلعب مستخلص القرفة دور وقائي لتأثيرها.