Journal of Pharmacology and Toxicology

ISSN 1816-496X

Academic Journals Inc.

www.academicjournals.com

Journal of Pharmacology and Toxicology 4 (2): 58-69, 2009
ISSN 1816-496X

Dibutylnitrosamine Induces Histopathological Changes in Rat: Possible Protective Effects of Cinnamon Flavonoid Extract

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Abstract: The aim of this study was to investigate the protective role of Cinnamon Flavonoid Extract (CFE) against histopathological changes in albino rats of Wistar strain treated with Dibutylnitrosamine (DBNA) for 12 weeks. The results indicated that rats treated with DBNA recorded decreasing in the total body and liver weights and increasing in spleen and kidney weights with significant values when compared with the control group all over the experiment period 4, 8 and 12 weeks. Addition of CFE by 150 and 300 mg kg^{-1} b.wt./day in the presence of nitrosamine induced significant improvements in all organs weights. Also, DBNA treated group had histopathological changes on liver through degeneration hyperemia, inflammatory reaction, kidney through hemorrhages renal casts hyperemia, inflammatory reaction and also urinary bladder through papillary hyperplasia with papillary projection formation in the cell layer of the lining epithelium. The co-treatment of CFE with DBNA leads to prevent some of the previous histopathological changes mainly on liver and urinary bladder and secondary on the kidney. It could be concluded that CFE was effective in protecting against DBNA-induced histopathological changes. These results supported present hypothesis that CFE contains several compounds that are able to prevent or inhibit DBNA toxicity.

Key words: Dibutylnitrosamine, cinnamon flavonoid extract, liver, spleen, kidney

INTRODUCTION

Although, efforts in cancer diagnosis and therapy have intensified over the years, many cancers still remain difficult to cure. Several recent studies become more conscious and enthusiastic to improve the process of treatment by searching about new compound, which might be effective toward cancer cells at the same time less harmful for the human health (Newmark *et al*., 1992).

Nitrosamines are known carcinogenic agents. However, their mechanism of action is not well understood. Generation of reactive oxygen species could be an important cause of toxicity of nitrosamines (Masuda *et al*., 2000). The presence of Nitrose 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). The maternal consumption during pregnancy of meat cured with sodium nitrite increases the risk for brain tumors in their offspring. Intake of vitamins C and E inhibit endogenous formation of N-Nitrose compounds and was accepted to be protective (Preston-Martin *et al*., 1996).

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Cinnamon (*Cinnamon vernum*) is a famous beverage in several countries all over the world including Egypt. It has a long history of medicinal use and is one of the world's most important spices (Stavric *et al*., 1992). Extract of cinnamon have also been shown to have antioxidant effects in part through activating antioxidant enzymes in various tissues (Anilakumar *et al*., 2001). That prevent free radical formation, remove radicals before damage can occur, repair oxidative damage, eliminate damaged molecules, or prevent mutations are important mechanisms in cancer prevention (Gordon, 1996).

Changes in histopathological parameters as the result of sodium nitrate intake have been studied by National Toxicology Program (2001). The mean body weight of females at the highest dose were lower than those of control groups and showed the incidences of squomas cell papilloma and carcinoma (combined) in the forestomach of female mice. The incidence of hyperplasia the glandular epithelium was significantly greater in males at the highest dose than in controls. Szemes and Szamado (1991) found that, rats receiving dibutylnitrosamine were showed histological changes in striated muscle, liver, intact rat urinary bladder suggested that including deep and homogenous necrosis in tract and tumorous tissues. Also, Alam *et al*. (2005) observed that in mice exposed to hepatocarcinogen N-nitrosodibutylamine an enhanced expression of high molecular weight glycoprotein TAA (Tumor-Associated Antigen) in liver cells and in histological examination DBNA treated animals showed cell distortion and extensive necrosis. Therefore, the present study was aimed to investigate the protective role of Cinnamon Flavonoid Extract (CFE) against histopathological changes in albino rats of Wistar strain treated with dibutylnitrosamine (DBNA) for 12 weeks.

MATERIALS AND METHODS

Materials

The bark of cinnamon (*Cinnamomum verum*) was obtained from Medical Plants Institute, Agricultural Research Center, Giza, Egypt. N-dibutylamine and sodium nitrite recommended by Hashimoto *et al*. (1976) as nitrosamine precursors were purchased from Sigma Chemical Co., St. Louis, MO. All chemicals and solvents used for cinnamon extract preparation were obtained from El-Nasser Chemical Company, Cairo, Egypt.

Experimental Animals

Ninety female albino rats of Wistar strain weighting 110±10 g per each were obtained from the Laboratory of Animal Colony, Ministry of Health and Population, Helwan, Egypt. All animals were subjected to acclimatization for weeks, during which the rats were maintained in Biology Lab, Nutrition and Food Science Department, Faculty of Home Economics, Minufiya University, Shebin El-Kom, Egypt. Rats were housed in plastic cages with galvanized iron filter taps.

Diets

The basal diet consisted of: casein 10%, corn oil 10%, vitamins mixture 1%, salt mixture 4% and starch up to 100%. The salt mixture used was according to Hegested *et al*. (1941) and the vitamins mixture was that of Campbell (1963).

Cinnamon Flavonoid Extract (CFE) Preparation

The Cinnamon Flavonoid Extract (CFE) was prepared according to the procedure described by Sauvesty *et al*. (1992). The bark of *Cinnamomum verum* was chopped. A known weight of the dried powdered plant tissue was extracted three times with ethanol, 50%, (1:4) at 40°C for 4 h and the mixture was centrifuged for 15 min at 3000 rpm at room temperature. The clear supernatants were combined and the solvent was removed by using rotary evaporator (type 349/2, Corning Limited Laboratory Division Stone Staffordshire, England) at room temperature. The final extract contained free and conjugated flavonoids.

Experimental Design

The experiments were conducted in accordance with the provisions of the guide for care and use of laboratory animals (NIH, 1985). The male albino rats weighting 100±10 g were kept in the laboratory under constant conditions of temperature (24±2°C) for at least 1 week before and through the experimental study, being maintained on a standard diet and water were available *ad libitum*. The experiment rats were divided into 6 groups as follow:

Group 1: Fed on the basal diet and normal water and left in normal condition for 12 weeks which served as control group

Group 2: Fed on the control diet and drink tap water containing 20 ml L^{-1} dibutylamine (DBA) and 5 g L^{-1} sodium nitrite daily for 12 weeks

Group 3: Fed the basal diet supplied with 150 mg kg^{-1} body weight of powdered Cinnamon Flavonoid Extract (CFE)

Group 4: Fed the basal diet supplied with 300 mg kg^{-1} body weight of powdered CFE

Group 5: Fed the basal diet supplied with 150 mg kg^{-1} body weight of CFE and drink tap water containing 20 ml L^{-1} DBA as well as 5 g L^{-1} sodium nitrite

Group 6: Fed the basal diet supplied with 300 mg kg^{-1} CFE and drink tap water containing 20 ml L^{-1} DBA as well as 5 g L^{-1} sodium nitrite

All the diet prepared daily just before the feeding time. The doses of CFE, 150 and 300 mg kg^{-1}, were calculated according to the recommended dose of human. DBN and sodium nitrite were mixed with the drinking water using magnetic stirrer for a period of 20 min. The experiment was terminated at 12 weeks after the dietary supplementation of cinnamon flavonoid extract.

Histopathological Examination

Five rats taken every 4 weeks from the experimental groups were sacrificed then the target organs such as liver, spleen and kidney were excised and weighted. Specimens from liver, kidney and urinary bladder were fixed in 15% neutral buffered formalin (pH 7.0), dehydrated in ethyl alcohol, cleared in xylol and embedded in paraffin 4-6 mM. Sections were prepared and stained with hematoxyl and eosin according to Carleton (1976) and Banchraft *et al*. (1996).

Statistical Analysis

Statistical analysis was performed with the student's t-test and MINITAB 12 computer program (Minitab Inc., State College, PA).

RESULTS AND DISCUSSION

The Effect of CFE and DBNA on Total Body, Liver, Spleen and Kidney Weights of Rats

The changes in total body weight (g) of rats during different periods of feeding diet contains 150 or 300 mg CFE/kg b.wt./day and drinking nitrosamine precursors (DBA+NO_2) (Table 1). In control group (G1), the total body weight at 12 weeks reached to the highest value 143.40 g. Animal maintained on nitrosamine precursors (G2) showed continuous significant decreasing during all sampling periods 4, 8 and 12 weeks by the ratio of 17.48, 28.15 and 51.60% (as a percent of control group), respectively. The feeding on 150 mg CFE/kg b.wt./day induced continuous increasing during all sampling periods 4, 8 and 12 weeks by the ratio of 3.80, 18.60 and 32.20% (as a percent of control group), respectively. With the raising of CFE feeding dose, 300 mg kg^{-1} b.wt./day, the total body weight was decreased after 4 weeks by the rate of 3.83%, then started to continuous increasing during the rest of the sampling periods 8 and 12 weeks by the ratio 5.18 and 21.06% (as percent of control

Table 1: The effect of feeding CFE on total body weight (Mean±SD, g) of rats drinking DBNA

Period of treatment (weeks)	Control (G1)	DBN (G2)	CFE feeding		DBN drinking+CFE feeding	
			150 mg kg^{-1} (G3)	300 mg kg^{-1} (G4)	150 mg kg^{-1} (G5)	300 mg kg^{-1} (G6)
0	121.51±8.039*	121.51±8.039	121.51±8.039	121.51±8.039	121.51±8.039	121.51±8.039
4	130.40±13.79^{b}**	107.60±5.55^{a}	134.20±2.77^{b}	125.40±11.28^{b}	102.80±11.10^{a}	107.20±14.64^{a}
8	142.80±20.13^{b}	102.60±18.86^{a}	161.40±12.44^{b}	150.20±14.87^{b}	113.00±19.39^{a}	111.00±17.73^{a}
12	143.40±20.21^{c}	69.40±9.89^{a}	175.60±9.29^{d}	173.60±14.81^{d}	113.80±11.48^{b}	124.80±17.18^{bc}

*Each value represents the Mean±SD of five rats, **In the same raw, different letter(s) mean significant variations at $p<0.05$ in comparison to control

Table 2: The effect of feeding CFE on liver weight (Mean±SD, g) of rats drinking DBNA

Period of treatment (weeks)	Control (G1)	DBN (G2)	CFE feeding		DBN drinking+CFE feeding	
			150 mg kg^{-1} (G3)	300 mg kg^{-1} (G4)	150 mg kg^{-1} (G5)	300 mg kg^{-1} (G6)
0	4.12±0.25*	4.12±0.25	4.12±0.25	4.12±0.25	4.12±0.25	4.12±0.25
4	4.28±0.67^{c}**	3.11±0.01^{a}	3.80±0.61^{bc}	3.39±0.35^{ab}	3.50±0.24^{ab}	3.35±0.32^{ab}
8	4.36±1.31b^{c}	2.82±0.18^{a}	4.89±0.30^{bc}	5.22±0.77^{c}	3.81±0.81^{ab}	2.86±1.79^{a}
12	4.22±0.43^{bc}	3.06±0.53^{a}	5.05±0.31^{cd}	5.99±1.63^{d}	3.88±0.99^{ab}	3.84±0.62^{ab}

*Each value represents the Mean±SD of five rats, **In the same raw, different letter(s) mean significant variations at $p<0.05$ in comparison to control

group), respectively. In contrary, feeding with 150 mg CFE/kg b.wt./day in the presence of nitrosamine induced continuous decreasing in total body weight during all sampling periods 4, 8 and 12 weeks by the ratio of 21.17, 20.87 and 20.64% (as a percent of control group), respectively. With the raising of CFE feeding dose in the presence of nitrosamine, 300 mg kg^{-1} b.wt./day, the rate of decreasing recorded 17.79, 22.27 and 12.97% (as a percent of control group) for the same previously sampling periods, respectively.

The changes in total body weight (g) of rats during different periods of feeding diet contains 150 or 300 mg CFE/kg b.wt./day and drinking nitrosamine precursors (DBA+NO_2) (Table 2). In control group (G1), the liver weight at 12 weeks reached to the highest value 4.22 g. Animal maintained on nitrosamine precursors (G2) showed continuous significant decreasing during all sampling periods 4, 8 and 12 weeks by the ratio of 27.34, 35.32 and 27.49% (as a percent of control group), respectively. The feeding on 150 mg CFE/kg b.wt./day not induced any significant changes during all sampling periods 4, 8 and 12 weeks, while the raising of that dose to 300 mg kg^{-1} b.wt./day, the total body weight was decreased after 4 week by the rate of 20.79%, then started to continuous increasing during the rest of the sampling periods 8 and 12 weeks by the ratio 19.72 and 41.94 % (as percent of control group), respectively. In contrary, feeding with 150 mg CFE/kg b.wt./day in the presence of nitrosamine induced decreasing in liver weight during all sampling periods 4, 8 and 12 weeks by the ratio of 18.22, 12.61 and 8.06% (as a percent of control group), respectively. With the raising of CFE feeding dose in the presence of nitrosamine, 300 mg kg^{-1} b.wt./day, the rate of decreasing recorded 21.73, 34.40 and 9.00% (as a percent of control group) for the same previously sampling periods, respectively.

The opposite directions were observed for the changes in spleen and kidney weights. In control group (G1), the spleen weight at 12 weeks reached to the highest value 0.46 g (Table 3). Animal maintained on nitrosamine precursors (G2) showed continuous significant increasing during all sampling periods 4, 8 and 12 weeks by the ratio of 21.95, 25.00 and 30.43% (as a percent of control group), respectively. The feeding on 150 mg CFE/kg b.wt./day not induced any significant changes during all sampling periods 4, 8 and 12 weeks, while the raising of that dose to 300 mg kg^{-1} b.wt./day, the spleen weight was increased after 4, 8 and 12 weeks by the rates of 9.76, 4.55 and 2.17%, (as a percent of control group), respectively. In contrary, feeding with 150 mg CFE/kg b.wt./day in the

Table 3: The effect of feeding CFE on spleen weight (Mean±SD, g) of rats drinking DBNA

Period of treatment (weeks)	Control (G1)	DBN (G2)	CFE feeding		DBN drinking+CFE feeding	
			150 mg kg^{-1} (G3)	300 mg kg^{-1} (G4)	150 mg kg^{-1} (G5)	300 mg kg^{-1} (G6)
0	0.39±0.03*	0.39±0.03	0.39±0.03	0.39±0.03	0.39±0.03	0.39±0.03
4	0.41±0.01^{cd}**	0.50±0.04^{a}	0.45±0.05^{abd}	0.45±0.04^{abd}	0.51±0.06^{ab}	0.38±0.04^{c}
8	0.44±0.06^{c}	0.55±0.07^{a}	0.51±0.02^{ad}	0.46±0.01^{cd}	0.36±0.06^{b}	0.36±0.01^{b}
12	0.46±0.04^{bc}	0.60±0.01^{a}	0.54±0.12^{ac}	0.47±0.01^{bc}	0.39±0.01^{b}	0.50±0.10^{c}

*Each value represents the Mean±SD of five rats, **In the same raw, different letter(s) mean significant variations at $p<0.05$ in comparison to control

Table 4: The effect of feeding CFE on kidney weight (Mean±SD, g) of rats drinking DBNA

Period of treatment (weeks)	Control (G1)	DBN (G2)	CFE feeding		DBN drinking+CFE feeding	
			150 mg kg^{-1} (G3)	300 mg kg^{-1} (G4)	150 mg kg^{-1} (G5)	300 mg kg^{-1} (G6)
0	0.71±0.02*	0.71±0.02	0.71±0.02	0.71±0.02	0.71±0.02	0.71±0.02
4	0.74±0.04^{d}**	1.03±0.08^{a}	0.82±0.03^{cd}	0.80±0.10^{cd}	0.99±0.11^{a}	0.86±0.06^{c}
8	0.81±0.03^{d}	1.21±0.05^{a}	0.92±0.07^{b}	0.96±0.06^{b}	0.96±0.09^{b}	1.10±0.07^{c}
12	0.78±0.02^{d}	1.21±0.11^{a}	0.83±0.05^{bc}	1.08±0.13^{bc}	1.09±0.11^{b}	1.04±0.07^{bc}

*Each value represents the Mean±SD of five rats, **In the same raw, different letter(s) mean significant variations at $p<0.05$ in comparison to control

presence of nitrosamine induced increasing in spleen weight after 4 weeks of feeding by the ratio of 24.39%, while elongation of the feeding period and/or increasing of the CFE dose leads to decreasing of the spleen weight by different rates.

For kidney weight, data in Table 4 indicted that the kidney weight of the control group (G1) at 12 weeks reached to the highest value 0.78 g. Animal maintained on nitrosamine precursors (G2) showed continuous significant increasing during all sampling periods 4, 8 and 12 weeks by the ratio of 39.19, 49.38 and 55.13% (as a percent of control group), respectively. The feeding on 150 mg CFE/kg b.wt./day not induced any significant changes during all sampling periods 4, 8 and 12 weeks, while the raising of that dose to 300 mg kg^{-1} b.wt./day, the spleen weight was increased after 4, 8 and 12 weeks by the rates of 8.11, 18.52 and 38.46%, (as a percent of control group), respectively. In contrary, feeding with 150 mg CFE/kg b.wt./day in the presence of nitrosamine induced increasing in kidney weight after 4 weeks of feeding by the ratio of 33.78% which continuously increased by the elongation of the feeding period and/or increasing of the CFE dose by different rates.

Finally, a comparison between the data of DBN group and DBN drinking +150 mg CFE feeding group or DBN group and DBN drinking+300 mg CFE feeding (Table 1-4) indicated that CFE feeding were significantly effective in protecting against DBNA-induced body, liver, spleen and kidney weight changes.

The Effect CFE and DBNA on Liver, Kidney and Urinary Bladder Histopathology

Liver Histopathology

Liver of rats fed the control diet showed the normal histological structure of the central veins and surrounding hepatocytes (Fig. 1a). Rats treated with DBNA for 4 weeks showed severe dilatation and congestion in the central and portal veins (Fig. 1b, c) and mononuclear leucocytes inflammatory cells infiltration in the portal area (Fig. 1d). With the elongation of treating period of DBNA, hyperemic central veins and surrounding sinusoids with degeneration in the hepatocytes were observed for 8 weeks (Fig. 1e) and hyperemia in the central veins and sinusoids with diffuse kupffer cells proliferation for 12 weeks (Fig. 1f). Feeding on diet containing 300 mg kg^{-1} b.wt./day CFE for 8 weeks showed hyperemia in the central veins with degeneration in the hepatocytes (Fig. 1g). Rats fed diet

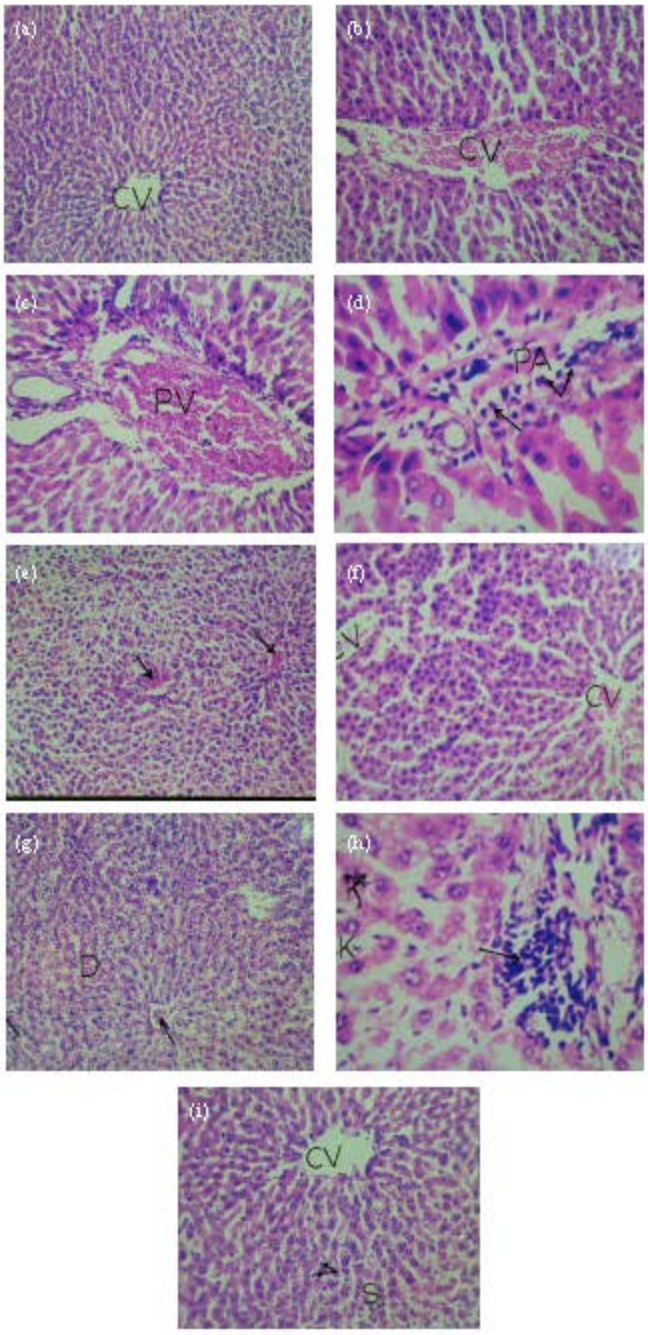

Fig. 1: The effect of CFE on liver histopathological changes induced by DBNA in rats, (a) normal (control diet), (b, c, d) fed the control diet and administrated DBNA for 4 weeks, (e) fed the control diet and administrated DBNA for 8 weeks, (f) fed the control diet and administrated DBNA for 12 weeks, (g) fed diet containing 300 mg kg^{-1} CFE for 8 week, (h) fed diet containing 150 mg kg^{-1} CFE and administrated DBNA for 4 weeks and (i) fed diet containing 300 mg kg^{-1} CFE and administrated DBNA for 8 weeks (H and E, X40)

containing 150 mg kg^{-1} CFE and administrated DBNA for 4 weeks showed mononuclear leucocytes inflammatory cells infiltration in the portal area with diffuse kupffer cells proliferation in between the hepatocytes (Fig. 1h). The increasing of CFE dose to 300 mg kg^{-1} b.wt./day in the same earlier treatment showed dilatated of the central vein and sinusoids with diffuse kupffer cells proliferation in between the hepatocytes (Fig. 1i).

Kidney Histopathology

Kidney of rats fed the control diet showed the normal histological structure of the glomeruli and renal tubules (Fig. 2a). Rats treated with DBNA for 4 weeks showed hyperemia in the glomerular tuft of the glomeruli with periglomerular inflammatory cells infiltration and focal hemorrhages between the tubules (Fig. 2b), swelling of the lining endothelium (Fig. 2c), mononuclear leucocytes inflammatory cells infiltration in between the degenerated renal tubules (Fig. 2d) and focal haemorrhages the corticomedullary portion (Fig. 2e). Kidney of rats fed diet containing 150 mg/kg b.wt./day CFE for 8 weeks showed hyperemia of the glomerular tuft of the glomeruli and intertubular stromal blood vessels (Fig. 2f) and increasing the previous dose of CFE to 300 mg kg^{-1} b.wt./day and elongation of the exposure period induced hyperemia in the glomerular tufts of the glomeruli (Fig. 2g) and hyperemia of the intertubular blood vessels (Fig. 2h). The group treated with DBNA for 8 weeks showed hyperemia in the glomerular tufts of the glomeruli with focal inflammatory cells infiltration in between the renal tubules in the cortical portion (Fig. 2i). Rats fed diet containing 150 mg kg^{-1} CFE and administrated DBNA for 4 weeks showed degenerative and necrobiotic changes of the epithelial cells lining the tubules (Fig. 2j). With the elongation of earlier treating period hyperemia of the glomerular tufts, focal haemorrhages, degeneration in the renal tubules and focal perivascular oedema with inflammatory cells infiltration were observed for 8 weeks (Fig. 2k) and focal haemorrhages in the medullary portion for 12 weeks (Fig. 2*l*). On the other side, increasing of the CFE dose to 300 mg kg^{-1} b.wt./day and administrated DBNA induced degeneration in the epithelial cells lining the renal tubules for 4 weeks (Fig. 2m), focal haemorrhages of the medullary portion for 8 weeks (Fig. 2n) and hyperemia in the glomerular tuft of the glomeruli, perivascular oedema and inflammatory cells infiltration and degeneration in the renal tubules for 12 weeks (Fig. 2o).

Urinary Bladder Histopathology

Urinary bladder of rats fed the control diet showed the normal histological structure of the lining mucosal epithelium with underlying lamina propria and muscularis (Fig. 3a). In rats maintained on DBNA for 8 weeks, papillary hyperplasia was noticed in the mucosal layer (Fig. 3b). For rats treated with DBNA and fed diet containing 150 mg kg^{-1} CFE for 8 weeks papillary hyperplasia in the lining mucosal layer was observed (Fig. 3c).

Changes in histopathological parameters as the result of nitrosamine subjection have been studied by many authors. Several universities and academic centers have paid attention towards the methods could be used successively in reducing like of these changes. One of the most effective methods commonly tested in the last decade is the using of origin- plant extract such herbs. Some of these herbs exhibited significant roles in reducing the adverse effects of nitrosamines including the histopathological changes. In the present study the effect of flavonoids containing diet on rats treated with nitrosamines have been investigated. Such as shown in the earlier data (Table 1-4) rats treated with DBN recorded decreasing in the total body and liver weights and increasing in spleen and kidney weights with significant values when compared with the control group all over the experiment period. All of these results are partially consistent with the finding of Lee *et al.* (2003), who stated that treatment with dimethylnitrosamine (DMN) caused a significant decrease in body and liver weight. The decreasing weight of these organs could be attributed to one or more of the following reasons. Nitrosamines

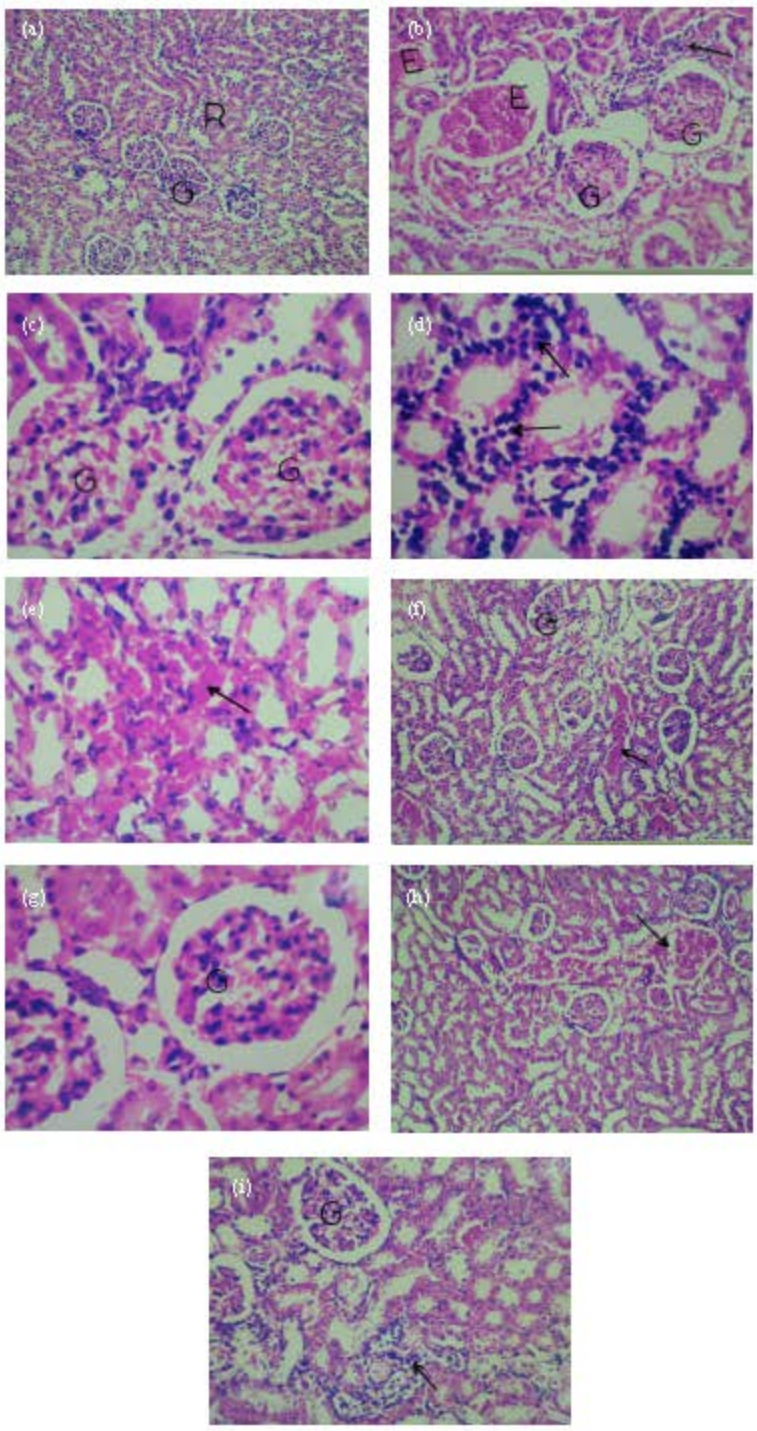

Fig. 2: Continued

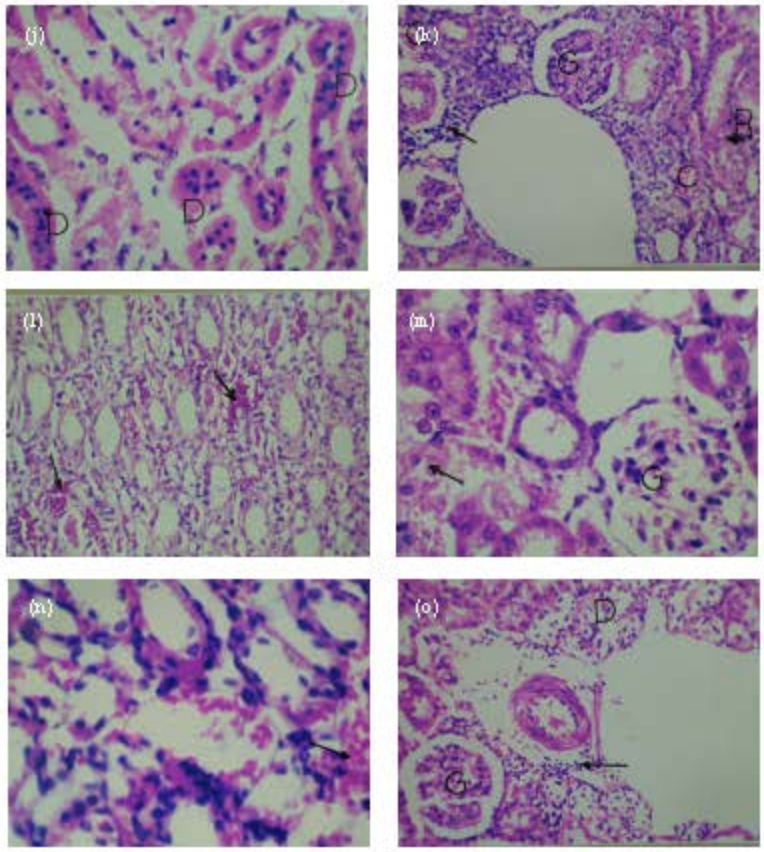

Fig. 2: The effect of CFE on kidney histopathological changes induced by DBNA in rats, (a) normal (control diet), (b-e) fed the control diet and administrated DBNA for 4 weeks, (f) fed diet containing 150 mg kg^{-1} b.wt./day CFE for 8 weeks, (g) fed diet containing 300 mg kg^{-1} b.wt./day CFE for 4 weeks, (h) fed diet containing 300 mg kg^{-1} b.wt./day CFE for 8 weeks, (i) treated with DBNA for 8 weeks, (j) fed diet containing 150 mg kg^{-1} CFE and administrated DBNA for 4 weeks, (k) fed diet containing 150 mg kg^{-1} CFE and administrated DBNA for 8 weeks, (l) fed diet containing 150 mg kg^{-1} CFE and administrated DBNA for 12 weeks, (m) fed diet containing 300 mg kg^{-1} CFE and administrated DBNA for 4 weeks, (n) fed diet containing 300 mg kg^{-1} CFE and administrated DBNA for 8 weeks and (o) fed diet containing 300 mg kg^{-1} CFE and administrated DBNA for 12 weeks (H and E, X40)

produce oxidative stress due to generation of reactive oxygen species which alter the antioxidant defense system in the tissues consequently brought hepatic degeneration as evidenced by the significant decrease in liver weight index (Rockey, 2005; Mittal *et al*., 2006). Also, Rajeshkumar *et al*. (2003) indicated that N-nitrosodiethylamine (NDEA) induced liver tumors in rats and the liver weights were reduced. Otherwise, both doses of CFE increased significantly the body weight and non-significantly the liver, kidney and spleen weights when compared with the control group all over the experimental periods. These results are partially consistent with the finding of Okazaki *et al*. (2002), who noticed that cinnamon component such isoflavones increased liver weight of rats

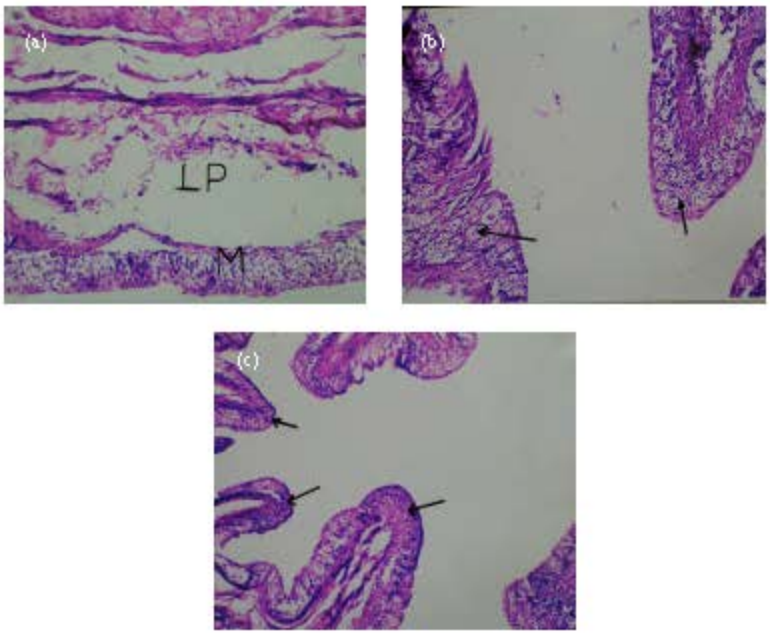

Fig. 3: The effect of CFE on urinarybladder histopathological changes induced by DBNA in rats, (a) normal (control diet), (b) treated with DBNA and fed control diet for 8 weeks and (c) treated with DBNA and fed diet containing 150 mg kg^{-1} CFE for 8 weeks (H and E, X40)

and Badary *et al*. (2005), who found that flavanone produced significant reduction in body weight loss. Also, Duarte *et al*. (2002) found that other cinnamon component i.e., quercetin markedly inhibited the development hypertension. This effect was accompanied by full prevention of most of the effects such as increases kidney weight indices. On the other side, Bruneton (1995) stated that cinnamon bark is used to treat symptoms of digestive disorder, functional asthenias and also for loss of appetite to facilitate weight gain or cinnamon's constituents eugenol, eugenol acetate and methyl eugenol have been reported to help digestion by enhancing trypsin activity and lipolytic (ability to hydrolyze fats) action (Leung and Foster, 1996). Finally, Yokozawa *et al*. (2005) stated that the improvement of kidney weight induced by CFE could be attributed to different cinnamon,s constituents in particular polyphenols.

The present study also revealed that nitrosamine alone had a toxic effect on liver through degeneration hyperemia, inflammatory reaction, kidney through haemorrhages reral casts hyperemia, inflammatory reaction and also urinarybladder through papillary hyperplasia with papillary projection formation in the cell layer of the lining epithelium. This finding was confirmed by Zakhary *et al*. (1994) and Alam *et al*. (2005), who found that drinking water contain 0.05% DBNA during 3 weeks showed cell distortion, extensive necrosis in the liver and bladder papillomas. Other studies have shown that treatment with DBNA was enhanced the incidences of preneoplastic and neoplastic lesions in the upper digestive tract, liver, pancreas, as well as papillomas of the urinary bladder (Szemes and Szamado, 1991; Shibata *et al*., 1994) and tumor incidence of other organs, such as the kidney and thyroid (Jang *et al*., 1991). Recently, Omotosho *et al*. (2000) have reported that, the N-nitrosation reaction may occur in the human bladder in addition to gastrointestinal tract. This reaction occurs in

the presence of bacteria and a precursor secondary amine that associated with toxicity for the liver (as a consequence of the urinary retention) and papillomas of the urinary bladder.

On the other hand, in the present study CFE act as a protector against nitrosamine toxicity mainly on liver and urinary bladder and secondary on the kidney. This finding agreed with that noticed by Wu *et al*. (2006), who found that administration of flavonoid in the initiation or post initiation phase significantly decreased the incidences of bladder neoplasms and preneoplastic lesions, induced by DBNA. Also, they indicated that all histopathological changes in liver such as fatty infiltration, variation in mitotic figures and focal necrosis, which induced, by DBNA were reversed by the administration of CFE.

In conclusion, CFE was effective in protecting against DBNA-induced histopathological changes. These results supported present hypothesis that CFE contains several free and conjugated compounds that are able to prevent or inhibit DBNA toxicity. Therefore, we recommended the cinnamon to be included in the daily diets and drink but by a moderate amount.

ACKNOWLEDGMENT

This study was financially supported by the Research Box (2005-2007), Minufiya University, Shebin El-Kom, Egypt.

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