EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 2
Issue: 7
57-66
Year: 2015

PROTECTIVE EFFECT OF PETROSELINUM CRISPUM OR ERUCA SATIVA EXTRACTS ON TESTES AGAINST DIOXIN INTOXICATION IN RATS

Gamal Mohamed Edrees[1], Hussam Ahmed El-Gayar[2*], El-Sayed Mohamed El Habibi[1], Eman Taha Salem[1]

[1]Zoology Department, Faculty of Science, Mansoura University, Mansoura, Egypt.
[2] Urology and Nephrology Center, Mansoura University, Mansoura, Egypt.

***Author for Correspondence: Dr. Hussam Ahmed El-Gayar**
Urology and Nephrology Center, Mansoura University, Mansoura, Egypt.

Article Received on 15/10/2015 **Article Revised on 03/11/2015** **Article Accepted on 26/11/2015**

ABSTRACT
This study was conducted to investigate the effect of ethanolic extract of either *Petroselinum crispum* (PC) or *Eruca sativa* (ES) on 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) – induced toxicity on reproductive system of male Albino rats. Forty two male rats were allocated to one of seven groups of six rats each: Control (NC), Corn oil (CO), PC, ES, TCDD, PC+ TCDD and ES+ TCDD. All treatments continued for 5 weeks. Body, testicular and epididymal weights were recorded, epididymal sperms were counted, free testosterone (FT), dehydroepiandrosterone (DHEA), follicle-stimulating hormone (FSH), luteinizing hormone (LH) levels, testicular 3β- hydroxysteroid dehydrogenase (3β-HSD) activity and apoptosis as assessed by flow cytometry were estimated. The results showed that TCDD exposure resulted in a significant decrease in body weight, testicular and epididymal weights, sperm count, FT, DHEA and 3β-HSD activity associated with significant increase in FSH and LH as well as apoptosis in testes. However, when ethanolic extracts of either PC or ES were administered in concomitant with TCDD, the estimated parameters tend to be nearly normalized. In general it could be concluded that ethanolic extract of *Eruca sativa* has greater protective effect than alcoholic extract of *Petroselinum crispum* in TCDD induced reproductive toxicity.

KEYWORDS: TCDD, Testis, Eepidydimis, *Eruca sativa*, *Petroselinum crispum*, Apoptosis.

INTRODUCTION
2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) is the most potent man-made toxicant known compound among halogenated aromatic hydrocarbons, which is widespread and persistent in the environment.[1] It is the unintentional byproduct of various industrial processes. The main source of dioxin in the environment comes from combustion processes, such as metal smelting and refining as well as waste-burning incinerators of various sorts (e.g. municipal, hazardous and hospital waste). From all these sources, dioxins are released into air, land and water.[2] The main sources of dioxin intake include dairy products, meat, fish and fish products,[3] TCDD was classified as a human carcinogen (Group I) by the International Agency for Research on Cancer (IARC) of the World Health Organization (WHO).[4] Due to its fat-soluble, dioxin is bio-accumulated in tissue lipids and in food chain. Dioxin induces adverse effects on the reproductive endocrine and immune systems. In addition, it causes liver toxicity, teratogenesis, tumor promotion and cancer.[5] Growing evidence suggests that the reproductive system is one of the most sensitive targets for the adverse effects of TCDD. Administration of TCDD caused reduced fertility, delayed puberty, decreased reproductive organ weights and decreased sperm counts,[6] as well as increased number of abnormal sperm.[7] Also, exposure of rats to TCDD induces distortion in gonadotropins hormones.[8]

The medicinal plants and herbs have been used in the treatment of various diseases in animals and human being. Currently utilization of these medicinal plants is increasing. WHO estimates, around 80% of the especially developing world is indigent on complementary and alternative medicines which are prodigiously derived from herbal material.[9]

Petroselinum crispum (PC) and *Eruca sativa* (ES) are vegetables that are enriched with phenolic compounds. They have been determined to exhibit a variety of pharmacological activities including antimicrobial,[10] antioxidant activity,[11] and antitumor,[12] through inhibition of cell proliferation and induction of apoptosis.[13]

PC the world's most popular culinary herb belongs to the Umbelliferae family that has been employed in the food, pharmaceutical, perfume, and cosmetics industries.[14] Previous studies on the chemical composition of parsley have revealed the presence of flavonoids,[15]

coumarins,[16] and terpenes.[17] In popular medicine, parsley is used to treat various illnesses such as Alzheimer disease, thrombosis and strokes.[18] It is a source of α-linolenic acid, an important fatty acid for growth and reproduction. [19] Also, it has been used as an aphrodisiac, improved productive performance in broiler[14] and used to treat impotance.[20]

Eruca sativa, a member of the Brassicacae family, is one of the medicinal plants, called "Jarjeer" in Arabic, is an edible plant and is widely used in folk medicine.[21] It has several antioxidant constituents including glucosinolates, flavonoids and carotenoids.[22] Salem and Moustafa [23] and Hussein [24] demonstrated that ES has beneficial effects on fertility and male reproductive system.

The present study was designed to investigate the protective effects of ethanolic extracts of either *Petroselinum crispum* or *Eruca sativa* against the TCDD-induced reproductive disorders in male Albino rats.

MATERIALS AND METHODS

Chemicals

TCDD (Cas No. 1746-01-6) was purchased from Sigma-Aldrich (Saint Louis, Missouri, USA). Ethanol was purchased from (Al-Gomohria Company for chemicals, Abou-Zaabal, Egypt). The corn oil was purchased from local store. All other chemicals were of analytical grade.

Animals

This study was performed on male rats (*Rattus rattus*) initially weighing (160±10g). Rats were obtained from the Institute of Ophthalmic Disease Research, Cairo, Egypt. They were housed in stainless steel cages in an artificially illuminated and thermally controlled room (22- 25°C and 12 h light / dark cycle). They were fed on normal laboratory rodent diet and given water *ad libitum* for one week of acclimation. All animals received human care in compliance with the guidelines of the Animal Care and Use Committee of Mansoura University.

Preparation of plant Extract.

PC and ES were purchased from a local market in Mansoura City, Egypt. Ethanolic extract of PC and ES was prepared according to Al-Howiriny et al.,[25] and Alqasoumi.[26] Briefly, five hundred grams of coarse powder of aerial parts of the plant were macerated in 3 liters of 96% ethanol and allowed to stand at room temperature for about 72 hours using percolation method. The percolate was collected and dried under reduced pressure in vacuum. The obtained extract was suspended in distilled water before administration.

Experimental protocol

Forty two male rats were allocated to one of seven groups of 6 rats each. Normal control (NC) group was fed on standard diet without any supplementation. Corn oil (CO) group treated with corn oil at dose of 2 ml/ kg b.wt. The PC group treated with PC alcoholic extract at a dose 2g/ kg b.wt.[25] The ES group treated with ES alcoholic extract at a dose 500 mg/kg b.wt.[26] The TCDD group received 100 ng/ kg b.wt./day [27] diluted in 2 ml corn oil. The PC + TCDD group was treated with 2g/kg b.wt PC extract and 100 ng/kg b.wt/day TCDD. The ES + TCDD group was treated with 500 mg/kg b.wt ES and 100ng /kg/day TCDD. All treatments were made by gastric intubation daily for 5 weeks. Body weights were recorded weekly to obtain body weight changes.

At the end of the experimental period, the animals were fasted overnight, weighed and sacrificed under slight anesthesia with diethyl ether. Blood samples were collected. Sera were separated by centrifugation at 860 Xg for 20 min at 4ºC and then quickly frozen at – 20ºC for future biochemical analysis. Immediately after collecting blood, rats were dissected, testes and epididymis were removed and cleared from the adhering tissues, then weighed. The relative weights of testes and epididymis were calculated as the ratio of organs weight to animal body weight. A known weight of right testis was weighed and homogenized at 4 ºC in 20% spectroscopic grade glycerol containing 5 mmol potassium phosphate and 1 mmol EDTA at tissue concentration of 100 mg/ml, then centrifuged at 10000 Xg for 30 min at 4ºC the supernatant was taken for the assay of 3β- hydroxysteroid dehydrogenase (3β-HSD) activity.[28] While the left testis was fixed in 10% neutral formaldehyde for histopathological examination.

Preparation of testes homogenate for quantification of apoptosis by flow cytometry

A portion of the right testes was minced in Tris buffer (pH=7.4), centrifuged at 860 Xg for 5 min at 4°C and the resultant supernatants were frozen at -20 °C until use.[29]

DNA staining for determination of sub G_1 apoptosis$1x10^6$ cells were fixed with 1ml ice cold absolute alcohol and preserved at 4°C until analysis, after incubation overnight of fixation the sample was again centrifuged and excess of ethanol was removed.[30] 200 µl of cell suspension in citrate buffer were put in a 15 ml Falcon tube. 1 ml of propiduim iodide (5mg/mL PI in PBS) was mixed and the sample was incubated overnight in dark at 4°C, filtered through a 30µm pore diameter nylon mesh filter to eliminate nuclear clumps. The samples were run in the flow cytometry within 1 hr after the addition of propidium iodide. Flow cytometry analysis was performed using FACS Clibur flow cytometry using Cell Quest software (Becton Dickinson, San Jose, CA) equipped with a compact air-cooled low power 15 mwatt argon ion laser beam (488 nm). The dot plot diagram derived from flow cytometry was obtained with a computer program for Dean and Jett mathematical analysis.[31]

A total of 50 000-120 000 event were collected for each analysis. Cells gates were set on live cells using forward (FSC) and side scatter (SSC). Data analysis was conducted using DNA analysis program MODFIT

(verity software house, Inc. Po Box 247, Topsham, ME 04086 USA, version: 2.0 power Mac with 131072 KB Registration No: 42000960827-16193213 Date made: 16-Sep., 1996). This software estimate DNA ploidy and cell cycle analysis, which was calculated the (sub G1 apoptosis percentage) for each sample.

Epididymal sperm count

Epididymal sperms were collected by chopping the epididymis in 5 ml saline (0.9%) and were incubated for 5 min at 37º C. The epididymal sperm counts were obtained by the method of Adeeko and Dada. [32]

Hormonal assay and determination of 3β-hydroxysteroid dehydrogenase (3β-HSD) activity

Serum free testosterone (FT) was determined by the enzyme linked immunosorbent assay (ELISA) method according to Stephen et al. [33] using (Diagnostic Biochem, Canada, Inc). Serum dehydroepiandrosterone (DHEA) was determined using Immuno-enzymatic assay (EIA) method according to Labrie et al. [34] using DRG® ELISA DHEA (DRG Diagnostic, GmbH, Marburg, Germany). Serum follicle stimulating hormone (FSH) and luteinizing hormone (LH) levels were measured using EIA system (IMMULITE and IMMULITE 1000, Siemens Healthcare Diagnostics Products Ltd, UK) according to Babson. [35] 3β- hydroxysteroid dehydrogenase (3β-HSD) activity was determined according to the method of Talalay et al. [28]

Histopathological examination

The fixed testes tissues in neutral formalin was dehydrated through ascending series of ethyl alcohol, cleared in xylene, infiltrated and embedded in paraffin wax. Transverse sections of testes were cut at thickness of 5 µm and stained with Mayer's Haematoxylin and Eosin (H&E) stains according to Weesner[36] for further examination.

Statistical analysis

All values are presented as mean ±SEM. Differences were considered to be significant at $p<0.05$. One-way analysis of variance (ANOVA) and post-hoc LSD test were used to determine differences between groups using the SPSS/PC program (version 17; SPSS, Chicago, Illiniois, USA).[37]

RESULTS

Body weight changes

Table 1 showed changes in body weight over the period of the experiment. Although treatment with PC or ES alone had no significant effect on the body weight evaluated, TCDD treatment alone significantly decreased body weight ($P<0.05$) compared with the control group. A significant increase in body weight ($P<0.05$) was observed in the PC+ TCDD or ES+TCDD groups, compared with values for the TCDD treated group. Also, such data showed that body weight changes are still significantly lower than the control levels.

Table (1): Body weight gain in normal control and different treated rat groups.

NO week	Animal groups						
	C	CO	PC	ES	TCDD	PC+ TCDD	ES+ TCDD
Base line	165.5 ^{a}±2.86	163.3 ^{a}±2.40	164.8 ^{a}±6.01	168 ^{a}±3.80	160.3 ^{a}±3.65	162 ^{a}±3.82	161.3 ^{a}±6.02
1st wk % of Wt gain	181.2 ^{a}±2.59 9.49%	179.8 ^{a}±3.26 10.1%	181.8 ^{a}±6.09 10.3%	185.9 ^{a}±3.76 10.6%	167.5 ^{a}±5.85 04.5%	173.6 ^{a}±3.54 07.1%	175 ^{a}±5.84 08.5%
2nd wk % of Wt gain	200.2 ^{a}±2.77 20.97%	199.1 ^{a}±4.25 22.0%	202.1 ^{a}±6.21 22.6%	204.7 ^{a}±4.28 21.8%	180 ^{b}±5.91 12.3%	189 $^{a\&b}$±3.78 16.7%	189.3 $^{a\&b}$±3.67 17.4%
3rd wk % of Wt gain	220.5 ^{a}±2.61 33.05%	218.8 ^{a}±4.50 34.0%	222 ^{a}±6.56 34.7%	224 ^{a}±4.89 33.3%	191 ^{b}±5.87 19.1%	206.2 $^{a\&b}$±4.24 27.3%	207.9 $^{a\&b}$±3.79 28.9%
4th wk % of Wt gain	238.3 ^{a}±3.55 43.99%	235.3 ^{a}±5.08 44.1%	239 ^{a}±5.42 45.0%	242 ^{a}±5.51 44.1%	199.2 ^{b}±4.76 24.3%	218^{c}±4.30 34.6%	221^{c}±3.95 37.0%
5th wk % of Wt gain	253.1 ^{a}±3.47 53%	250.4 ^{a}±5.02 53.3%	255 ^{a}±5.70 54.7%	257 ^{a}±6.00 53.0%	205 ^{b}±5.11 28.0%	228 ^{c}±4.42 41.0%	231 ^{c}±4.00 43.2%

Values are means ± SEM (n = 6); with each row, values superscripts with different letters (a-c) were significantly different (P≤ 0.05). C: control. CO: corn oil. PC: petroselinum crispum. ES: Eruca sativa. TCDD: 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin.

Testicular and Epididymal weights

Testes and epididymis weights are presented in table 2. The data were expressed as a percentage of testes to body weight. Although treatment with TCDD alone significantly ($P<0.05$) decreased weights of testes and epididymis, compared with the control group, Co-administration of either PC or ES to TCDD-treated rats significantly ($P<0.05$) increase these weights compared with the TCDD group. Treatment with either PC or ES did not show any significant change in testes and epididymis weights when compared with control group.

Sperm count

Administration of TCDD caused a significant ($P<0.05$) reduction in the epididymal sperm count compared with the corresponding group of the control rats. When either PC or ES was administered to TCDD treated rats a significant ($P<0.05$) increase in the sperm counts, compared with the TCDD group were observed. Treatment with either PC or ES showed non-significant increase in sperm counts when compared with control group. Such data showed that sperm count is still significantly lower than the control levels except

ES+TCDD group which returned back nearly to the normal range (Table 2).

Table (2): Relative testicular weight, epididymal weight and epididymal sperm count in normal control and different treated rat groups.

Groups	Testis (g/100 g b.wt)	Epididymis (g/100 g b.wt)	Epididymal sperm count (x 10^5 g^{-1})
NC	0.5330.01[a]	0.190±0.007 [a]	5.16±0.08[a]
CO	0.522±0.01[a]	0.188±0.008 [a]	5.13±0.18[a]
PC	0.527±0.016[a]	0.189±0.006 [a]	5.22±0.24[a]
ES	0.535±0.01[a]	0.200±0.008 [a]	5.30±0.14[a]
TCDD	0.414±0.01[b]	0.143±0.004[b]	1.98±0.16[b]
PC+TCDD	0.5100.02[a]	0.178±0.008 [a]	3.81±0.14[c]
ES+TCDD	0.520±0.03[a]	0.186±0.005 [a]	4.5±0.21[a&c]

Each value represent the mean ± SEM (n=6), values superscripts with different letters (a-b) were significantly different at p≤0.05.

Hormonal changes

The obtained results in table (3) reveal that, rats exposed to TCDD had significantly lower serum FT and DHEA concentrations and significantly higher serum FSH and LH concentrations when compared with the control group. Also, a significant decrease in the activity of testicular 3β-HSD was observed. However, when either PC or ES was administered to TCDD treated rats significantly (P<0.05) increase in FT and DHEA concentrations as well as a significant increase in 3β-HSD activity and significantly decrease serum FSH and LH concentrations compared with the TCDD group. Treatment with either PC or ES did not show any significant change in the measured hormones or 3β-HSD activity when compared with control group. Moreover, comparing the protected groups with the control one, the serum FT & DHEA still significantly lower than in the control group except serum FSH & LH in ES +TCDD group which returned nearly to the normal level.

Table (3): Serum free testosterone (FT), dehydroepiandrosterone (DHEA), Follicle-stimulating hormone (FSH), Luteinizing hormone (LH) and testicular 3β- hydroxysteroid dehydrogenase (3β-HSD) activity in normal control and different treated rat groups.

Groups	FT (pg/ml)	DHEA (ng/ml)	FSH (mIU/ml)	LH (mIU/ml)	3β-HSD (U/mg)
NC	32.6±1.05 [a]	2.34±0.07 [a]	1.32±0.03 [a]	1.2±0.06 [a]	4.53 ± 0.16[a]
CO	32.7±1.53 [a]	2.31±0.04 [a]	1.3±0.06 [a]	1.21±0.09 [a]	4.42±0.10[a]
PC	34.9±0.84 [a]	2.5±0.12 [a]	1.16±0.08 [a]	1.07±0.06 [a]	4.86±0.15[a]
ES	37.4±1.15 [a]	2.57±0.10 [a]	1.01±0.04 [a]	0.99±0.05 [a]	5.05±0.15[a]
TCDD	10.9±1.1[b]	0.88±0.05[b]	2.76±0.24[b]	2.57±0.18[b]	2.06±0.17[b]
PC+TCDD	22.2±1.5[c]	1.46±0.1[c]	1.83±0.06[c]	1.56±0.07[c]	3.69±0.10[c]
ES+TCDD	25.8±1.84[c]	1.72±0.17[c]	1.74±0.07[a&c]	1.67±0.18[a&c]	3.96±0.16[a&c]

Each value represent the mean SEM (n=6), values superscripts with different letters (a-c) were significantly different at p≤0.05.

Apoptosis

As shown in table 4 and figure 1, administration of TCDD caused a significant (P<0.05) elevation in the apoptotic cells in the testicular tissue compared with the corresponding group of the control rats. When either PC or ES was administered to TCDD treated rats significantly (P<0.05) decrease in the apoptotic cells compared with the TCDD group. Treatment with PC did not show any significant change in the apoptotic cells when compared with control group, while treatment with ES showed significant reduction (P<0.05) in the apoptotic cells when compared with control group.

Table (4): Apoptosis in adult testis in in normal control and different treated rat groups.

Groups	% of apoptosis	N
NC	20.22±1.38[a]	6
CO	17.42±1.62[a]	6
PC	16.48±1.28[a]	5
ES	13.80±1.04[c]	4
TCDD	34.03±2.46[b]	6
PC+TCDD	20.16±1.73[c]	5
ES+ TCDD	15.88±0.54[a&c]	5

Each value represents the mean ± SEM values superscripts with different letters (a-c) were significantly different at p≤0.05.

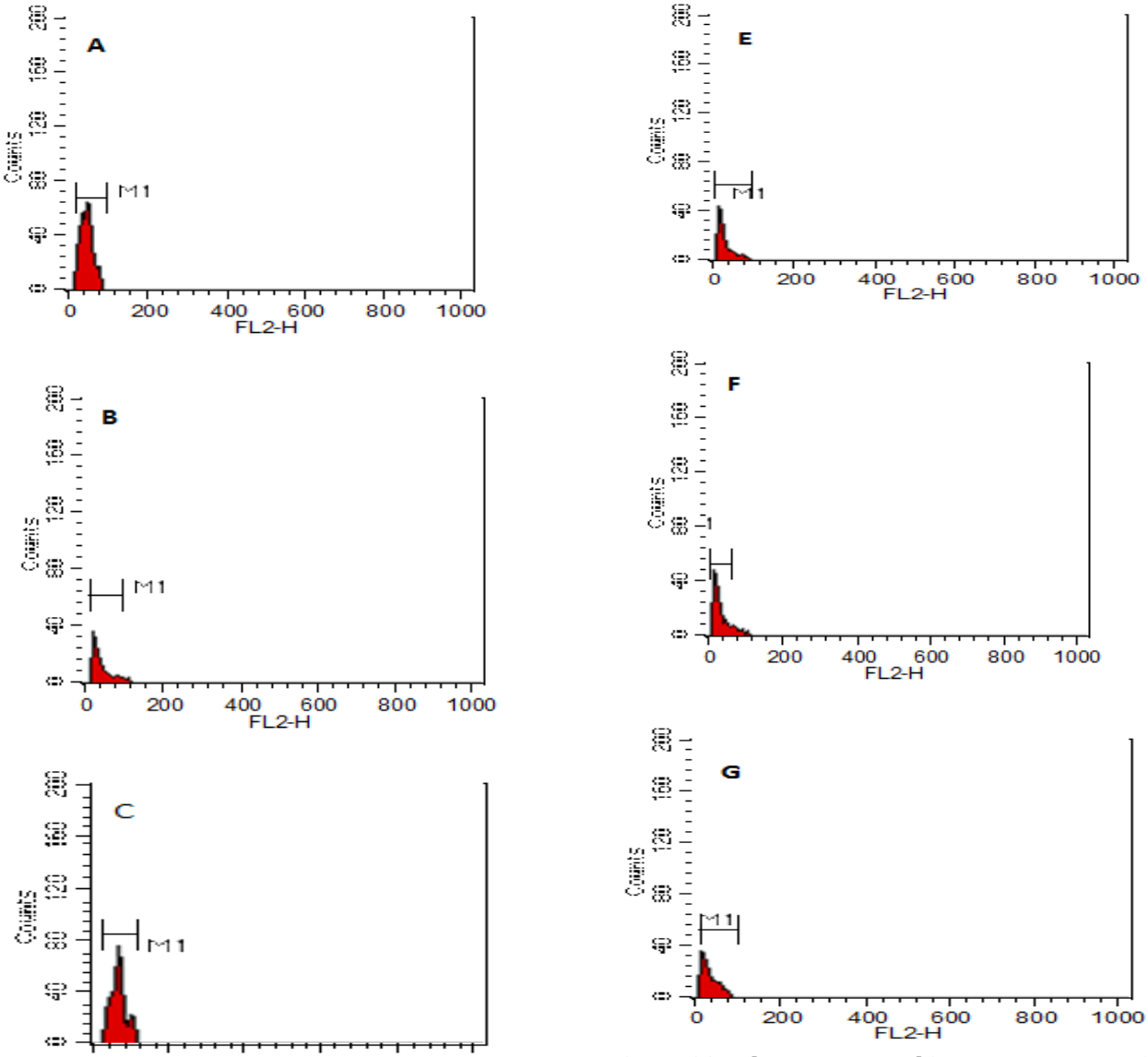

Figure (1): Flow cytometry histograms representing the frequency of apoptosis (sub G1) % in adult rat testes in control and different treated rat groups A: NC, B: CO: C: PC, D: ES, E: TCDD, F: PC+ TCDD, and G: ES+TCDD, (M1= +ve population of stained cells with propidium iodide from total No of count 10000 cells/ml).

Histopathological examination

As shown in figure (2), histological observations of the control, corn oil, ES, and PC groups testes (A, B, C, and D, respectively) showed normal morphological appearance of seminiferous tubules in various shapes and sizes with stratified epithelium. Each tubule showed regular arrangement of the spermatogenic cells (spermatogonia, spermatocytes and spermatids). Many metamorphosed spermatids and spermatozoa were observed near the lumen of the tubules. However, in the group treated with TCDD, there was focal disorganization of the seminiferous tubules associated with a marked loss of many of the germ cells and the lack of sperms were noted. Also, degenerated Leydig cells and mild sloughing of germ cells from the basement

membrane were observed as shown in figure (3 A and B). On the other hand, concomitant administration of either alcoholic extract of either ES or PC with TCDD ameliorated the histopathological changes in the testes compared with TCDD treated group, where the testes showed normal spermatogenic cells (figure 3C, and D, respectively).

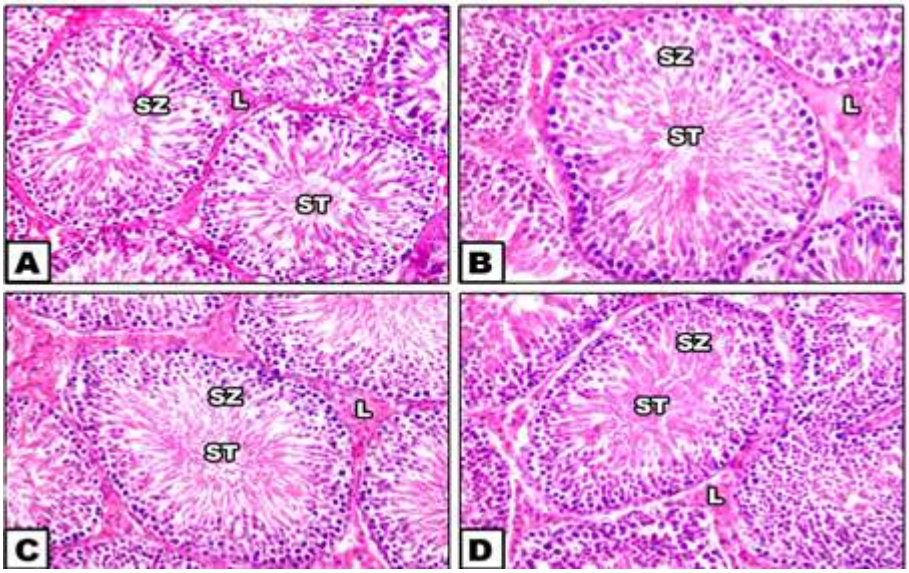

Figure 2(A-D): Photomicrographs of histological sections of rat testes. A: Control showing normal arrangement of germ cells. B: Corn oil treatment, C: Eruca sativa and D: Petroselinum Crispum treatment showing normal histological features. ST- seminiferous tubules, SZ-spermatozoa, L- Leydig cells.

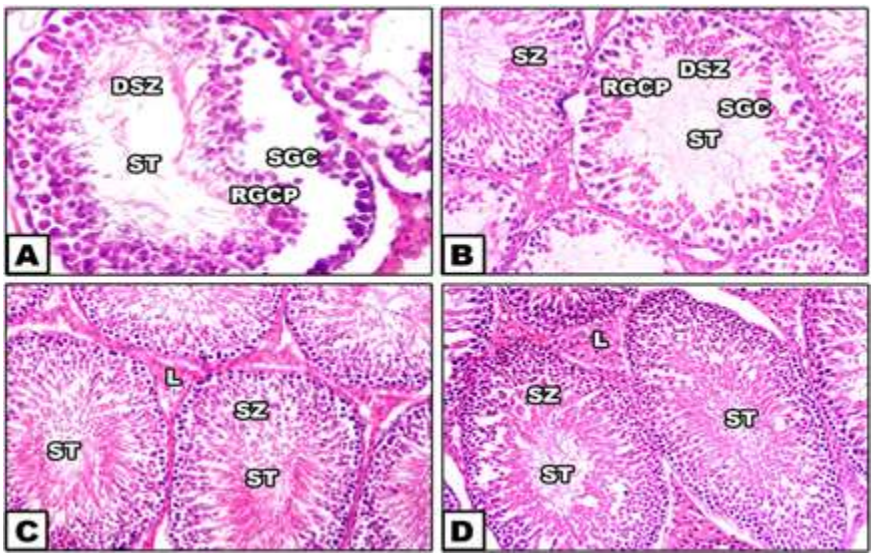

Figure 3(A-D): Photomicrographs of histological sections of rat testes. A&B dioxin-treatment, showing massive reduction of spermatogenic cells, sloughing germ cells (SGC), devoid of spermatozoa (DSZ) and reduced germ cell population (RGCP), C&D: Eruca sativa and Petroselinum crispum with dioxin treatment showing normal morphological appearance with seminiferous tubules in various shapes and sizes, ST- seminiferous tubules, SZ-spermatozoa, L- Leydig cells.

DISCUSSION

Administration of TCDD caused decrease in the body weight. This result is in agreement with [38] and may be attributed to the decreased food, water consumption in both feed and water intake[39], loss of muscles and decrease bone mass due to decline in the anabolic testosterone as reported by.[40] When alcoholic extract of either PC or ES administered to TCDD treated rats, the body weight tends to be near to those of control group. These results may be attributed to apigenin and, isothiocynates which are found in high quantities in PC and ES, respectively, a promising testosterone booster, through inhibiting the activity of the enzyme cyclooxygenase -2 (COX2) and enhancing the steroidogenic acute regulatory (StAR) protein which plays a key role in the synthesis of testosterone.[41, 42]

There was a marked reduction in weights of testes and epididymis in TCDD treated rats. These results are in agreement with.[43] This reduction may be a result of reduced number of Leydig cell, germ cell and steroidogenic enzyme activity, as well as impaired Leydig cell LH responsiveness [44], inhibition of the expression of androgen receptor and proliferating cell nuclear antigen and apoptosis as reported by [45], and/or induction of oxidative stress that lead to cell damage by lipid peroxidation and decreasing antioxidant enzymes, an explanation which agrees with. [43] The treatment of rats with either alcoholic extract of PC or ES in the presence of TCDD resulted in significant improvements in the weights of testes and epidydmyes compared with control group where the phytochemicals found in PC and ES break the chain reactions of free radicals responsible for peroxidation of lipids, that have the ability to maintain membrane fluidity and integrity by protecting the lipids.[46]

Toxicants that target the male reproductive system can affect sperm count or shape, alter sexual behavior, and/or increase infertility.**[47]** Testosterone is necessary for normal sperm development. Although small amounts are also secreted by the adrenal glands, testosterone hormone is primarily secreted in the testicles of males and the ovaries of females. It activates genes in Sertoli cells, which promote differentiation of spermatogonia.**[48]**

The present work showed that TCDD administration decreased testosterone concentration and consequently sperm count. These results are in agreement with.[38] The reduction in testosterone level in TCDD treated rats may be due to the increase in the activity of the enzyme cyclooxygenase-2 (COX2) in testes which plays a role in the conversion of metabolites of n-6 fatty acids into thromboxane A_2. Since, the testosterone producing Leydig cells in the testes have receptors for thromboxane A2 that activates the protein DAX-1 via these receptors, which in turn inhibits the steroidogenic acute regulatory (StAR) protein that plays a key role in the conversion of cholesterol into testosterone.[40] Also, the observed decrease in testosterone concentration in TCDD treated rats may be due to a decrease dehydroepiandrosterone (DHEA) level and 3β-HSD activity as presented in the present study which essential in testosterone synthesis.

TCDD may acts by binding to specific cytosolic receptor (AhR) and travels into the nucleus forming a dimer ARNT transcription factor to activate CYP1A1 gene thus triggering gene expression, a view which in accordance with. [49] Most of the biological adverse effects including the reproductive toxicity of TCDD are thought to be mediated by aryl hydrocarbon receptor (AhR) dependent gene expressions. [50] Once TCDD enters cells, it binds AhR, a cytosolic transcription factor. The liganded AhR translocates to the nucleus, where it heterodimerizes with the aryl hydrocarbon receptor nucleus translocator (ARNT). The AhR:ARNT complex then binds to the specific enhancer, the dioxin responsive element (DRE), and alters the expressions of various genes.**[49]**

Concomitant supplementation of either alcoholic extract of PC or ES to TCDD treated rats, restored testosterone concentration and sperm count to control level where apigenin and isothiocynates are naturally occurring substances that scuppers the working of the thromboxane A2 receptors.[41, 42] Adverse effects induced by TCDD are reversed by restoration of DHEA level and 3β-HSD activity to be similar that of control group may be responsible for the inhibition of the adverse effect induced by TCDD.[51] The present work also showed elevation of FSH and LH in TCDD treated rats, these results may be due to decreased testosterone via negative feedback mechanism to stimulate GnRH by hypothalamus which in turn stimulates pituitary gland to release FSH and LH.[52] The toxicated rats were protected when alcoholic extracts of either PC or ES were administered in concomitant with TCDD, since FSH, LH and testosterone concentrations were not significantly changed as compared with control group.

Cell death takes two distinct forms, necrosis and apoptosis. Although necrosis is considered to be degenerative phenomena commonly associated with chemical injury, apoptosis in contrast, appears to be an active endogenous process implicated in the regulation of normal as well as preneoplastic and neoplastic tissues.[53] The present work revealed significant increase in apoptosis induced by TCDD, this result is in agreement with [54], apoptosis may be attributed to endocrine disruptor as mentioned by [55], and/or oxidative damage, apoptosis in germinal cells via *bax* gene expression through AhR receptor.[56] Also, our results revealed a significant reduction in apoptotic cells in ES treated rats compared to control group (13.80 vs 20.22, respectively). This result may be due to essential polyunsaturated fatty acids content of ES extract, linolenic acid and oleic acid [57], reserve the mitochondrial integrity and have a role in the structure and function of cell membrane [58] which prevent cell membrane from oxidation and apoptosis

Administration of either alcoholic extract of PC or ES with TCDD ameliorates the apoptotic effect of TCDD. These effect may be related to its relation with phenolic composition, mostly caffeic, ferulic acid, and p-coumaric acid in addition to ascorbic acid that found in either PC or ES are responsible for free radical scavenging and activity of antioxidant [59], or may be due to binding of caffeic acid to the AhR receptor, being an inhibitor of its action thus decreasing the transcription and activity of CYP1A1.[13]

CONCLUSIONS

In conclusions, this study supports that PC and ES have protective effects against reproductive toxicity induced by TCDD. It seems that alcoholic extract of ES has greater protective effect than alcoholic extract of PC in TCDD toxicity.

ACKNOWLEDGMENTS

The authors are grateful to Dr. Mona Samy Othman Gouida, Assistant professor of molecular immunology and head of flow cytometry unit, Genetic unit, Mansoura University Children hospital, Mansoura, Egypt for measurements of samples by flow cytometry analysis. Also, many thanks to Dr. Mamdouh Ghoneim for supplying the TCDD from USA.

DISCLOSURE STATEMENT

No competing financial interests exist.

REFERENCES

1. Kulkarni PS, Crespo JG, Afonso CA. Dioxins sources and current remediation technologies--a review. Environ Int, 2008; 34(1): 139-153.
2. Hays S M, Aylward L L. Dioxin risks in perspective: past, present, and future. Regul Toxicol Pharmacol, 2003; 37: 202-217.
3. Kiviranta H, Hallikainen A, Ovaskainen ML, Kumpulainen J, Vartiainen T. Dietary intakes of polychlorinated dibenzo-p-dioxins, dibenzofurans and polychlorinated biphenyls in Finland. Food Addit Contam, 2001; 18: 945-953.
4. IARC. Polychlorinated dibenzo-p-dioxins and polychlorinated dibenzofurans. IARC Monographs on the evaluation of carcinogenic risk to humans, 1997; 69.
5. 5-Pohjanvirta R. Tuomisto J. Short-term toxicity of 2,3,7,8-tetrachlorodibenzo-p-dioxin in laboratory animals: effects, mechanisms, and animal models. Pharmacol Rev, 1994; 46: 483-549.
6. Kwon YI, Yeon JD, Oh SM, Chung KH. Protective effects of ursodeoxycholic acid against 2,3,7,8-tetrachlorodibenzo-p-dioxin-induced testicular damage in mice. Toxicol Appl Pharmacol, 2004; 194(3): 239-247.
7. Faqi AS, Dalsenter PR, Merker HJ, Chahoud I. Reproductive toxicity and tissue concentrations of low doses of 2,3,7,8-tetrachlorodibenzo-p-dioxin in male offspring rats exposed throughout pregnancy and lactation. Toxicol Appl Pharmacol, 1998; 150: 383–392.
8. Petroff BK, Croutch CR, Hunter DM, Wierman ME, Gao X. 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) stimulates gonadotropin secretion in the immature female Sprague-Dawley rat through a pentobarbital- and estradiol-sensitive mechanism but does not alter gonadotropin-releasing hormone (GnRH) secretion by immortalized GnRH neurons in vitro. Biol Reprod, 2003; 68(6):2100-2106.
9. Mahmood S, Hussain S, Malik F. Critique of medicinal conspicuousness of Parsley (Petroselinum crispum): A culinary herb of Mediterranean region. Pak J Pharm Sci., 2014; 27(1):193-202.
10. Wahba NM, Ahmed AS, Ebraheim ZZ. Antimicrobial effects of pepper, parsley, and dill and their roles in the microbiological quality enhancement of traditional Egyptian Kareish cheese. Food borne Pathog Dis, 2010; 7(4): 411-418.
11. Ramkissoon J, Mahomoodally M, Ahmed N, Subratty A. Antioxidant and anti-glycation activities correlates with phenolic composition of tropical medicinal herbs. Asian Pac J Trop Med, 2013; 6(7): 561-569.
12. Lefort EC, Blay J. The dietary flavonoid apigenin enhances the activities of the anti-metastatic protein CD26 on human colon carcinoma cells. Clin Exp Metastasis, 2011; 28 (4):337-349.
13. Kampa M, Alexaki VI, Notas G, Nifli AP, Nistikaki A, Hatzoglou A, Bakogeorgou E, Kouimtzoglou E. Antiproliferative and apoptotic effects of selective phenolic acids on T47D human breast cancer cells: potential mechanisms of action. Breast Cancer Res, 2004; 6 (2): R63-74.
14. López MG, Sánchez-Mendoza IR, Ochoa-Alejo N. Compartive study of volatile components and fatty acids of plants and in vitro cultures of parsley (Petroselinum crispum (Mill) nym ex hill). J Agric Food Chem, 1999. 47(8): 3292-3296.
15. Chaves DSA., de Almeida AP, Assafim M, Frattani F, Zingali RB, Costa SS. Chemical study and potential antithrombotic evaluation of medicinal species Petroselinum crispum (APIACEAE) . Drugs of the Future, 2007; 32: 66-67
16. Cherng J M, Chiang W, Chiang LC. Immunomodulatory activities of common vegetables and spices of Umbelliferae and its related coumarins and flavonoids. Food Chemistry, 2008; 106 (3): 944–950.
17. Larsen E, Christensen LP. Simple saponification method for the quantitative determination of carotenoids in green vegetables. J Agric Food Chem, 2005; 53(17): 6598-6602.
18. Gadi D, Bnouham M, Aziz M, Ziyyat A, Legssyer A, Legrand C, Lafeve FF, Mekhfi H. Parsley extract inhibits in vitro and ex vivo platelet aggregation and prolongs bleeding time in rats. J Ethnopharmacol, 2009; 125 (1):170-174.
19. Bradley PR. Parsley herb. In: British Herbal compendium. Volume1. A hand book of Scientic information widely used plant drugs. British Herbal Medicine Association, Bournemouth: 1992. Dorst, pp. 168-169.
20. Jassim AM. Protective effect of petroselinum crispum (parsley) on histopathological changes in liver, kidney and pancreas induced by sodium valproate- in male rats. *Kufa* Journal For Veterinary Medical Sciences, 2013; 4(1): 20-27.
21. Zohara Y, Schafferman D, Amarisrael Z. Traditional uses and biodiversity of rocket (Eruc sativa, Brassicaceae) in Israel. Economic Botany, 1998; 52: 394-400.
22. Barillari J, Canistro D, Paolini M, Ferroni F, Pedulli GF, Iori R, Valgimigli L. Direct antioxidant activity of purified glucoerucin,the dietary secondary metabolite contained in rocket (Eruca sativa Mill.) seeds and sprouts. J. Agric. Food Chem, 2005; 53: 2475–2482.

23. Salem MAR, Moustafa NA. Histological and Quantitative Study of the Effect of Eruca Sativa Seed Oil on The Testis of Albino Rat. Egypt J. Hosp. Med, 200; 2: 148 – 162.
24. Hussein, Z F. Study the Effect of Eruca Sativa Leaves Extract on Male Fertility in Albino Mice. J Al-Nahrain Univ, 2013; 16 (1): 143-146
25. Al-Howiriny T, Al-Sohaibani M, El-Tahir K, Rafatullah S. Prevention of experimentally-induced gastric ulcers in rats by an ethanolic extract of "Parsley" Petroselinum crispum. Am. J. Chin. Med, 2003; 31(5): 699-711.
26. Alqasoumi S. CarbonTetrachloride-Induced Hepatotoxicity: Protective Effect of 'Rocket' Eruca sativa L. in Rats. Am. J. Chin. Med, 2010; 38 (1):75–88.
27. Latchoumycandane C, Mathur PP. Effects of vitamin E on reactive oxygen species-mediated 2,3,7,8-tetrachlorodi-benzo-p-dioxin toxicity in rat testis. J Appl Toxicol, 2002; 22(5):345-351.
28. Talalay P. Hydroxysteroid dehydrogenase. In: colowick, kaplan (eds). "Methods in enzymology". New York: Academic press: 1962; 5, pp. 512-516.
29. Tribukait B, Moberger G, Zetterberg A. Methodological aspects for rapid flow cytofluorometry for DNA analysis of human urinary bladder cells. In Haenen, C., Hillen, H., Wessels, S, eds. Pluse cytophotometry, part 1. European Press Medicon, Ghent: 1975, pp. 55-60.
30. Vindelov LL. Flow microfluorometric analysis of nuclear DNA in cells from solid tumors and cell suspensions. A new method for rapid isolation and straining of nuclei. Virchows Arch B Cell Pathol, 1977; 24(3):227-242.
31. Dean PN, Jett JH. Mathematical analysis of DNA distributions derived from flow microfluorometry. J Cell Biol, 1974; 60(2): 523-527.
32. Adeeko AO, Dada OA. Chloroquine reduces fertilizing capacity of epididyma sperm in rats. Afr J Med Med Sci, 1998; 27(1-2): 63-64.
33. Stephen JW, David EK, Bret G. The analog free testosterone assay: Are the results in men clinically useful. Clin. Chem, 1998; 44(10): 2178-2182.
34. Labrie F, Luu-The V, Belanger A, Lin SX, Simard J, Pelletier G, Labrie C. Is dehydroepiandrosterone a hormone?. J Endocrinol, 2005; 187 (2): 169-196.
35. Babson AL, Olson DR, Palmieri T, Ross AF, Becker DM, Mulqueen PJ. The immulite assay tube: a new approach to heterogeneous ligand assay. Clin. Chem, 1991; 14: 1521-1522.
36. Weesner FM. General Zoological Microtechinques Scientific Book Agency, Calcutta, India, 1968; 86 p.
37. Snedecor CW, Cochran WC. Statistical methods (7th edn). Iowa state Univ. press, Ames, Iowa: 1980, 593 p.
38. Choi J.S, Kim IW, Hwang SY, Shin BJ, Kim S K. Effect of 2,3,7,8-tetrachlorodibenzo-p-dioxin on testicular spermatogenesis-related panels and serum sex hormone levels in rats. BJU Int, 2007; 101(2): 250-255.
39. Kelling CK, Christian BJ, Inhorn SL, Peterson RE. Hypophagia-induced weight loss in mice, rats, and guinea pigs treated with 2,3,7,8-tetrachlorodibenzo-p-dioxin. Fundam Appl Toxicol, 1985; 5(4): 700-712.
40. Dhanabalan S, Jubendradass, R, Latha P, Mathur PP. Effect of restraint stress on 2,3,7,8 tetrachloro dibenzo-p-dioxin induced testicular and epididymal toxicity in rats. Hum Exp Toxicol, 2011; 30 (7): 567-578.
41. Rose P, Won YK, Ong CN, Whiteman M. Beta-phenylethyl and 8-methylsulphinyloctyl isothiocyanates, constituents of watercress, suppress LPS induced production of nitric oxide and prostaglandin E2 in RAW 264.7 macrophages. Nitric Oxide, 2005; 12(4): 237-243.
42. Li W, Pandey AK, Yin X, Chen JJ, Stocco DM, Grammas P, Wang X. Effects of apigenin on steroidogenesis and steroidogenic acute regulatory gene expression in mouse Leydig cells. J Nutr Biochem, 2011; 22(3): 212-218.
43. Latchoumycandane C, Chitra C, Mathur P. Induction of oxidative stress in rat epididymal sperm after exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin. Arch Toxicol, 2002;76 (2):113-118.
44. Johnson L, Dickerson R, Safe SH, Nyberg C.L, Lewis RP, Welsh TH Jr. Reduced Leydig cell volume and function in adult rats exposed to 2,3,7,8-tetrachlorodibenzo-p-dioxin without a significant effect on spermatogenesis. Toxicology, 1992; 76(2): 103-118.
45. Jin MH, Ko HK, Hong CH, Han SW. In utero exposure to 2,3,7,8-Tetrachlorodibenzo-p-Dioxin affects the development of reproductive system in mouse. Yonsei Med J, 2008; 49(5): 843-850.
46. Arora A, Byrem TM, Nair MG, Strasburg GM. Modulation of liposomal membrane fluidity by flavonoids and isoflavonoids. Arch Biochem Biophys. 2000; 373(1):102-109.
47. Sönmez M, Türk G, Çeribaşı AO, Sakin F, Ateşşahin A. Attenuating effect of lycopene and ellagic acid on 2,3,7,8-tetrachlorodibenzo-p-dioxin-induced spermiotoxicity and testicular apoptosis. Drug Chem Toxicol, 2011; 34(4):347-356.
48. Ostrer H. Sexual differentiation. Semin Reprod Med, 2000; 18(1): 41-49.
49. Denison MS, Fisher JM, Whitlock JP Jr. Protein-DNA interactions at recognition sites for the dioxin-Ah receptor complex. J Biol Chem, 1989; 264 (28): 16478-16482.
50. Kim KH, Park HJ, Kim JH, Kim S, Williams DR, Kim MK, Jung YD, Teraoka H, Park HC, Choy HE, Shin BA, Choi SY. Cyp1a reporter zebrafish reveals target tissues for dioxin. Aquat Toxicol, 2013; 134-135: 57-65.
51. Ciolino HP, Yeh GC. The steroid hormone dehydroepiandrosterone inhibits CYP1A1 expression in vitro by a post-transcriptional mechanism. J Biol Chem, 1999; 274(49):35186-35190.

52. Håkonsen LB, Thulstrup AM, Aggerholm AS, Olsen J, Bonde JP, Andersen CY, Bungum M, Ernst EH, Hansen ML, Ernst EH, Ramlau-Hansen CH. Does weight loss improve semen quality and reproductive hormones? Results from a cohort of severely obese men. Reprod Health, 2011; 17: 8-24.
53. Kroemer G, Petit P, Zamzami N, Vayssière JL, Mignotte B. The biochemistry of programmed cell death. FASEB J, 1995; 9(13):1277-1287.
54. Xu G, Liu J, Yoshimoto K, Chen G, Iwata T, Mizusawa N, Duan Z, Wan C, Jiang J. 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) induces expression of p27(kip¹) and FoxO3a in female rat cerebral cortex and PC12 cells. Toxicol Lett, 2014; 226(3): 294-302.
55. Chen SC, Liao TL, Wei YH, Tzeng CR, Kao SH. Endocrine disruptor, dioxin (TCDD)-induced mitochondrial dysfunction and apoptosis in human trophoblast-like JAR cells. Mol Hum Reprod, 2010; 16(5): 361-372.
56. MatikainenT, Perez GI, Jurisicova A, Pru J K, Schlezinger JJ, Ryu HY, Laine J, Sakai T, Korsmeyer SJ, Casper RF, Sherr DH, Tilly JL. Aromatic hydrocarbon receptor-driven Bax gene expression is required for premature ovarian failure caused by biohazardous environmental chemicals. Nat Genet, 2001. 28(4): 355-360.
57. Solana M, Boschierob I, Dall'Acquab S, Bertucco A. Extraction of bioactive enriched fractions from Eruca sativa leaves bysupercritical CO2technology using different co-solventsM. J. of Supercritical Fluids, 2014; 94: 245–251
58. Mclennan PI, Dallinore J A. Dietary canola oil modifies myocardial fatty acids and inhibist cardiac arrgythmias in rats in rats J. Nutr, 1995; 125(4):1005-10099
59. Tiveron AP, Melo PS, Bergamaschi KB, Vieira TM, Regitano-d'Arce MA, Alencar SM. Antioxidant activity of brazilian vegetables and its relation with phenolic composition. Int J Mol Sci, 2012; 13(7): 8943-8957.