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Protective effects of some fruit juices with low-fat diet on rat testis damaged by carbon tetrachloride: A genetic and histological study

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Oxidative stress, free radical, lipid peroxidation and antioxidant have become a common expression with most disease and methods for protection. Carbon tetrachloride (CCl_4) is an industrial solvent which has destructive effects on a cell while most fruit juices have antioxidant effects. The aim of this study was to investigate the protective role of fruit juice on testis after toxic effect with CCl_4 through oxidative stress with basal diet and low-fat diet. Seventy-five male albino rats were used for this study in which the juices of three fresh fruit, yellow apples (*Malus domestica*, L.), red grapes containing seeds (*Vitis vinifera,* L.) and pomegranates (*Punica granatum*, L.) were used as therapeutic agents. Histological sections of testis indicated that low-fat diet has obvious effects than basal diet in both the low-fat diet with CCl_4 "LdC""con++", LdC with grape juice 2 ml "grpL2", LdC with pomegranate juice 2 ml "pomL2" and hyper effect in LdC with pomegranate juice 4 ml "pomL4" while it was equal in effect with basal diet in the other treatments. Low-fat diet gave significant effects (about 75% recovery in con++, LdC with Apple juice 2 ml and 4 ml "appL2, appL4" , LdC with grape juice 4 ml "grpL4" and pomL2) while 25% began to recover as shown in basal diet with pomegranate juice 4 ml "pomB4" and grpL2. Treatment of rats with pomegranate juice ameliorated the toxic effects of CCl_4 with low-fat diet on Semi-random RAPD-DNA profile. Low-fat diet with fruit juice had positive effect against toxicity induced by CCl_4 in testes of rats on the level of histological and DNA-RAPD studies.

Key words: CCl_4, rat testis, yellow apples, red grapes, pomegranates, low-fat diet, oxidative stress.

INTRODUCTION

By alteration of protein and nucleic acid structure, oxidative stress has a destruction effect on cells. Moreover, oxidative stress increases the intracellular free calcium, destruction of cells by lipid peroxidation thereby

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damaging the membrane ion transport and permeability (Reckmage et al., 1989). Because of its association with some of the abnormal physiological processes, lipid peroxidation has attracted much attention in recent years (Hartley et al.,1999; Reckmage et al., 1989). Due to the abundance of highly unsaturated fatty acids and the presence of potential reactive oxygen species generating systems, Testicular micro-environment characterize by low oxygen tensions, but this tissue remains vulnerable to oxidative stress. Testicular micro-environment generated from the mitochondria and a variety of enzymes including the xanthine and NADPH-oxidases (Banfi et al., 2001; Kumagai et al., 2002) and the cytochrome P450s (Zangar et al., 2004).

Carbon tetrachloride (CCl_4) is an industrial solvent that cause damages in experimental animals especially in kidney, lungs and testicular (Abraham et al., 1999). CCl_4 is xenobiotic which can be metabolized by hepatic microsomal cytochrome P450 to trichloromethyl free radical which reacts with sulfhydryl groups and antioxidant enzymes. CCl_4 can induce chemical liver injury and over production of trichloromethyl free radicals which initiate a membrane lipid peroxidation that is viewed as a complicated biochemical reaction. These biochemical reactions involving metal ions, free radicals, oxygen and a host of other factors in the biological system and leading to various pathological changes (Bast, 1993; Cemek et al., 2010; Szymonik-Lesiuk , et al., 2003).

CCl_4-induced reproductive toxicity in male rats, free radicals of CCl_4 bind with polyunsaturated fatty acid of sperm membrane to produce alkoxy and peroxy radicals that generate lipid peroxides that are highly reactive, alters hormonal levels, reduces enzyme activity, change sperm concentration and induce injury or necrosis (Sikka et al., 1995;Ogeturk et al., 2005). Protected the testes and other origins against antioxidant status induced by CCl_4 can be achieved by natural products containing antioxidant as shown through some studies (Khan et al., 2009;Cemek et al., 2010).

Apples may play a large role in reducing the risk of a wide variety of chronic disease, the phrase that says "An apple a day keeps the doctor away" is quite popular but many studies have provided the scientific backing for both of these very common phrases. Apples also, maintain a healthy lifestyle in general (Boyer and Liu, 2004).

Many studies reported that grapes have antioxidant properties (Park et al., 2003; Dani et al., 2007; Buchner et al., 2014) and this antioxidant properties could be at least attributed to the high phenolic content present in grape juice (Dani et al., 2008) where others refer to grape juice modulates as apoptosis but not oxidative stress (Oshima et al., 2015). Many cultures indicated that pomegranate fruit has been used as a natural medicine; the antioxidant defense mechanism was augmented by pomegranate juice against CCl_4-induced reproductive toxicity, pomegranate juice provides evidence that it may have a therapeutic role in free radical mediated diseases (Al-Olayan et al., 2014). In these days, pollution is widespread with chemical materials which cause oxidative stress in the human body and has an effect on reproduction. So the aim of this study is to investigate these effects, and reduce it with a natural material like fresh fruit juice.

MATERIALS AND METHODS

Fruits, rats and chemicals

Fruits [fresh yellow apples (*Malus domestica*, L.), red grapes containing seeds (*Vitis vinifera,* L.) and pomegranates (*Punica granatum*, L.)] were obtained from the local market, Tanta city, El-Gharbia governorate, Egypt. Normal male albino rats (n = 75) of Sprague Dawley strain weighing 200±10 g used in this experiment were obtained from the laboratory animal colony, Helwan Farm, Vaccine and Immunity Organization, Cairo, Egypt. Casein, DL-methionine, choline chloride, vitamins, minerals, cellulose, CCl_4 and other required chemicals were obtained from Elgomhorya Company for Chemicals and Drugs, Cairo, Egypt. Corn starch and soybean oil were purchased from the local market, Tanta city, El-Gharbia governorate, Egypt.

Preparation of fruit juices

Samples of fruits were cleaned and free from evidence of insect infestation and objectionable materials. Afterwards, grape and pomegranate fruits were homogenized in the blender individually without water or sugar. In contrast, apple juice was prepared using water (30g/100 ml) and with zero sugar. Pomegranate peels, in particular, were removed before homogenization and homogenized with their seeds, whereas apple homogenized with their peels and without seeds. After that, each juice was cleared and administered to rats immediately as a fresh juice.

Biological experiment

Male albino rats (n =75) of Sprague Dawley strain weighing (200± 10 g) were housed in well aerated cages under hygienic conditions and fed on basal diet for one week for adaptation. Basal diet was prepared from fine ingredients per 100 g. The diet had the following composition: Casein (>85% protein) 14%, soybean oil 10%, cellulose 5%, salt mixture (Hegsted et al., 1941) 3.5%, vitamin mixture (Campbell, 1961) 1%, choline chloride 0.25%, DL-methionine 0.3% and corn starch up to 100g (AIN , 1993). Low-fat diet was prepared from fine ingredients per 100 g. The diet had the following composition: Casein (>85% protein) 14%, soybean oil 4%, cellulose 5%, salt mixture, Hegsted et al.,1941, 3.5%, vitamin mixture (Campbell, 1961) 1%, choline chloride 0.25%, DL-methionine 0.3% and corn starch up to 100 g (Reeves et al., 1993). Dextrose was added to complete the weight of vitamin mixture to one kilogram. Vitamins A and D were supplied by adding 0.5 g of cod liver oil to the diet. Vitamin E was supplied from sunflower oil to give the final concentration 50 mg/kg diet. After this period, rats were divided into two main groups. The first main group (5 rats) was kept as a negative control group (con-) and fed on basal diet only. The second main group (70 rats) was injected subcutaneously with CCl_4 in paraffin oil (50% v/v, 2 ml/kg body weight) twice a week for two weeks to induce chronic damage in the liver according to Jayasekhar et al. (1997). To examine the effectiveness of induction, blood samples were withdrawn from eye plexus of veins and the activities of transaminases (AST "SGOT" and ALT "SGPT") were

Table 1. The treatments and its abbreviations.

No.	Treatment	Abbreviation
1	Basal diet only "Bd"	con-
2	Bd with CCl4 "BdC'	con+
3	BdC with Apple juice 2 ml	appB2
4	BdC with Apple juice 4 ml	appB4
5	BdC with grape juice 2 ml	grpB2
6	BdC with grape juice 4 ml	grpB4
7	BdC with pomegranate juice 2 ml	pomB2
8	BdC with pomegranate juice 4 ml	pomB4
9	low-fat diet with CCl_4 "LdC"	con++
10	LdC with Apple juice 2 ml	appL2
11	LdC with Apple juice 4 ml	appL4
12	LdC with grape juice 2 ml	grpL2
13	LdC with grape juice 4 ml	grpL4
14	LdC with pomegranate juice 2 ml	pomL2
15	LdC with pomegranate juice 4 ml	pomL4

determined in serum (Reitman and Frankel, 1957). AST activity in CCl_4 –injected group was 160.57±5.68 U/L versus 61.71±9.99 U/L in normal control group, while ALT activity in CCl_4–injected group was 85.88±5.98 U/L versus 21.65±3.45 U/L in normal control group. After that, injected rats were divided into 14 equal subgroups. Of which, 7 groups were fed on basal diet only (positive control group "I" or basal diet) plus either apple, grape or pomegranate juices in two doses (2 and 4 ml), while the others were fed on low-fat diet (4% soybean oil) only (positive control group "II" or low-fat diet) plus either apple, grape or pomegranate juices in two doses (2 and 4 ml) Table 1. Juices were administered using a stomach tube and given once daily. After 28 days, animals were sacrificed under other anesthetized and testes were removed by careful dissection, and one of two testes was immersed in formalin solution (10%) to be examined histopathologically, while the others were taken for DNA analysis.

Histopathological examination

Testis of rats were taken and immersed in 10% buffered neutral formalin solution. After immersing in formalin, the fixed specimens were trimmed, washed and dehydrated by passing in serial concentrations in ascending grades of alcohol. Furthermore, testis were then cleared in xylol and embedded in paraffin. They have now been cut in sections with thickness of 4-6 microns and stained with haematoxylin and eosin as described by Drury and Wallington (1980).

Total Genomic DNA Extraction

To extract the total genomic DNA, aTIAamp genomic DNA Kit (Cat. no. DP304,TianGen) was used according to the manufacture's protocol. Briefly, rat tissue (10 mg) was washed, resuspended and centrifuged at 10.000 rpm for 1 min. Proteinase K were added in 20 µl to samples and incubated at 56°C until completely lyses. Finally, DNA was dissolved in 80 µl TE buffer.

Semi-Random Amplified Polymorphic DNA-Polymerase Chain Reaction (Semi-random RAPD)

Seven Semi- Random RAPD (intron-exon splice junctions (ISJ)) primers were screened against the pooled rats DNA. The list of primers and their sequences are presented in Table 2.

Table 2. Primers, their codes and sequences used in this study.

Number	Primer Code	Sequence (5`→3`)
1	ISJ-3	TGC AGG TCA G
2	ISJ-4	GTC GGC GGA CAG GTA AGT
3	ISJ-5	CAG GGT CCC ACC TGC A
4	ISJ-6	ACT TAC CTG AGC CAG CGA
5	ISJ-7	TGC AGG TCA GGA CCC T
6	ISJ-11	TGC AGG TCA AAC GTC G
7	ISJ-12	GGA CTG GAG CAG GTA AGT

The optimization of PCR conditions for each primer was performed in a 20 µl reaction volume including 1 µl of isolated template DNA. Final concentration of each reaction was 1x master mix (MyTaq™ Red Mix, Bioline, England), 0.8 µM primers. Amplifications were carried out in a thermal cycler PCR machine according to the instructions of the manufacturer as follows: the initial amplification program started with 95°C to denaturation for 2 min, followed by 35 cycles consisting of denaturation at 95°C for 15 s, annealing at 30 - 50°C according to the primer for 20 s and elongation at 72°C for 1 min.

The program ended with a final elongation step for 5 min at 72°C. The amplified products were separated on 1.2 % agarose gel, stained with ethidium bromide and photographed with UV-Gel documentation. A known DNA Ladder (O'GeneRuler DNA Ladder Mix ready-to-use, Cat-no: #SM1173, Thermo Scientific) was run against the PCR products, Weining and Langridge (1991), Sawicki and Szczecinska (2007).

Data analysis

Genomic template stability (GTS) has been calculated as follows: GTS (%) = (1 - a/n) ×100; where *n* is the number of total bands detected in the control and *a* is the number of polymorphic bands detected in each treated sample. Polymorphism observed in the semi-RAPD profile included appearance of a new band and disappearance of a normal band in comparison to control semi-RAPD profile (Luceri et al., 2000; Atienzar et al., 2002; Qari, 2010).

RESULTS AND DISCUSSION

Histological studies

A number of reports clearly demonstrated that CCl_4 does not only induce free radical attack against liver cells, but also against many tissues such as kidney, heart, lung, testis and brain, and may induce oxidative injury in these tissues (Dashti et al., 1989; Adewole et al., 2007). In this present study, histological and genetic investigations were carried out on rat testis to evaluate high toxicity on reproductive tissue and the role of natural juice in protecting against the side effects of CCl_4.

Microscopically, testis of rat from negative control "con-" revealed normal seminiferous tubules (Figure1a). Rat testis from positive control "con+" appeared to be spermatogoneal cells lining seminiferous tubules

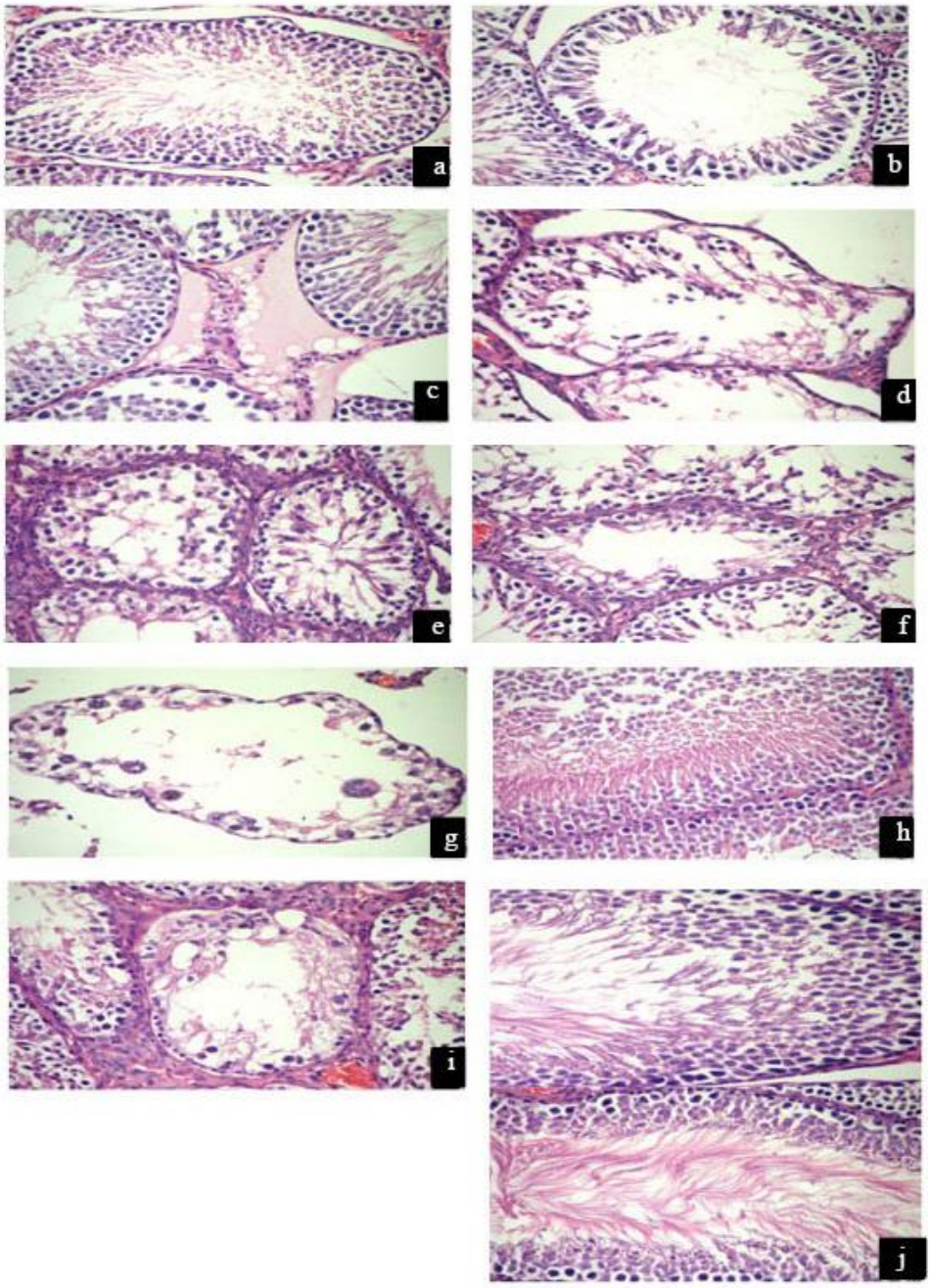

Figure 1 (a-j). Histological studies on rat testis damaged by carbon tetrachloride and treated with fruit juice. "a" con- showing normal seminiferous tubules; "b" con+ showing degeneration of spermatogenial cells lining seminiferous tubules; "c" con+ showing interstitial oedema; "d" grpB2 showing necrosis of spermatogoneal cells lining seminiferous tubules; "e" grpB4 showing necrosis of spermatogoneal cells lining seminiferous tubules; "f" pomB2 showing degeneration and necrosis of spermatogoneal cells lining seminferous tubules, "g" pomB2 showing spermatide giant cells in the lumen of seminiferous tubules; "h" con++ showing hyperactivation of spermatogoneal cells lining seminiferous tubules; "i" grpL4 showing necrosis of spermatogoneal cells lining seminiferous tubules; "j" pomL4 showing hyperactivation and hyperplasia of spermatogoneal cells lining seminiferous tubules.

degeneration (Figure1b) in addition to interstitial oedema (Figure1c), whereas no histological changes were noticed in testis of rats from appB2 and appB4 groups. Examined sections from grpB2 and some sections from grpB4

revealed spermatogoneal cells lining seminferous tubules necrosis (Figure 1d and 1e) respectively, whereas no histological changes has been detected from other sections from grpB4. Tests of rat from pomB2 revealed necrosis and degeneration in spermatogoneal cells lining seminferous tubules (Figure 1f) and spermatid giant cells in the lumen of seminiferous tubules (Figure 1g). However, testis of rat from pomB4 and some sections from low-fat diet with CCl_4 treatment "con++" revealed no histological changes whereas, other sections from con++ showed hyper-activation of spermatogoneal cell lining seminiferous tubules (Figure 1h). More also, testis of rat from appL2, appL4, grpL2 and some sections from grpL4 revealed no histological changes whereas, other sections from grpL4 showed necrosis of spermatogoneal cells lining seminiferous tubules (Figure 1i). No histological changes were noticed in testes of rat from pomL2. Meanwhile, tests of rat from pomL4 revealed hyper-activation of spermatogoneal cells lining seminiferous tubules (Figure 1j).

From previous results, we noticed that CCl_4 destroy testis in positive control, this result agree with Khan (2012) who reported that CCl_4 caused loss of germ cells, interruption in meiosis, sperm with abnormal shape, abnormality of germinative epithelium, fibroblast and inflammatory cells, as well as those caused by atrophy of somniferous tubules. Whereas CCl_4 with low-fat diet showed recovery in some section, the others did not. Orally, treatment with apple juice revealed a recovery of testicular abnormalities induced by CCl_4 in both concentrations 2 and 4 ml in both of basal diet and low-fat diet. Treatment with grape juice did not show any positive effect with concentration 2 ml in basal diet but it was more effective in low-fat diet. In concentration, 4 ml in some sections were recovered while others did not in both of basal and low-fat diet.

Pomegranate did not show any effect at a level of 2 ml but 4 ml had positive effect in basal diet. In low-fat diet, pomegranate at 2 ml was more effective and 4 ml was hyper effective as shown in Figure 1j.

From previous results, we can conclude that low-fat diet has obvious effects than basal diet in both of con++, grpL2, pomL2 and hyper effect in pomL4 while low-fat diet was in equal effect with basal diet in the other treatments, so it could be indicated that low-fat diet gave significant effects (about 75% in con++, appL2, appL4, grpL4, pomL2 recovery) while 25% began to recover as shown in pomB4 and grpL2.

Yang et al. (2010) reported that mice which had acute hepatotoxicity induction by CCl_4, gave a significant protective effect when treated with apple polyphenols and this effect may be due to inhibition of lipid peroxidation, its free radical scavenging effect, and ability to increase antioxidant activity. On the other hand, Khan (2012) examined the protective effects of *Launaea procumbens* on testis against oxidative stress of CCl_4 in male rat which also improved the levels of antioxidant enzymes in CCl_4 administered rats as a result of the presence of phenolic and polyphenolic constituents.

RAPD polymorphism and GTS % among treatments in testes

DNA alteration can be detected by many laboratory technique, one of them is random amplified polymorphism DNA " RAPD', which was developed by Williams et al. (1990) and Welsh and McClelland (1990). It can be used with any organism without prior information on the nucleotide sequences, and this is one of its advantages.

In this experiment, we study the protective role of fruit juice on testis after exposure to CCl_4 as a toxin through oxidative stress. Seven (intron-exon splice junctions (ISJ)) primers have been used with random sequences as shown in Table 2. These primers gave total of fragments 714, and total of 58 bands (Table 3). Twenty five bands out of them were polymorphic with the percentage of 43.1%, whereas thirty three bands were monomorphic (common) for all treatments. The highest level of polymorphism (75 %) was observed with primer ISJ-4. Moreover, the lowest level of polymorphism was 0% with primer 3 as shown in Table 3.

Results from semi-RAPD profile which appeared in Table (3) refer to changes between control and other treatments, while negative control showed in total 45 bands resulted from seven primers, number of these bands ranged from three bands with primer ISJ-3 and 4 to twelve bands with primer 5. The positive control gave 6 variable bands (polymorphic bands include appearance of new bands and disappearance of normal bands) and GTS% was 86.7 in addition to treatments 2 and 12 where showed results similar to positive control. Treatments appB4, appL4, grpL2, pomL2 and pomL4 showed lowest polymorphic bands 5 and GTS% was 88.9 compared with negative control. The highest polymorphic bands were in grpB2 which gave thirteen polymorphic bands, GTS % was 71.1% compared with the negative control.

The results showed high increase in band intensity in treatment pomL2 when compared with negative control, positive control and other treatments, which recorded 13 bands, followed by appL2 and appB4 treatments that recorded 16 bands increase in its intensity for both treatments (Figure 2). On the other hand, the decrease in band intensity was detected in 8 bands in the positive control whereas the recorded high number of other treatments was compared with negative control followed by treatment grpL4 which decrease in 6 bands than negative control.

In this study, the results showed that the decrease in polymorphic bands (5) and increase in GTS % (Table 4) which was 88.9% and increase in intensity was in treatments appB4, appL4, grpL2, pomL2 and pomL4 in rats with toxicity in testis which was induced by CCl_4, the treatment appB4 with basal diet and the others were in

Table 3. Level of polymorphism among treatments in testes compared with control- on RAPD analysis.

Primer	TAF	PB	MP	P%	con-	con+	appB2	appB4	grpB2	grpB4	pomB2	pomB4	con++	appL2	appL4	grpL2	grpL4	pomL2	pomL4
3	3	0	3	0	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
4	4	3	1	75	3	4	4	3	2	4	3	4	3	3	3	3	3	3	3
5	17	8	9	47.1	12	12	12	12	12	12	12	15	11	13	12	12	12	11	11
6	12	3	9	25	10	9	12	12	12	12	12	12	12	12	12	12	12	12	12
7	7	1	6	14.3	7	6	7	7	7	7	7	7	7	7	7	7	7	7	7
11	8	5	3	62.5	4	4	5	3	5	4	5	7	4	4	5	3	4	3	3
12	7	5	2	71.4	6	7	6	6	7	7	6	8	6	9	6	4	6	7	7
Total	58	25	33	43.1	45	45	49	46	48	49	48	56	46	51	48	44	47	46	46
		Total										714							

TAF, Total amplified fragment; PB, polymorphic bands; MP, monomorphic bands; P%, polymorphism %.

Table 4. Changes in DNA semi-RAPD profile in rat's testes with CCl4 induced toxicity and treated with fruit juices.

Primer	No. of bands in cont-	con+				appB2				appB4				grpB2				grpB4				pomB2				pomB4			
		a	b	c	d	a	b	c	d	a	b	c	d	a	b	c	d	a	b	c	d	a	b	c	d	a	b	c	d
3	3	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	2	0	0	0	1	0	0	0	1	0
4	3	1	0	1	0	1	0	1	0	0	0	1	0	1	2	0	0	1	0	0	0	0	0	0	1	1	0	0	0
5	12	0	0	1	2	1	1	0	1	1	1	0	0	2	2	0	0	3	3	0	0	1	1	0	1	3	0	0	0
6	10	0	1	5	1	2	0	9	0	2	0	10	0	2	0	10	0	2	0	10	0	2	0	10	0	2	0	10	0
7	7	0	1	0	4	0	0	1	0	0	0	3	0	0	0	3	0	0	0	1	0	0	0	0	0	0	0	0	0
11	4	1	1	0	0	1	0	0	0	0	1	0	0	2	1	0	0	1	1	0	0	2	1	0	0	4	1	0	0
12	6	1	0	0	0	0	0	0	0	0	0	2	0	1	0	0	1	1	0	0	0	0	0	1	0	2	0	0	0
Total	45	3	3	7	8	5	1	11	1	3	2	16	0	8	5	13	2	8	4	13	0	5	2	12	2	12	1	11	0
a+b		6				6				5				13				12				7				11			
GTS %		86.7				86.7				88.9				71.1				73.3				84.4				75.6			

Primer	No. of bands in cont-	con++				appL2				appL4				grpL2				grpL4				pomL2				pomL4			
		a	b	c	d	a	b	c	d	a	b	c	d	a	b	c	d	a	b	c	d	a	b	c	d	a	b	c	d
3	3	0	0	1	0	0	0	1	0	0	0	1	0	0	0	1	0	0	0	1	1	0	0	1	1	0	0	1	1
4	3	1	1	0	0	0	0	1	0	0	0	1	0	0	0	1	0	1	1	0	2	0	0	1	0	0	0	0	0
5	12	1	2	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	2	0	0	1	0	0
6	10	2	0	10	0	2	0	10	0	2	0	10	0	2	0	10	0	2	0	10	0	2	0	10	0	2	0	10	0
7	7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
11	4	1	1	0	0	1	1	0	0	2	1	0	0	0	1	1	0	1	1	1	0	0	1	1	0	0	1	1	0
12	6	1	1	0	3	3	0	4	0	0	0	0	0	0	2	2	0	0	0	0	3	1	0	2	0	1	0	2	0

Table 4. Contd.

Total	45	6	5	11	3	7	1	16	0	4	1	12	0	2	3	15	0	4	2	12	6	3	2	17	1	3	2	14	1		
a+b				11				8				5				5				6				5				5			
GTS %				75.6				82.2				88.9				88.9				86.7				88.9				88.9			

a: appearance of new band, b: disappearance of normal band, c: increase in band intensity, d: decrease in band intensity, a+b: polymorphic bands, GTS: genomic template stability.

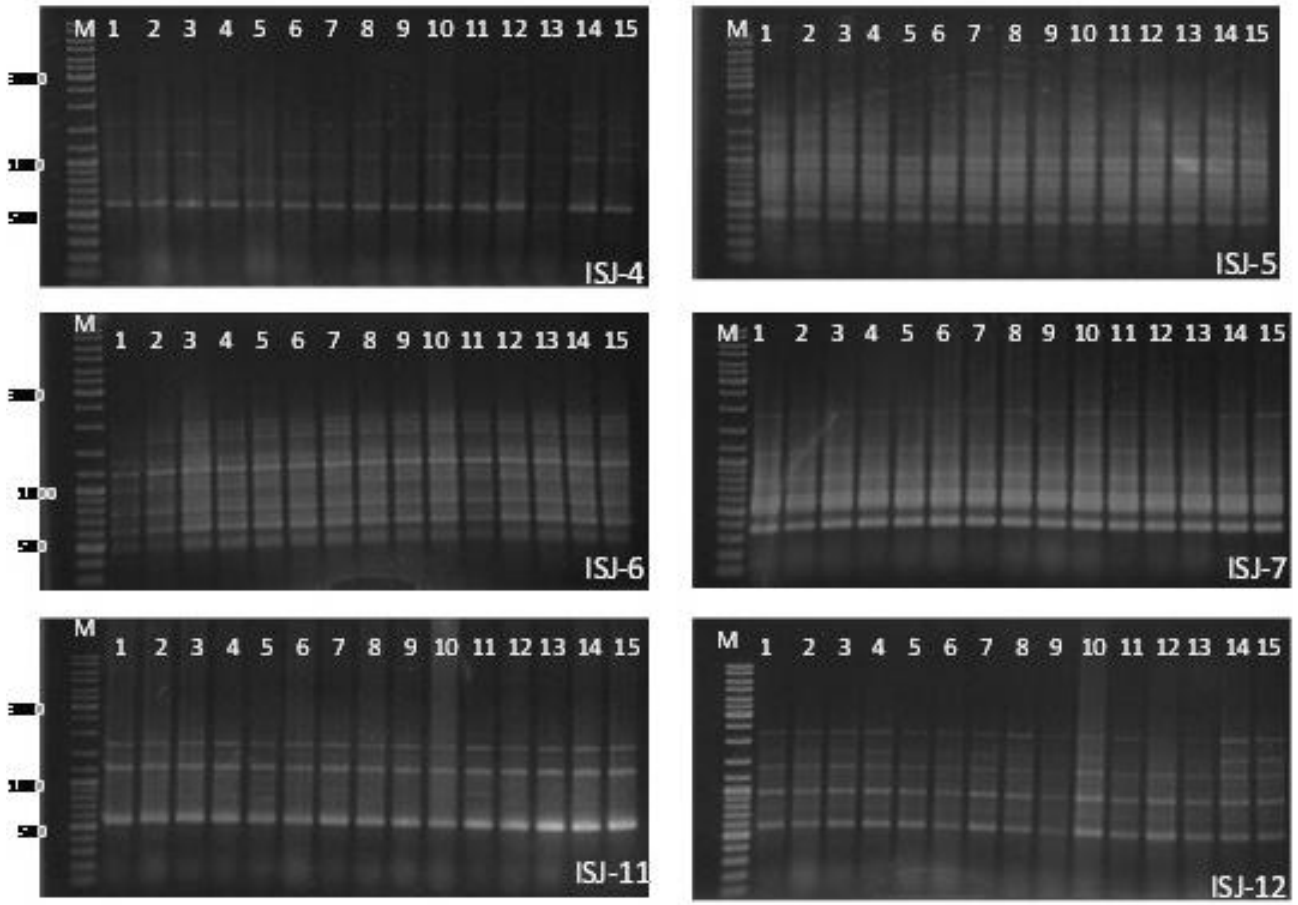

Figure 2. DNA-semi-random RAPD patterns generated by seven arbitrary primers (ISJ) with fifteen sample (1= con-; 2= con+; 3= appB2; 4= appB4; 5= grpB2; 6= grpB4; 7= pomB2; 8= pomB4; 9= con++; 10= appL2; 11= appL4; 12= grpL2; 13= grpL4; 14= pomL2; 15= pomL4; (M) refers to the DNA ladder.

low-fat diet and this indicated that low-fat diet had a positive effect against CCl_4 induced toxicity with fruit juice. Low-fat diet only with CCl_4 gave negative effects, and this agreed with Gomes et al. (2014), who refer to low-fat diet as one that can change the metabolic parameter and cause changes in hormonal milieu which in turn affects reproduction.

The increase of apple juice dose in both basal and low-fat diet increased GTS to 88.9 % in comparison with grape juice in basal diet and increment of dose in low-fat diet (Table 4). The results refer to negative effect by increasing the dose of grape juice, whereas, pomegranate juice gave positive effect than grape juice which may be attributed to its antioxidant potency in pomegranate juice than in grape juice. Pomegranate juice sugar-containing polyphenolic anthocyanins and polyphenols were considered to confer pomegranate juice the antioxidant capacity (Rozenberg et al., 2006).

With regard to pomegranate juice, this result agrees with Ebtesam et al. (2014). in the low-fat diet, it gave changes less than positive control and this didn't happen in basal diet, it was reported that the effects of *P. granatum* juice on lipid peroxidation, and nitric oxide contents in testes of rats treated with CCl4 were low in treatment about positive control and high about negative control, but this results disagree with basal diet. The protective effects of pomegranate on carbon tetrachloride mediated reproductive toxicity come from tannins (Amakura et al., 2000; Yehia et al., 2011), phenols (Lansky et al., 2005) and flavonoids (Van Elswijk et al., 2004). These components in pomegranate can directly or indirectly reduce oxidative damage by preventing the excessive generation of free radicals (Ebtesam et al., 2014).

These results agree with histological studies, where more effects have been observed in low-fat diet. This indicated that fruit juice has positive effect in low-fat diet against toxicity induced by CCl_4 in testes of rats.

Conflict of Interests

The authors have not declared any conflict of interests.

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