Investigation of the Genetic Toxicology of Dill and Fennel Extracts and Cyclophosphamide in Male Rats by RAPD-PCR Assay

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Abstract: Volatile compounds from the phenylpropanoid pathway represent an interesting class of extremely bioactive that have been found in a number of genera or families especially in the Lauraceae, Myrtaceae and Apiaceae families. Genotoxic properties of the essential oils extracted from dill (*Anethum graveolens* L.) and fennel (*Foeniculum vulgare* L.) seeds were studied using random amplified polymorphism DNA (RAPD) method in male rats *in vivo*. Sixty adult male albino rats were classified into 6 groups and treated orally daily for 30 days. RAPD analysis was performed on DNA extracted from liver of animals after treatments with single dose of 25 mg/kg b.w. of cyclophosphamide as a positive control, and fennel or dill extract using two doses, 0.3 and 0.6 mg/kg b.wt., respectively comparing with the negative control. However, random amplified polymorphism of DNA (RAPD) showed that Feeding of animals with low dose (0.3 mg/kg b.w) of dill and fennel extract did not cause any damage on the DNA. In addition, feeding of animals on dill at the high dose (0.6 mg/kg b.w) induced slightly DNA damage in the rat samples. On the other hand, most DNA of the samples treated with cyclophosphamide revealed polymorphic bands including appearance of new bands, which did not appear in the DNA samples of control or dill and fennel treated rats. These new bands could be considered as "genus diagnostic" markers which attributed to cyclophosphamide treatment.
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1. Introduction

During the past decade, there has been great awareness of the antithrombotic potential of food extranutritional constituents. Indeed, several reviews have summarized the protective effects of secondary metabolites from plant foods against the serious health risks due to thromboembolic diseases, like coronary thrombosis, atherosclerosis and stroke, and numerous experimental studies have been carried out both *in vivo* and *in vitro* (Wang and Ng, 1999; Etherton *et al*., 2002). As a consequence, a number of phytochemicals have been isolated and secondary metabolites proposed as responsible, to some degree, for antithrombotic or antiplatelet action: plant-derived heparins, cathechins, ginkgolids, flavonoids, stilbenes, tocotrienols, statins, thiosulfinates, phenylpropanoids and phenolic compounds (Etherton *et al.*, 2002; Basila and Yuan, 2005; Tognolini *et al*., 2006). Such momentum is further enhanced by the growing interest of the consumers toward functional ingredients from natural sources and also by the increasing concerns toward adverse side effects, caused by synthesized drugs most commonly used to prevent thromboembolic diseases. These effects range from gastric erosion (aspirin) and agranulocytosis (ticlopidine) to the poor separation between therapeutic and hemorrhagic doses (glycoprotein IIb/IIIa receptor inhibitors) (Van De Graaff and Steinhubl, 2001). Hence, the recognition of herbal antithrombotic remedies devoid of noxious side effects begins to be considered an important goal for the herbal and pharmaceutical industries.

Volatile compounds from the phenylpropanoid pathway represent an interesting class of extremely bioactive phytochemicals (Jiang and Dusting, 2003; Kurkin, 2003) that have been found in a number of genera or families especially in the Lauraceae, Myrtaceae and Apiaceae families. Among them, *Foeniculum vulgare* (L.) is perhaps the plant most widespread in use.

Fennel is a small, hardy perennial herb widely used as food and with an established role as herbal remedy. Both infusions and essential oils obtained from the fruits and the aerial parts of the plant, in fact, are included in the herbalist armamentarium for their relaxant, estrogenic, analgesic, anti-inflammatory properties (Boskabady *et al*., 2004; Modaress and Asadipour, 2006), antioxidant and antimicrobial activity (Miguel *et al*., 2010).

Green leafy vegetables are good source of minerals as well as vitamins.

Dill (*Anethum graveolens L.*) is a green leafy vegetable that belongs to the carrot family and has an attractive flavour. It has been used as a basic component in canning, soups and sauces and also flavouring salads and seafood (Kmiecik *et al*., 2004). Dill is a sparse looking plant with feathery leaves and tiny yellow flowers. Some pharmacological effects have been reported, such as antimicrobial (Delaquis *et al*., 2002; Singh *et al*., 2005; Arora and Kaur, 2007; Kaur and Arora, 2008, 2009), antihyperlipidaemic and antihypercholesterolaemic (Yazdanparast and Alavi., 2001 ; Yazdanparast and Bahramikia, 2008) , anticancer (Zheng *et al.*, 1992); anti-diabetic (Panda, 2008); antioxidant (Bahramikia and Yazdanparast, 2009; Sushruta and Dong, 2011);antispasmodic (Naseri and Heidari, 2007) and insecticidal (Chaubey, 2008 and Seo *et al.*, 2009) activities. As a folk remedy, dill is considered for some gastrointestinal ailments such as flatulence, indigestion, stomachache and colic (Duke, 2001 and Yazdanparast and Bahramikia, 2008). Dill fruit has an antispasmodic effect on the smooth muscles of the gastrointestinal tract (Fleming, 2000 and Kaur and Arora, 2010).

Many studies indicated the usefulness of randomly amplified polymorphic DNA (RAPD) analysis for assessing the genotoxic effects of many tested substances and/or environments, in zebra fish (Rong and Yin, 2004), mice (Noel and Rath, 2006), rats (El-Rahim *et al*., 2008) as well as in plants (Enan, 2006; Cenkci *et al*., 2009) mice and Drosophila (Ebeed *et al*., 2010). The aim of this study was to investigate the safety of

Fennel (*Foeniculum vulgare* L.) and dill (*A. Graveolens*) seed extracts using random amplified polymorphism DNA (RAPD) method which was evaluated in male rats.

2. Material and Methods

The preparation of extracts

The fennel and dill seed powder was extracted using maceration withethano (80% v/v) or water for 3 days and, subsequently, the mixture was filtered and concentrated under reduced pressure (by a rotaevaporator) at 40°C. The yield (w/w) of the aqueous and ethanolic extracts was 6. 46% and 8.5%, respectively.

Animals:

Sixty adult male albino rats (100 - 125 g, purchased from the Animal House Colony, University of king Abdulaziz , Saudi Arabia) were maintained on standard laboratory diet (protein, 16.04%; fat, 3.63%; fiber, 4.1%; and metabolic energy, 0.012 MJ) and water *ad libitum* at the Animal House Laboratory, University of king Abdulaziz , Saudi Arabia. After an acclimation period for 1 week. Animals were divided into six groups (10 rats/ group) and housed individually in filter-top polycarbonate cages housed in a temperature-controlled (23 ± 1^{o}C) and artificially illuminated (12 h dark/light cycle) room free from any source of chemical contamination. All animals received humane care in compliance with the guidelines of the Animal Care and Use Committee of University of king Abdulaziz , Saudi Arabia.

Experimental design

Animals within different treatment groups were treated daily (at a 24-h interval) intragastrically Per orally for 30 days as follows: group 1, untreated control; groups 2 and 3 treated with 0.3 and 0.6 mg/kg b.w. of dill, respectively; whereas, groups 4 and 5 treated with 0.3 and 0.6 mg/kg b.w. of fennel, respectively and group 6, treated with single dose of 25 mg/kg b.w. of cyclophosphamide at the 30th day of treatment. At the end of the experimental period, all animals were sacrificed and dissected on day 31 Liver samples were collected from all animals for DNA extraction.

Molecular analysis

The genomic DNA was isolated using phenol/chloroform extraction and ethanol precipitation (Sambrook *et al*., 1989). The purity of the DNA preparation was judged by examining the ratio of absorbency at 260 to 280 nm (Aquardo *et al*., 1992).

RAPD-PCR analysis

To generate RAPD profiles from rat DNA, oligodecamers (10-mer random primers) A, B, C and D kits from the Operon Technologies were used. DNA amplification reactions were performed under conditions reported by Williams *et al*. (1990) and Plotsky *et al*. (1995). PCR amplification was conducted in 25 µl reaction volume containing 100 ng genomic DNA, 100 µM dNTPs, 40 nM primer (Operon, Almeda, CA, USA), 2.5 units of Taq DNA polymerase and 5 µl promega 10X Taq DNA polymearse buffer. The reactions were carried out in Thermocycler (Perkin-Elmer 9700) programmed with a first denaturation of 5 min at 94°C, followed by 45 cycles of 1 min at 94°C, 1 min at 36°C and 2 min at 72°C and finally, one cycle at 72°C for 5 min. The PCR product was analyzed by electrophoresing 15 µl of the amplified mixture on agarose gel. The Gel-Pro Analyzer (Media Cybernetics) was used to document ethidium bromide DNA gels.

3. Results

RAPD fingerprinting assay

The molecular genetic variability among the treated rat genomes and their control were evaluated using 4 random primer kits (A, B, C and D). Only sixteen of these primers (10-mer random primers: A02, A03, A04, A06, A20, B14, C03, C05, C06, C07, C09, C12, C15, D01, D03 and D04, Table 1) gave positive and detectable bands (Figs. 1-4). They provided a total of 326 different bands with an average of 20.36±3.3 bands per primer (Table 2). Nearly the same results were obtained when the PCR assay was performed for each sample within each group (10 animals). Feeding of animals on dill and fennel at the low dose did not cause any damage on the DNA. Where, 199 bands (61.04%) were monomorphic for the control and dill as well as fennel treated animals (Figs. 1-4).

Table 1. Sequence of primers employed.

Primer	Sequence	Primer	Sequence
A02	5'-TGCCGAGCTG-3'	C06	5'-GAACGGACTC-3'
A03	5'-AGTCAGCCAC-3'	C07	5'-GTCCCGACGA-3'
A04	5'-AATCGGGCTG-3'	C09	5'-CTCACCGTCC-3
A06	5'-GGTCCCTGAC-3',	C12	5'-TGTCATCCCC-3'
A20	5'-GTTGCGATCC-3'	C15	5'-GACGGATCAG-3'
B14	5'-TCCGCTCTGG-3'	D01	5'-ACCGCGAAGG-3'
C03	5'-GGGGGTCTTT-3'	D03	5'-GTCGCCGTCA-3'
C05	5'-GATGACCGCC-3'	D04	5'-TCGACTCTGG-3'

However, of all the scorable bands, only two bands (0.61%) were polymorphic, because it was present in the groups treated with high dose of fennel (primer D03 at 373 bp; primer C09 at 687 bp, Table 2). In addition, feeding of animals on dill at the high dose induced slightly DNA damage in the rat samples. Where, 23 (7.1%) new bands were found in the treated rats on the high dose of dill

On the other hand, most DNA of the samples treated with cyclophosphamide revealed polymorphic bands including appearance on new bands, which did not appear in the DNA samples of control or dill and fennel treated rats (Figs.1-4). These new bands could be considered as “genus diagnostic” markers which attributed to cyclophosphamide treatment.

Table 2: Size in base pair of detected rat markers

	Control	D (0.3)	F (0.3)	D (0.6)	F (0.6)	Cyclopho. (0.6)		Control	D (0.3)	F (0.3)	D (0.6)	F (0.6)	Cyclopho. (0.6)		Control	D (0.3)	F (0.3)	D (0.6)	F (0.6)	Cyclopho. (0.6)		Control	D (0.3)	F (0.3)	D (0.6)	F (0.6)	Cyclopho. (0.6)
A02-1514						+	A03-1567						+	A04-1292						+	A06-1011				+		+
A02-1496						+	A03-1511						+	A04-1050						+	A06-942	+	+	+	+	+	
A02-1456						+	A03-1478				+		+	A04-1033				+		+	A06-921	+	+	+		+	+
A02-1025				+		+	A03-1260						+	A04-1028	+	+	+	+	+		A06-911	+	+	+	+	+	
A02-860	+	+	+	+	+		A03-1218				+		+	A04-1015	+	+	+		+		A06-896	+	+	+	+	+	+
A02-977	+	+	+		+	+	A03-1198	+	+	+	+	+		A04-1002	+	+	+	+	+		A06-839	+	+	+	+	+	
A02-860	+	+	+	+	+		A03-1160	+	+	+	+	+		A04-733	+	+	+		+		A06-816	+	+	+		+	
A02-756	+	+	+	+	+	+	A03-1123	+	+	+	+	+		A04-587						+	A06-605	+	+	+	+		
A02-652	+	+	+	+	+		A03-1023	+	+	+	+	+	+	A04-578						+	A06-598	+	+	+	+	+	
A02-645	+	+	+		+		A03-1003						+	A04-530				+			A06-538	+	+	+	+	+	
A02-623	+	+	+	+			A03-999						+	A04-466	+	+	+	+	+	+	A06-478	+	+	+		+	+
A02-578	+	+	+	+	+		A03-989	+	+	+	+	+		A04-456	+	+	+		+		A06-465	+	+	+		+	
A02-499	+	+	+	+	+		A03-978	+	+	+	+	+		A04-446	+	+	+		+	+	A06-455	+	+	+	+	+	+
A02-462	+	+	+		+	+	A03-957	+	+	+		+		A04-428	+	+	+	+	+		A06-333	+	+	+	+		
A02-453	+	+	+		+		A03-718	+	+	+	+	+	+	A04-368	+	+	+	+	+		A06-231				+		
A02-446	+	+	+	+	+	+	A03-704						+	A04-360					+		A06-224				+		
A02-405	+	+	+	+			A03-517						+	A04-324	+	+	+	+	+		A20-1004						+
A02-328				+			A03-380	+	+	+	+	+		A04-268	+	+	+	+	+		A20-987						+
A02-325				+			A03-298	+	+	+	+	+		A04-160	+	+	+	+	+		A20-840						+
A02-320						+	A03-296	+	+	+		+		A04-124						+	A20-766						+
A02-315						+	A03-284	+	+	+	+	+	+	A06-1102						+	A20-682						+
A02-306						+	A03-254	+	+	+		+	+	A06-1071						+	A20-655						+
A02-299						+	A03-150						+	A06-1050						+	A20-603				+		+

D: Dill; F: Fennel, +, Each marker was found in control and treated samples

Table 2: Continued

	Control	D (0.3)	F (0.3)	D (0.6)	F (0.6)	Cyclopho. (0.6)		Control	D (0.3)	F (0.3)	D (0.6)	F (0.6)	Cyclopho. (0.6)		Control	D (0.3)	F (0.3)	D (0.6)	F (0.6)	Cyclopho. (0.6)		Control	D (0.3)	F (0.3)	D (0.6)	F (0.6)	Cyclopho. (0.6)
A20-508	+	+	+	+	+		B14-380	+	+	+	+	+		C03-328	+	+	+	+	+	+	C05-704	+	+	+	+	+	
A20-499	+	+	+	+	+		B14-298	+	+	+		+		C03-280	+	+	+	+	+	+	C05-517	+	+	+	+	+	
A20-462	+	+	+	+	+		B14-296	+	+	+	+	+		C03-271	+	+	+	+		+	C05-380						+
A20-453	+	+	+	+	+	+	B14-284	+	+	+		+		C03-136	+	+	+	+	+	+	C05-298						+
A20-436						+	B14-254						+	C03-105	+	+	+	+	+	+	C05-296	+	+	+	+	+	+
A20-405						+	**C03-1697**	+	+	+	+	+		C03-89	+	+	+	+	+	+	C05-284	+	+	+	+	+	+
A20-328	+	+	+	+	+		C03-1682	+	+	+		+		C03-76	+	+	+	+		+	C05-254	+	+	+	+	+	
A20-280	+	+	+	+	+		C03-1557						+	C03-57	+	+	+	+		+	C05-196	+	+	+	+	+	
A20-271	+	+	+		+		C03-1367						+	**C05-1647**						+	C05-124	+	+	+	+	+	+
B14-1860						+	C03-1159				+			C05-1608				+			C05-97	+	+	+	+	+	+
B14-1818						+	C03-1004	+	+	+	+	+	+	C05-1498	+	+	+	+	+	+	**C6-1592**						+
B14-1698				+		+	C03-987	+	+	+		+		C05-1360	+	+	+		+		C6-1571						+
B14-1660	+	+	+	+	+		C03-840	+	+	+		+	+	C05-1318	+	+	+		+	+	C6-1459						+
B14-1623	+	+	+		+		C03-766	+	+	+	+	+		C05-1298	+	+	+	+	+		C6-1319						+
B14-1323	+	+	+	+	+		C03-682	+	+	+	+	+		C05-1260	+	+	+	+	+		C6-1134	+	+	+	+	+	
B14-1293	+	+	+		+		C03-655					+		C05-1223					+		C6-1106	+	+	+	+	+	
B14-1154						+	C03-603	+	+	+	+	+		C05-1220	+	+	+	+	+		C6-929	+	+	+	+	+	+
B14-1128						+	C03-508	+	+	+	+	+		C05-1193	+	+	+	+	+		C6-910	+	+	+	+	+	
B14-1103	+	+	+	+	+		C03-499	+	+	+	+	+		C05-1154	+	+	+	+	+		C6-891	+	+	+	+		+
B14-839	+	+	+		+		C03-462	+	+	+	+	+		C05-1128						+	C6-837	+	+	+	+		+
B14-718						+	C03-453	+	+	+	+	+		C05-1103						+	C6-696				+		+
B14-704						+	C03-436	+	+	+	+	+		C05-839	+	+	+	+	+		C6-655	+	+	+	+	+	
B14-517				+		+	C03-405	+	+	+	+	+		C05-718	+	+	+	+	+		C6-641	+	+	+		+	

D: Dill; F: Fennel, +, Each marker was found in control and treated samples.

Table 2: Continued

	Control	D (0.3)	F (0.3)	D (0.6)	F (0.6)	Cyclopho. (0.6)		Control	D (0.3)	F (0.3)	D (0.6)	F (0.6)	Cyclopho. (0.6)		Control	D (0.3)	F (0.3)	D (0.6)	F (0.6)	Cyclopho. (0.6)		Control	D (0.3)	F (0.3)	D (0.6)	F (0.6)	Cyclopho. (0.6)
C6-628	+	+	+	+	+		C07-592						+	C9-428	+	+	+	+	+	+	**C15-1560**						+
C6-570	+	+	+	+		+	C07-571						+	C9-368	+	+	+	+	+	+	C15-1518						+
C6-405	+	+	+	+	+		C07-495				+		+	C9-360	+	+	+	+	+		C15-1498						+
C6-347	+	+	+	+	+		C07-339	+	+	+	+	+		C9-324	+	+	+	+	+		C15-1460				+		+
C6-341	+	+				+	C07-333	+	+	+	+	+		**C12-1223**						+	C15-1423	+	+	+	+	+	
C6-322	+	+	+	+		+	C07-327	+	+	+	+	+		C12-1087						+	C15-1323	+	+	+		+	+
C6-246	+	+	+	+	+		C07-321	+	+	+	+	+	+	C12-990				+		+	C15-1293	+	+	+	+	+	
C6-242	+	+	+	+	+		C07-233	+	+	+	+	+	+	C12-987	+	+	+	+	+		C15-1154	+	+	+	+	+	+
C6-238	+	+	+	+			C07-127	+	+	+	+	+	+	C12-840	+	+	+	+	+		C15-1128	+	+	+	+	+	
C6-83	+	+	+	+			C07-111	+	+	+		+		C12-766	+	+	+	+	+		C15-1103	+	+	+		+	
C07-1512						+	**C9-1423**						+	C12-682	+	+	+	+	+	+	C15-839	+	+	+	+		
C07-1279	+	+	+	+	+		C9-1319				+			C12-655	+	+	+	+	+	+	C15-718	+	+	+	+	+	
C07-1146	+	+	+	+	+		C9-1193	+	+	+	+	+	+	C12-603						+	C15-704	+	+	+	+	+	
C07-1123	+	+	+		+	+	C9-1078	+	+	+		+		C12-508	+	+	+	+	+		C15-517	+	+	+		+	+
C07-1078	+	+	+		+		C9-1055	+	+	+		+	+	C12-499	+	+	+	+	+	+	C15-380	+	+	+		+	
C07-1059	+	+	+	+	+	+	C9-1012	+	+	+	+	+		C12-462	+	+	+	+	+	+	C15-298	+	+	+		+	
C07-953	+	+	+	+			C9-733	+	+	+	+	+		C12-453						+	C15-296	+	+	+		+	
C07-936				+			C9-687					+		C12-436	+	+	+	+	+		C15-284	+	+	+	+	+	+
C07-919				+			C9-578	+	+	+	+	+		C12-405	+	+	+	+	+		C15-254	+	+	+	+		
C07-795						+	C9-530	+	+	+	+	+		C12-401	+	+	+		+	+	**D01-1279**						+
C07-781						+	C9-466	+	+	+	+	+		C12-328	+	+	+		+		D01-1246						+
C07-752						+	C9-456						+	C12-280	+	+	+	+	+	+	D01-1123						+
C07-603						+	C9-446						+	C12-271	+	+	+	+			D01-1078						+

D: Dill; F: Fennel, +, Each marker was found in control and treated samples.

Table 2: **Continued**

	Control	D (0.3)	F (0.3)	D (0.6)	F (0.6)	Cyclopho. (0.6)		Control	D (0.3)	F (0.3)	D (0.6)	F (0.6)	Cyclopho. (0.6)		Control	D (0.3)	F (0.3)	D (0.6)	F (0.6)	Cyclopho. (0.6)
D01-1059	+	+	+	+			D03-652	+	+	+	+	+		D04-491						+
D01-953	+	+	+	+			D03-627	+	+	+	+	+		D04-467	+	+	+	+	+	
D01-936	+	+	+	+	+		D03-491	+	+	+	+	+		D04-380	+	+	+	+	+	
D01-919	+	+	+	+	+	+	D03-467	+	+	+	+	+		D04-303	+	+	+	+	+	
D01-795	+	+	+	+	+		D03-380	+	+	+	+	+								
D01-781						+	D03-373					+								
D01-752						+	D03-301						+							
D01-603	+	+	+	+	+		D03-299	+	+	+	+	+								
D01-592	+	+	+	+			D03-298	+	+	+	+	+								
D01-571	+	+	+	+	+	+	D03-279	+	+	+	+	+								
D01-495	+	+	+	+	+		**D04-1655**			+			+							
D01-339	+	+	+	+	+		D04-1574						+							
D01-333						+	D04-1423				+		+							
D01-327						+	D04-990				+		+							
D01-321	+	+	+	+	+		D04-970	+	+	+	+	+								
D03-1329						+	D04-929						+							
D03-1103						+	D04-910	+	+	+	+	+								
D03-929						+	D04-891	+	+	+	+	+	+							
D03-910						+	D04-659	+	+	+	+	+								
D03-891						+	D04-652	+	+	+	+	+								
D03-664	+	+	+	+	+		D04-627						+							

D: Dill; F: Fennel, +, Each marker was found in control and treated samples

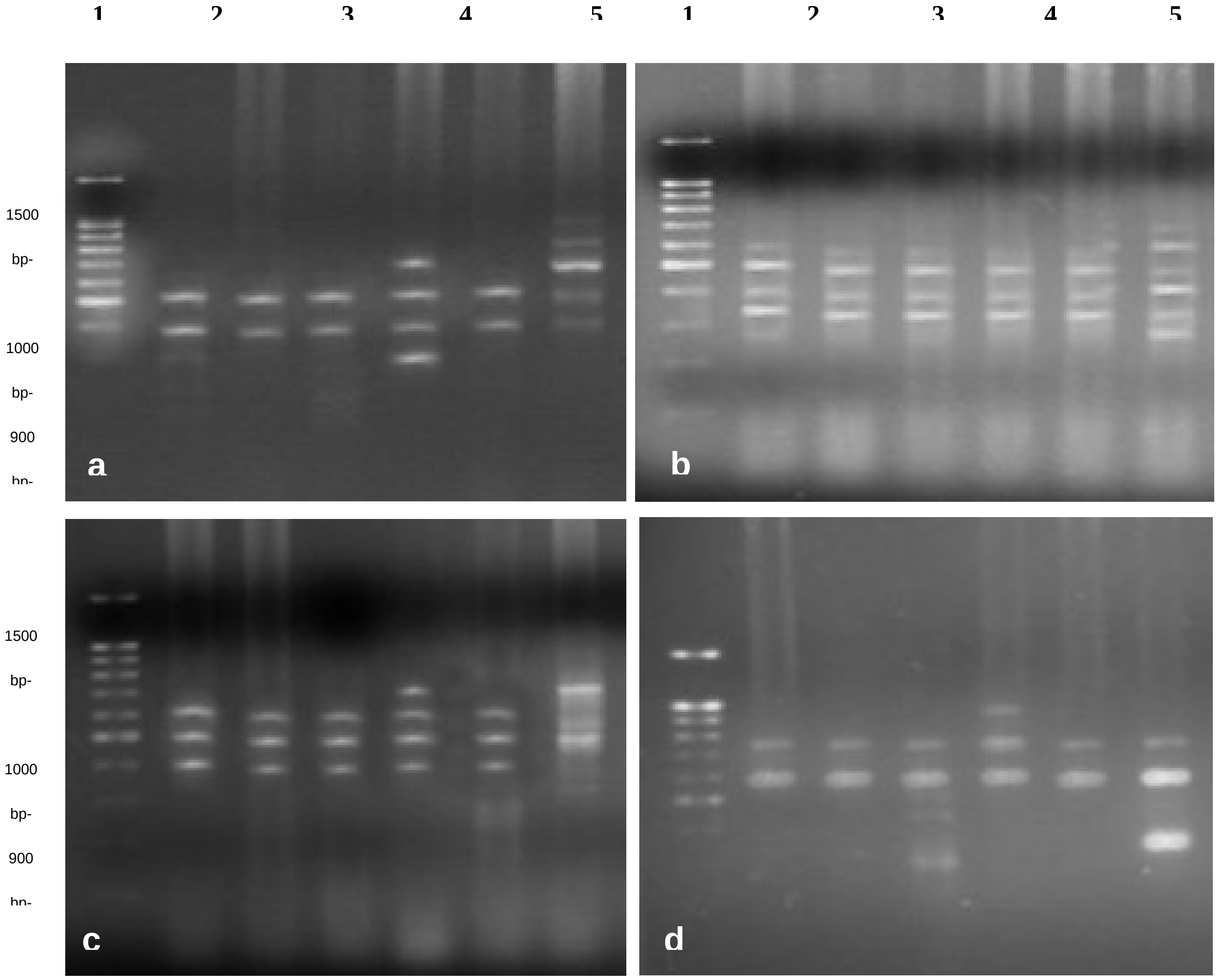

Figure 1: Comparison of RAPD fingerprinting profiles of different male rat genomic DNA treated with dill and fennel for 30 days. a) Represents PCR products with primer A06; b) represents PCR products with primer A20; c) represents PCR products with primer B14; and d) represents PCR products with primer C06. The DNA marker was in lane 1. Lane 2 represents PCR products of untreated control samples; lane 3 represents rats treated with 0.3 mg/kg b.w. of dill; lane 4 represents rats treated with 0.3 mg/kg b.w. of fennel; lane 5 represents rats treated with 0.6 mg/kg b.w. of dill; lane 6 represents rats treated with 6 mg/kg b.w. of fennel; lanes 7 represents rats treated with single dose of 25 mg/kg b.w. of

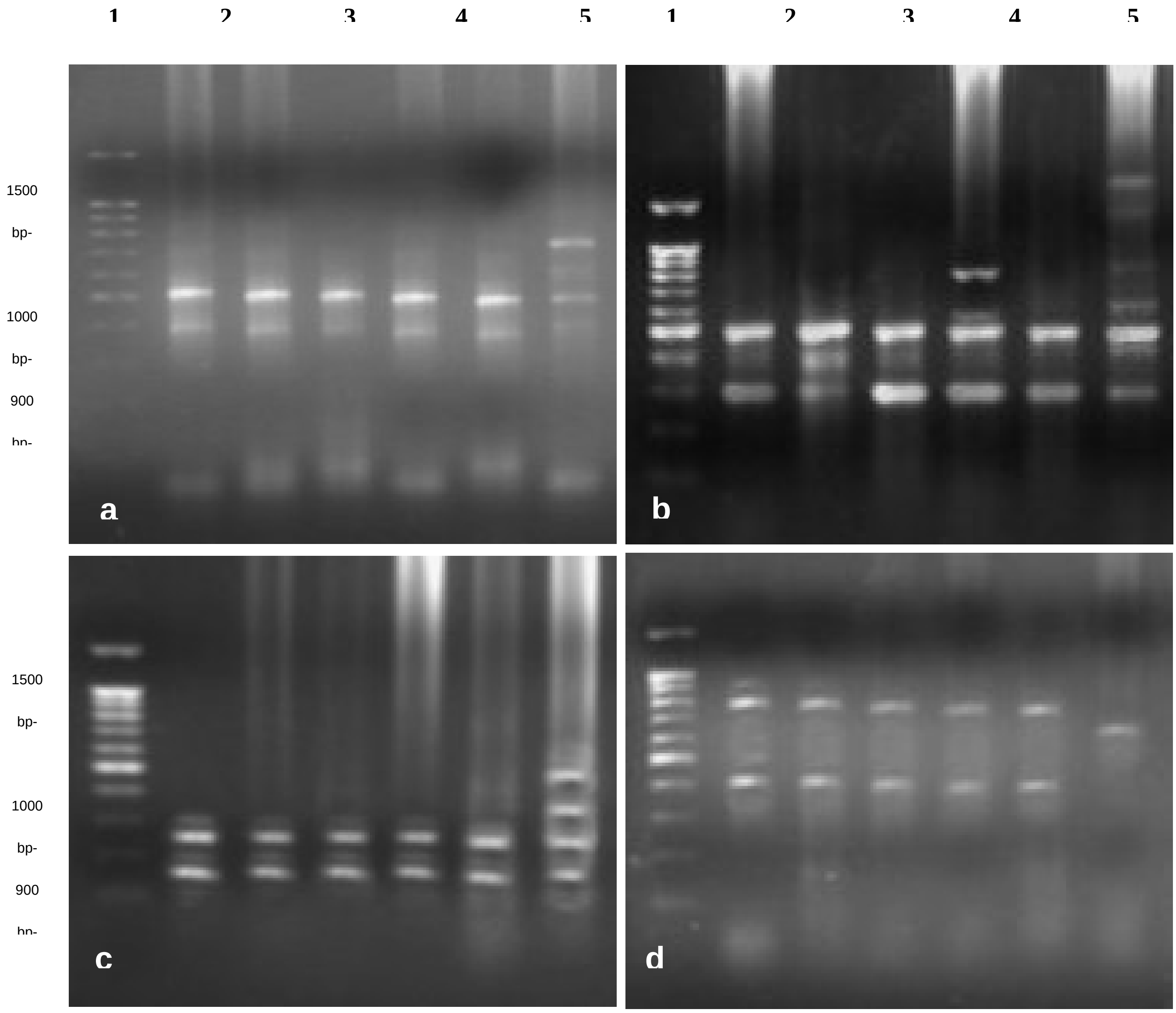

Figure 2: Comparison of RAPD fingerprinting profiles of different male rat genomic DNA treated with dill and fennel for 30 days. a) Represents PCR products with primer C09; b) represents PCR products with primer D01; c) represents PCR products with primer D03; and d) represents PCR products with primer D04. The DNA marker was in lane 1. Lane 2 represents PCR products of untreated control samples; lane 3 represents rats treated with 0.3 mg/kg b.w. of dill; lane 4 represents rats treated with 0.3 mg/kg b.w. of fennel; lane 5 represents rats treated with 0.6 mg/kg b.w. of dill; lane 6 represents rats treated with 0.6 mg/kg b.w. of fennel; lanes 7 represents rats treated with single dose of 25 mg/kg b.w. of

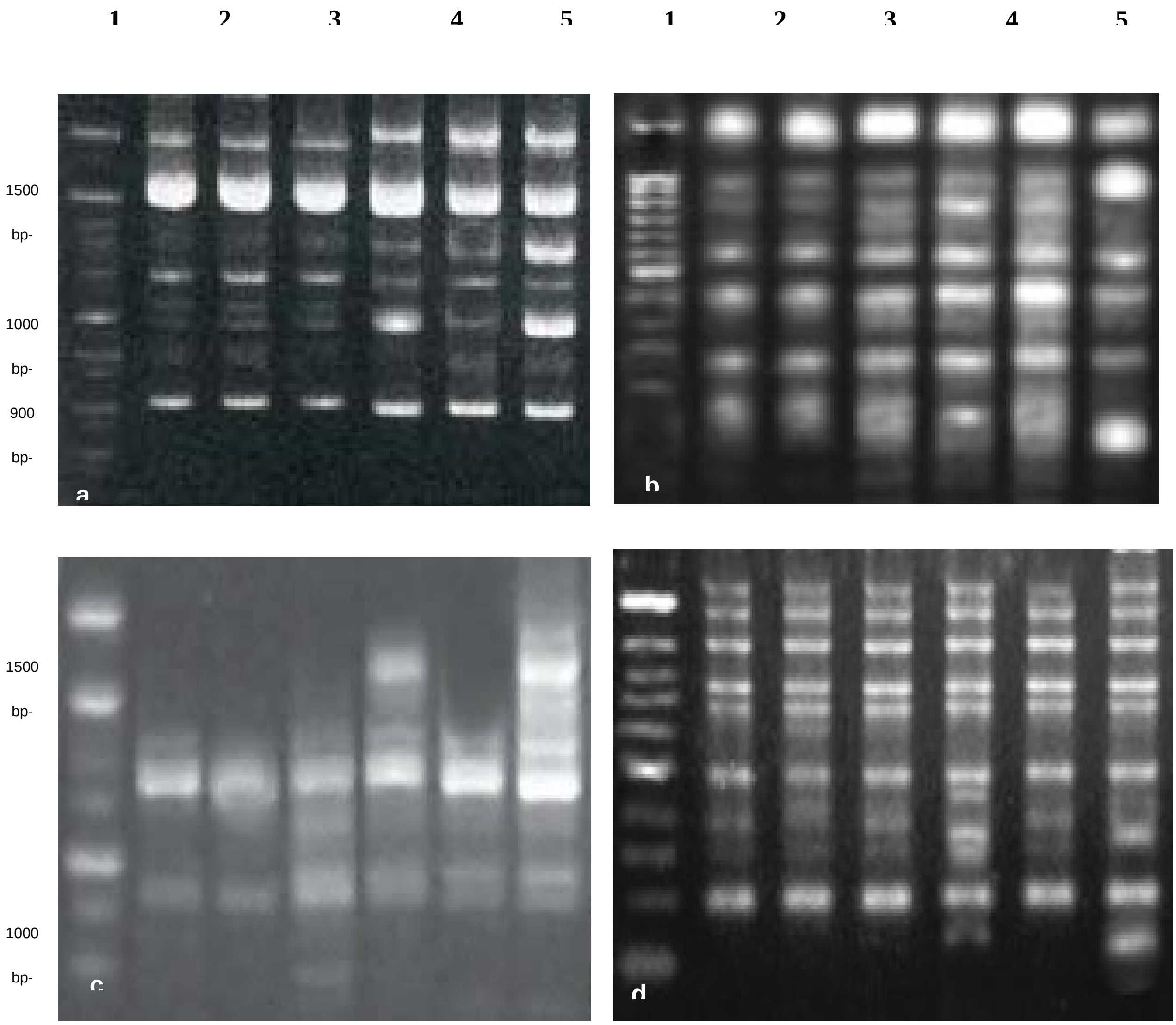

Figure 3: Comparison of RAPD fingerprinting profiles of different male rat genomic DNA treated with dill and fennel for 30 days. a) Represents PCR products with primer A02; b) represents PCR products with primer A03; c) represents PCR products with primer A04; and d) represents PCR products with primer C03. The DNA marker was in lane 1. Lane 2 represents PCR products of untreated control samples; lane 3 represents rats treated with 0.3 mg/kg b.w. of dill; lane 4 represents rats treated with 0.3 mg/kg b.w. of fennel; lane 5 represents rats treated with 0.6 mg/kg b.w. of dill; lane 6 represents rats treated with 0.6 mg/kg b.w. of fennel; lanes 7 represents rats treated with single dose of 25 mg/kg b.w. of

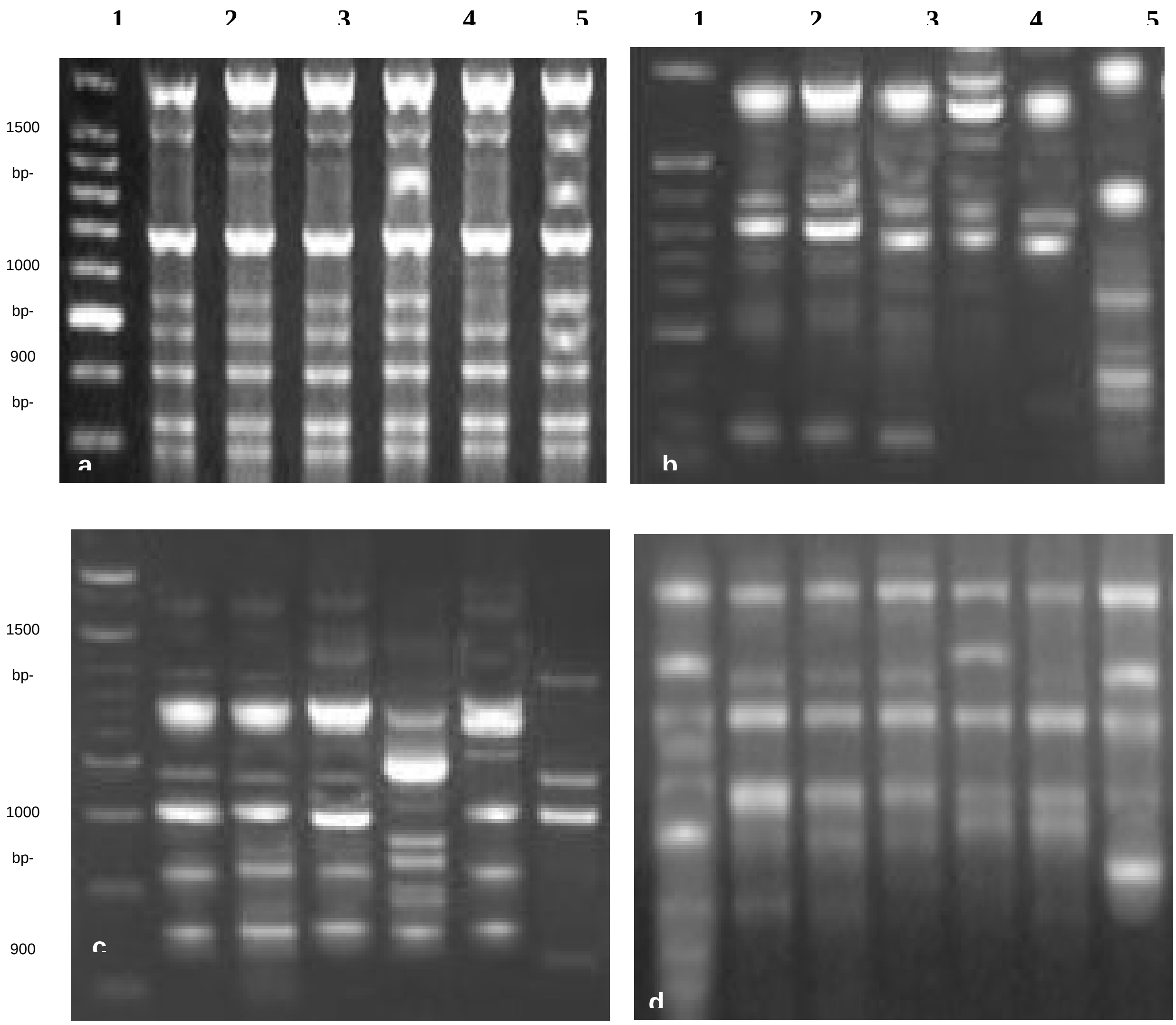

Figure 4: Comparison of RAPD fingerprinting profiles of different male rat genomic DNA treated with dill and fennel for 30 days. a) Represents PCR products with primer C05; b) represents PCR products with primer C07; c) represents PCR products with primer C12; and d) represents PCR products with primer C15. The DNA marker was in lane 1. Lane 2 represents PCR products of untreated control samples; lane 3 represents rats treated with 0.3 mg/kg b.w. of dill; lane 4 represents rats treated with 0.3 mg/kg b.w. of fennel; lane 5 represents rats treated with 0.6 mg/kg b.w. of dill; lane 6 represents rats treated with 0.6 mg/kg b.w. of fennel; lanes 7 represents rats treated with single dose of 25 mg/kg b.w. of cyclophosphamide.

4. Discussion

Essential extracts of plants are widely used as flavouring additives of food beverages, scenting agents of variety of household products and as constituents of some drugs. Investigation results of essential plant extracts are rather contradictory. Some reports indicated that essential oil of various plants may be genotoxic *in vitro a*s well as *in vivo* (Lazutka *et al*., 2001).Essential oils extracted from dill induced chromosome aberrations and sister chromatid exchanges in human lymphocytes *in vitro* as well as gene mutations in *Drosophila melanogaster* somatic cells *in vivo* (Mierauskiene *et al*., 2000). In agreement with these investigations, Results have also indicated that feeding of animals on standard laboratory diet mixed with dill at the high dose induced slightly DNA damage in the rat samples.

According to other authors, essential aromatic plant extracts from different plants such as *Mentha pulegium* L., *Origanum vulgare* subsp. *Hirtum Ietswaart*, *Coridothymus capitatus reichenb*. and *Satureja thymbra* L. were not mutagenic in *D. melanogaster* somatic mutation and recombination (SMART) test *in vivo* (Francioz *et al*., 1997; Karpouhtsis *et al*., 1998). Aromatic sagebrush (*Artimisia dracunculus* L.) essential oil was not genotoxic in *Salmonella*-microsomes reversion assay (Zani *et al*., 1991). In the present study, feeding of male rats on fennel at the low and high doses did not cause any damage in the DNA. Furthermore, Morkunas (2002) found that dill essential oil did not induce the formation of micronucleated polychromatic erythrocytes in the mouse bone marrow.

As mentioned above, results of essential plant extracts genotoxicty investigations are rather contradictory. It is even more interesting that essential oil extracted from different parts of the same plant might show different genotoxicity. For example, genotoxic properties than essential oil from dill seeds, which was almost inactive in *D. melanogaster* SMART test (Lazutka *et al*., 2001).

Essential extracts from dill and fennel seeds in the present investigation were not active at the low concentration in male rats to induce the DNA damage. Furthermore, the high dose of fennel cauld not induce any damage in the liver tissue of male rats. In addition, the damage in the DNA due to use high dose of dill was not high. This phenomenon probably could be explained by a different concentration of individual components in the essential extracts from different parts of plant. Furthermore, a seasonal variation in the chemicals composition of essential extracts of aromatic plants was indicated (Muller-Riebau *et al*., 1997). Thus, the genotoxic properties of essential extracts of the same plant may vary during seasons of the year.

Morkunas (2002) reported that dill extract was able to inhibit the mutagenicity of benzo(a) pyrene in mouse bone marrow. This antimutagenic effect of dill extract and other plants such as fennel *in vivo* probably could be caused by some of its compounds, for example, ß-myrcene. According to some reports, ß-myrcene, terpinol, mentholand and some other compounds of essential extracts are able to inhibit mono-oxygenases **responsible** for activation of polycyclic aromatic hydrocarbons pro-mutagenes (Morkunas, 2002).

The preservative effect of herbs suggests the presence of anti-genotoxic constituents in their tissues (Ebeed *et al.,* 2010). They indicated that fennel extract may have slight genotoxic effects on mice rather than Drosophila. In addition, the biochemical, chromosomal aberrations in mice bone marrow as well as aneuploidy and chromosomal aberration test in Drosophila male germ-lines confirmed the antimutagenic effects of fennel extract against MMC and colchicine induced mutations. However, the pre and post treatment analysis revealed that hot water crude extract of fennel may contain some compounds that can act as dis-antimutagen and some compounds can act as bio-antimutagen. The molecular studies using RAPD indicated the effect of fennel extract to induce DNA changes as confirmed by biochemical assays.

Thus, the results of the present investigation showed that essential dill and fennel extracts were not genotoxic for the rat genomic *in vivo*. These effects of dill and fennel can be attributed to their individual components which may have the ability to inhibit the enzymes responsible for any damage in the DNA.

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