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Full Length Research Paper

Antioxidant effect of celery against carbontetrachloride induced hepatic damage in rats

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Ethanolic extract of celery (*Apium graveolens*) and its diet were evaluated for antioxidant and hepatoprotective activities in rats. Celery is valuable in weight loss diets and regulate lipid metabolism. Albino male rats were used to evaluate its antioxidant and hepatoprotective activities against carbon tetrachloride induced toxicity. Chromosomal aberration, sperm abnormalities, biochemical and molecular assay were used to evaluate its antioxidant activity. Liver damage was assessed by estimating biochemical parameters such as total soluble protein, DNA and RNA contents. Celery showed significant antioxidant effect by reducing chromosomal aberration, sperm abnormalities and increasing DNA bands number and pattern as the control. It also showed a significant hepatorotective effect by readjusting the toxic effect of CCl_4 on the total soluble protein, DNA and RNA contents around that of the normal.

Key words: Celery (*Apium graveolens*), albino male rats, carbontetrachloride, DNA and RNA contents and biochemical parameters.

INTRODUCTION

The liver is a vital organ which regulates many important metabolic functions and it is responsible for maintaining homeostasis of the body. It is involved with all the biochemical pathways related to growth, fight against disease, nutrient supply, energy provision and reproduction (Ward and Daly, 1999). Liver is the major organ responsible for the metabolism of drugs and toxic chemicals; and thus, it is the primary target organ for nearly all toxic chemicals. Various pharmacological or chemical substances (such as acetaminophen, galactosamine, chloroform, dimethylnitrosamine, etc.) are known to cause hepatic injuries. Excessive exposure to chemicals may cause acute liver injury charachterized by abnormality of hepatic function, degeneration, necrosis or apoptosis of hepatocytes (Pang et al., 1992). In modern civilization drugs or chemicals induced liver injury has become a serious clinical problem. CCl_4-induced liver injury is one of the well established systems for xenobiotic-induced hepatotoxicity and is a commonly used model for screening of the anti-hepatotoxic and/or hepatoprotective activities of the drugs. Jundice and hepatitis are two major hepatic disorders that account for a high death rate (Pang et al., 1992).

Plant products play a beneficial role in the management of various liver disorders. In the absence of a reliable liver protective drug in the modern medicine, a number of medicinal preparations in ayurveda are recommended for the treatment of liver disorders. Celery (*Apium graveolens*, Family *Apiaceae*) is an excellent source of vitamin C. It is a very good source of dietary fiber, potassium, folate, manganese and vitamin B1, B2 and B6. It is also a good source of vitamin A, calcium, phosphorus and iron (Mitra et al., 2001). Aqueous extract of celery caused significant reduction in serum total cholesterol level in hypercholesterolemic rats (Tsi and Tan, 2000). Recently, medicinal plants have been paid more attention and used as herbs in most parts of the world due to their physiological functions (Geng et al., 2005).

The present study aims to evaluate the hepatoprotective and antioxidant activity of celery ethanolic extract and its diet against CCl_4-induced hepatotoxicity and hepatotoxicity and genotoxicity in rats. The study was designed to investigate its effects on

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Table 1. ISSR primer codes and sequences used for ISSR-PCR and their annealing temperature.

Primer set	Primer code	Primer sequence	Annealing temp.(°C)
1	HB 8	5'(GA) 6 GG 3'	48
2	HB 10	5' (GA) 6 CC 3'	48
3	HB 11	5' (GT) 6 CC 3'	48
4	HB12	5' (CAC)3 GC 3'	48
5	HB14	5' (CTC)3GC 3'	52

Table 2. RAPD primer codes and sequences.

Primer set	Primer code	Primer sequence
1	A 9	5'GGGTAACGCC 3'
2	A 12	5'TCGGCGATAG 3'
3	A 16	5'AGCCAGCGAA 3'
4	A 18	5'AGGTGACCGT 3'
5	B 11	5'GTAGACCCGT 3'

sperm abnormalities chromosomal aberration, molecular assay and biochemical analysis.

MATERIALS AND METHODS

Celery collection and extraction

Celery was purchased from the local market, cut and dried in an air oven at 50°C till complete dryness, and weighted to calculate its moisture content. Then the dried celery was ground to fine powder for analysis and was used in the experiment. The dried celery powder was mixed with ethanol (80%) for 2 days. The resulting ethanolic extract was filtered and subsequently concentrated with a rotary evaporator.

Chemicals

Carbon tetrachloride (CCl_4)

Carbon tetrachloride (CCl_4) is one of the most commonly used hepatotoxins in the experimental studies of liver diseases. CCl_4 induced hepatotoxicity as judged from serum marker enzymes and antioxidant levels in liver tissues (Palanivel et al., 2008).

Silymarin

The standard hepatoprotective silymarin, a plant extract with strong antioxidant activity against hepatotoxicity induced by CCl_4.

Celery plant

Celery diet and ethanolic extract used as hepatoprotective and antioxidant against CCl_4 induced hepatic damage.

Experimental animals

Male albino rats of Sprague-Dawley strain weighting 80-100 g were used for the experiment. The animals were obtained from the animal house, National Research Center and were housed in well ventilated hygienic experimental animal house under constant environmental and nutritional conditions. All rats were kept in cages and administered food and water *ad libitum*.

Experimental protocol

Carbon tetrachloride induced hepatotoxicity. Animals were divided into five groups of fifteen animals each. Group I served as normal control and received corn oil (1.0 mg/kg) twice a week and was fed on normal diet. Group II was administered orally with CCl_4 (10% CCl_4 / corn oil, 1.0 mg/kg) twice a week. Group III was treated with CCl_4 and received the standard drug Silymarin at a dose of (0.2 g/kg) four times a week. Group IV was treated with CCl_4 and fed on celery diet (5%). Group V was treated with CCl_4, fed on normal diet and received celery extract (0.3 g/kg) four times a week. The treatment was carried out for a period of 60 days.

Assay of sperm abnormalities

The sperm shape morphology assay is used as a short-term bioassay to investigate the mutagenic effects of agents responsible for significant human exposures (Wyrobek et al., 1983). Details of the test procedure and slid preparation are described by Wyrobek et al. (1983) and Bakare et al. (2005). At least 4000 sperms per group were assessed for morphological abnormalities (head and tail).

Chromosomal aberration assay

Animals were sacrificed 24 h. after the last treatment and chromosome smears of bone marrow cells were prepared according to Yosida and Amano (1965). In order to obtain the frequencies of chromosomal aberrations in bone marrow cells, at least 50 metaphases from each animal were examined for a total of 300 cells per each treatment and control.

Molecular assay

Genomic DNA extraction: DNA was extracted from the tested animals according to the method described by Sharma et al. (2000). The concentration of DNA and its relative purity were determined using a spectrophotometer based on absorbance at 260 and 280 nm, respectively. The integrity of extracted genomic DNA was checked by electrophoresis in 0.8% agarose gels using DNA molecular weight marker (Eurobio, Paris, France).

PCR conditions: Inter Simple Sequence Repeat (ISSR) analysis was performed using five different primers listed in Table 1. The RAPD primers codes and sequences were listed in Table 2.

PCR for both analyses (RAPD and ISSR) was performed in 25 µl volume containing 2.5 mM $MgCl_2$, 0.2 mM dNTPs, 20 µM primer, 50 ng genomic DNA and 1 unit Taq DNA polymerase (Bioron, Germany). All reactions were performed in a Perkin Elmer 2400 thermal cycler.

ISSR program was performed as 1 cycle of 94°C for 4 min and 35 cycles of 94°C for 30 s, the annealing temperature for each primer 72°C for 1.5 min. Then, a final extension step of 72°C for 10 min was done. For each primer, the annealing temprature was chosen after different trials with different temperatures (Table 1). RAPD

Program was performed as 1 cycle of 94°C for 4 min and 40 cycles of 94°C for 1 min, 35°C for 1 min, and 72°C for 2 min.

To visualize the PCR products, 15 µl of each reaction was loaded on 1.2% agarose gel. The gel was run at 90 V for 1 h and visualized with UV Transilluminator and photographed using UVP gel documentation system (GelWorks 1D advanced software, UVP).

For each amplification, a negative control reaction without DNA template was included. PCR reactions, those which generated high level of polymorphism across both types of analyses, were repeated twice in order to verify the reproducibility of polymorphic bands scored. This procedure allowed only those bands present in all replicated experiments to be scored as markers.

Biochemical assay

Determination of nucleic acid (DNA and RNA): total DNA and RNA contents were determined according to Pears (1985).

Statistical analysis

The one-way analysis of variance test (ANOVA) was applied to evaluate the distribution of abnormal spermatozoa and chromosomal aberrations. Differences between the negative control and the individual dosage group were analyzed by Duncan's test of significant at the P≤ 0.05 and P≤ 0.01 levels. The mean ± standard error was calculated.

Data of polymorphic and monomorphic bands for both analyses was scored using the UVP gel documentation system. Amplicon sizes were estimated using 100-bp and 1-kb DNA standards (Bioron, Germany).

RESULTS

Cytogenetic results

The results of celery plant against CCl_4 induced genotoxicity are shown in Tables 1 and 2. Administration of CCl_4 to rats caused severe damage as there was a high significant increase in abnormal sperm morphology and also chromosomal aberrations in rats. Rats treated with celery (diet or extract) exhibited a significant reduction in the percentage of abnormal sperms as well as in cells with chromosomal aberrations.

Analysis of sperm-shape abnormalities was made at the end of the experiment. Table 1 shows the effect of different treatments on rat sperm morphology. The normal control group showed 2.2% abnormalities, while the positive control group (CCl_4) induced statistically significant abnormal sperm morphology (20.7%). The different types of abnormal sperms observed were amorphous, hookless, banana, big and small heads coiled and divided tails. Rats treated with celery after treatment with CCl_4 exhibited a significant reduction in the percentage of abnormal sperms (13.13% diet, 8.2% extract).

The results of celery against CCl_4 induced chromosomal aberrations are shown in Table 2. Administration of CCl_4 to rats caused highly significant increase in chromosomal aberrations (P≤ 0.01). Celery administration, diet (5%) or extract at the dose of (0.3 g/kg) during CCl_4 treatment significantly lowered the cells with chromosomal aberrations and greatly improved their percentage near to the control, especially celery extract. The different types of chromosomal aberrations observed were gap, break, deletion, fragment and centromeric attenuations. Numerical aberration were also studied and recorded as polyploidy, hyperploidy and hypoploidy. Celery improved the percentage of numerical aberrations induced by CCl_4 significantly ($P < 0.05$). All these results indicated the antioxidant potential of celery, especially its extract.

Molecular results

ISSR analysis was performed on DNA extracted from liver of animals after treatment with carbon tetrachloride and celery (diet or extract) comparing with the normal control. Five anchor primers (HB8, HB10, HB11, HB 12 and HB 14) were used in the present study to analyze the genetic variation among the tested groups (Table 1). Data were considered for 5 primers (Figure 1). The molecular size of amplified bands ranged from 190 to 1650-bp. Each primer generated between 3 (primers HB12) and 10 (primer HB 10) bands.

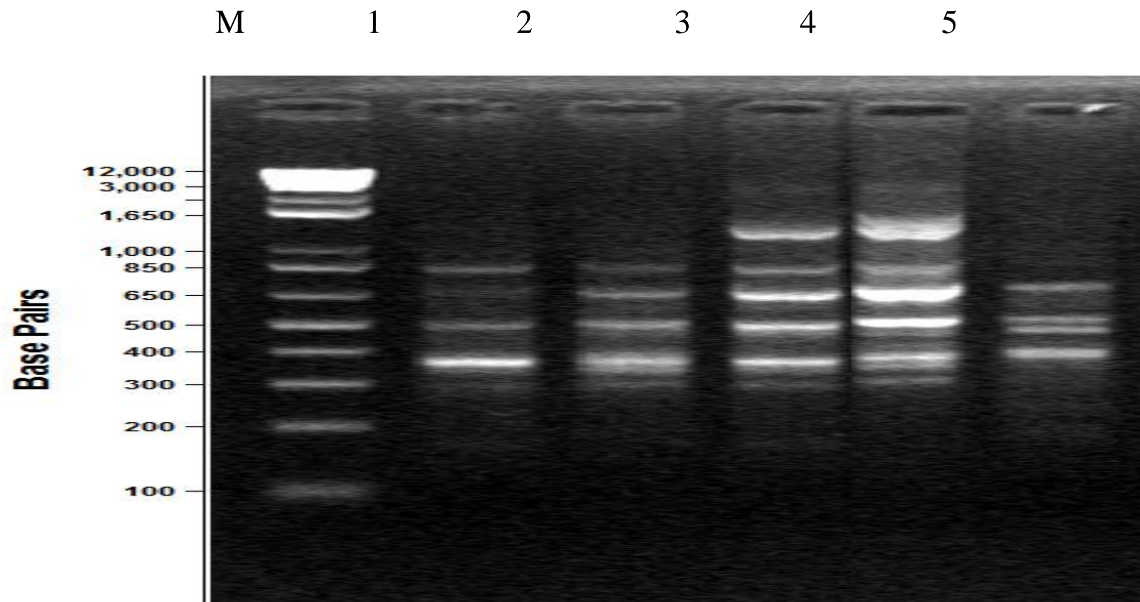
The results of ISSR analysis showed that primer HB8 produced 92% polymorphic bands while HB11 produced 67% polymorphic bands as shown in Table 5.

Animals treated with CCl_4 (group 2) showed the highest lost bands (total bands was 24 compared to 30 for control group), which could be a clear indication for the high genotoxic effect of CCl_4 due to losses of alleles compared with groups treated with silymarin and celery extract (groups 3, 5) (Table 6). Animals fed on celery diet after treatment with CCl_4 (group 4) showed an increase in bands number. The appearance of new fragments may attribute to some sites becoming accessible to the primer after point mutations and/or large rearrangements take place in genomic DNA. These results suggested that animals treated with silymarin and celery extract had less genotoxic effect compared with animals treated with CCl_4 or CCl_4 with celery diet. The obtained results of ISSR gave remarkable distinct characterization of each tested group.

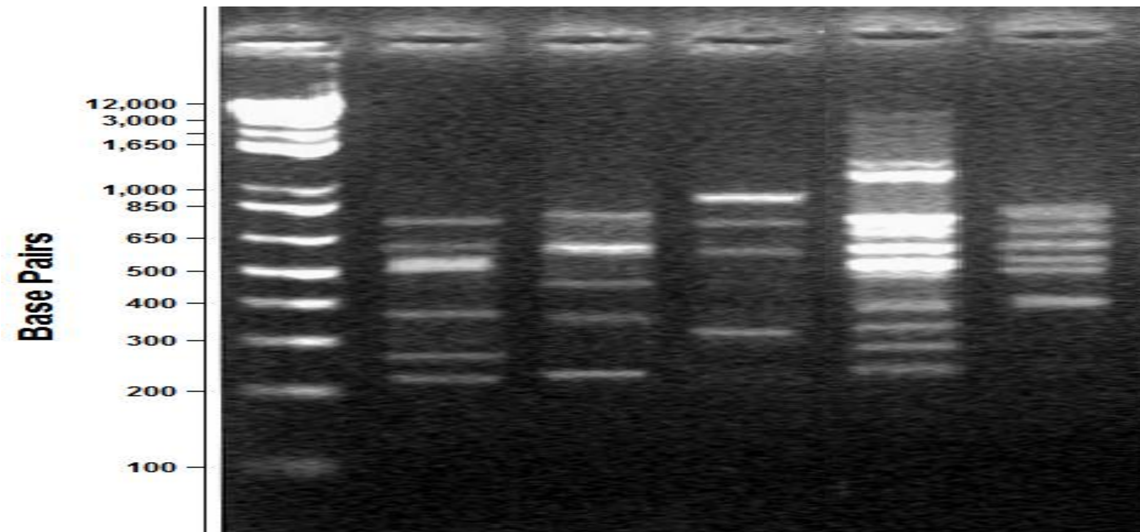
RAPD result

A high level of polymorphism was generated utilizing the five RAPD primers (Figure 2 and Table 2). A total number of 60 RAPD bands separated by electrophoresis on agaros gel across the experimental groups were obtained. The highest number of amplicons was generated from group 4 (33 amplicons) while group 2 generated the lowest (19 amplicons) (Table7). The molecular size of amplified bands ranged from 200 to 1000-bp. The obtained results affected DNA banding

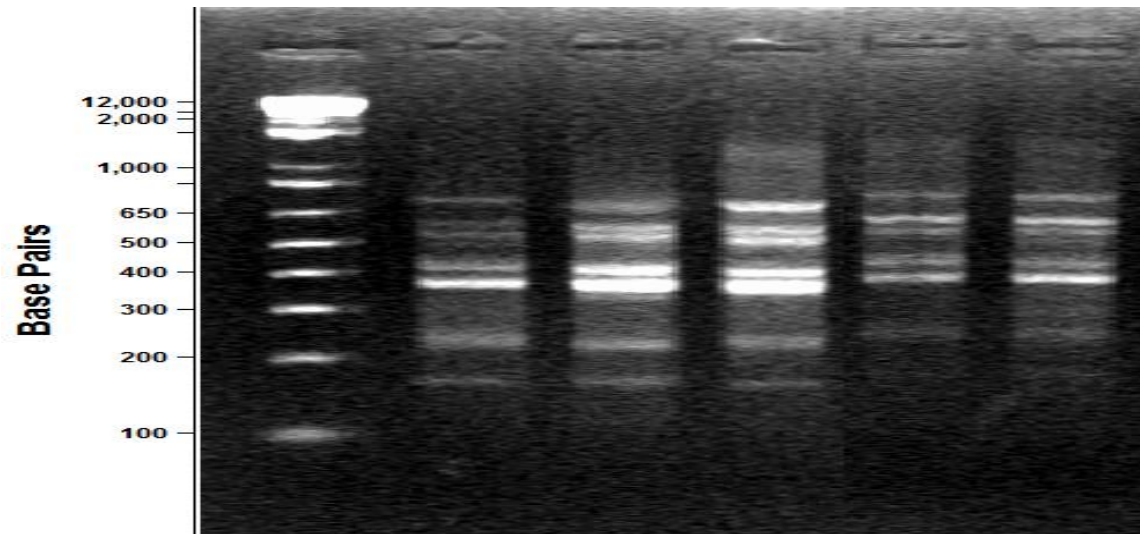
M 1 2 3 4 5



HB8



HB10



HB11

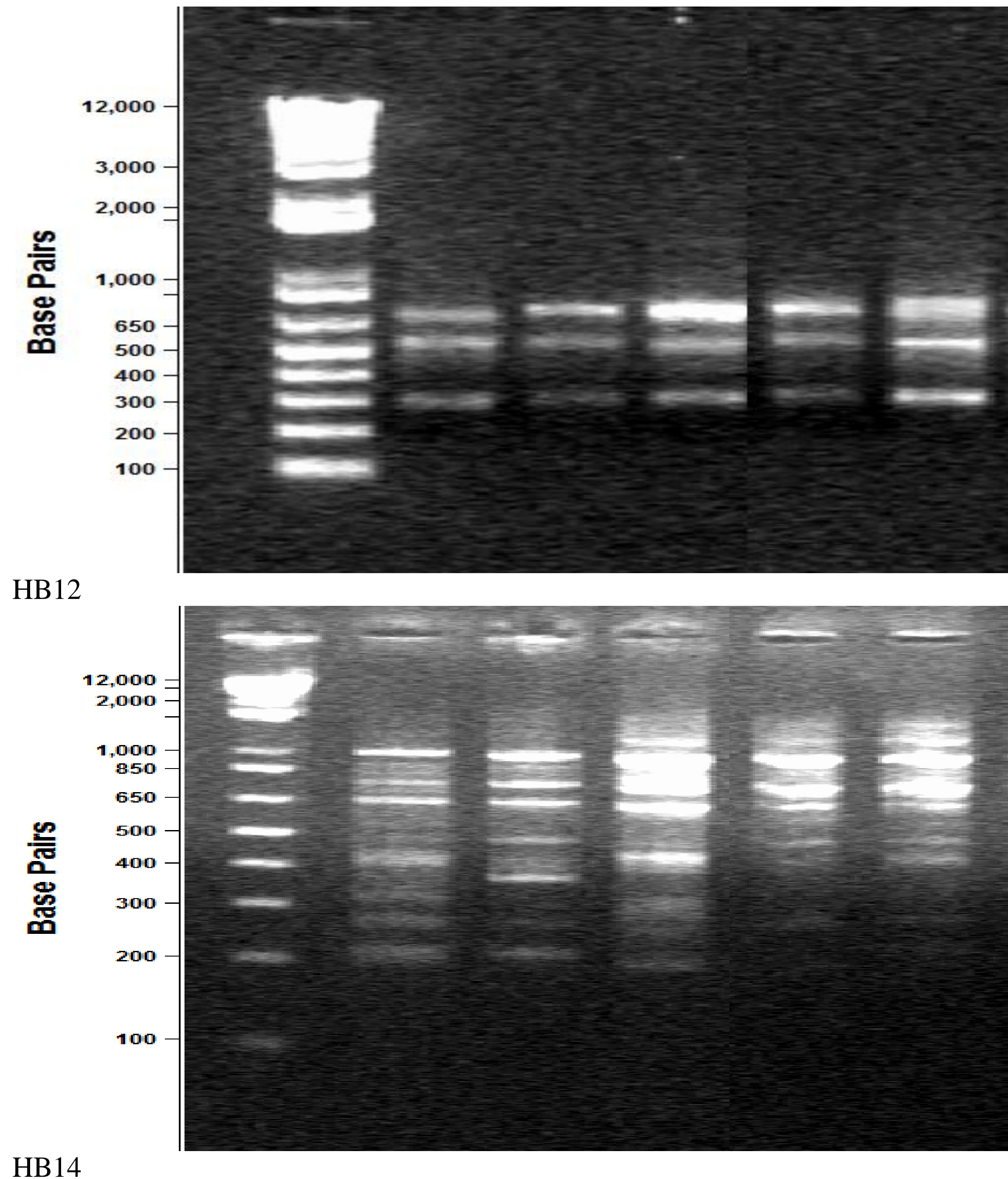


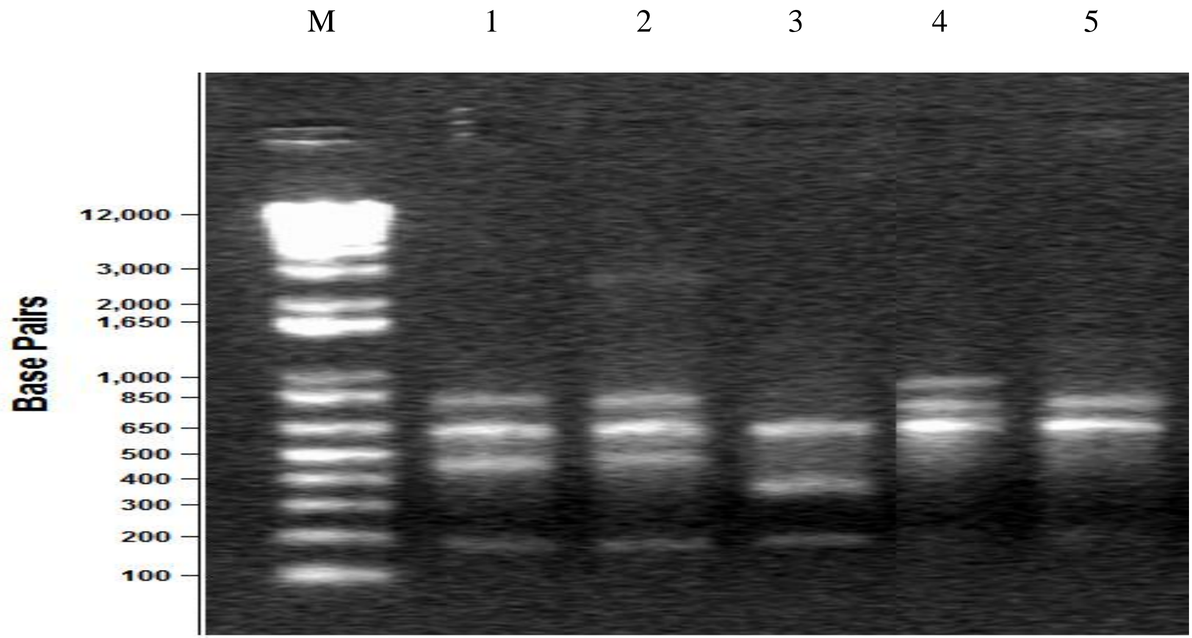
Figure 1. ISSR –PCR fragments for different rats genomic DNA. Groups 1, 2, 3, 4 and 5 with primers A9, A12, A16, A18 and B11, respectively. M represents DNA marker.

pattern in all treatments compared with control group (Figure 2).

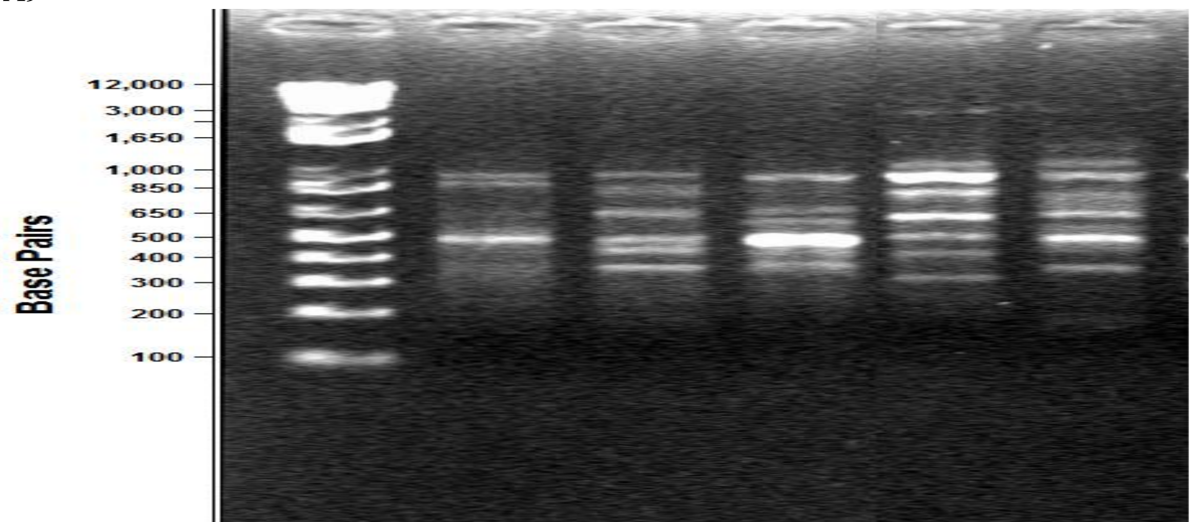
The results of RAPD and ISSR showed substantial differences between control and CCl_4 treated rats with apparent changes in the number and size of amplified DNA fragments for different primers. The disappearing of a normal band or appearing of a new band is the obvious changes in the RAPD and ISSR patterns generated by celery treatments. The animals treated with celery extract had almost the same bands pattern and numbers as control group.

Biochemical results

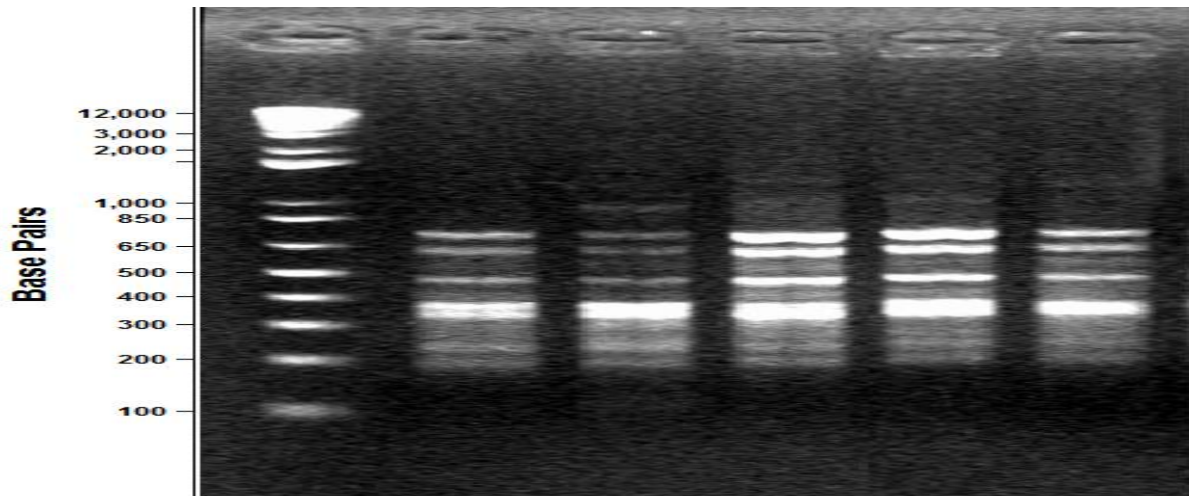
The biochemical studies of liver and testes tissues (total



A9



A12



A16

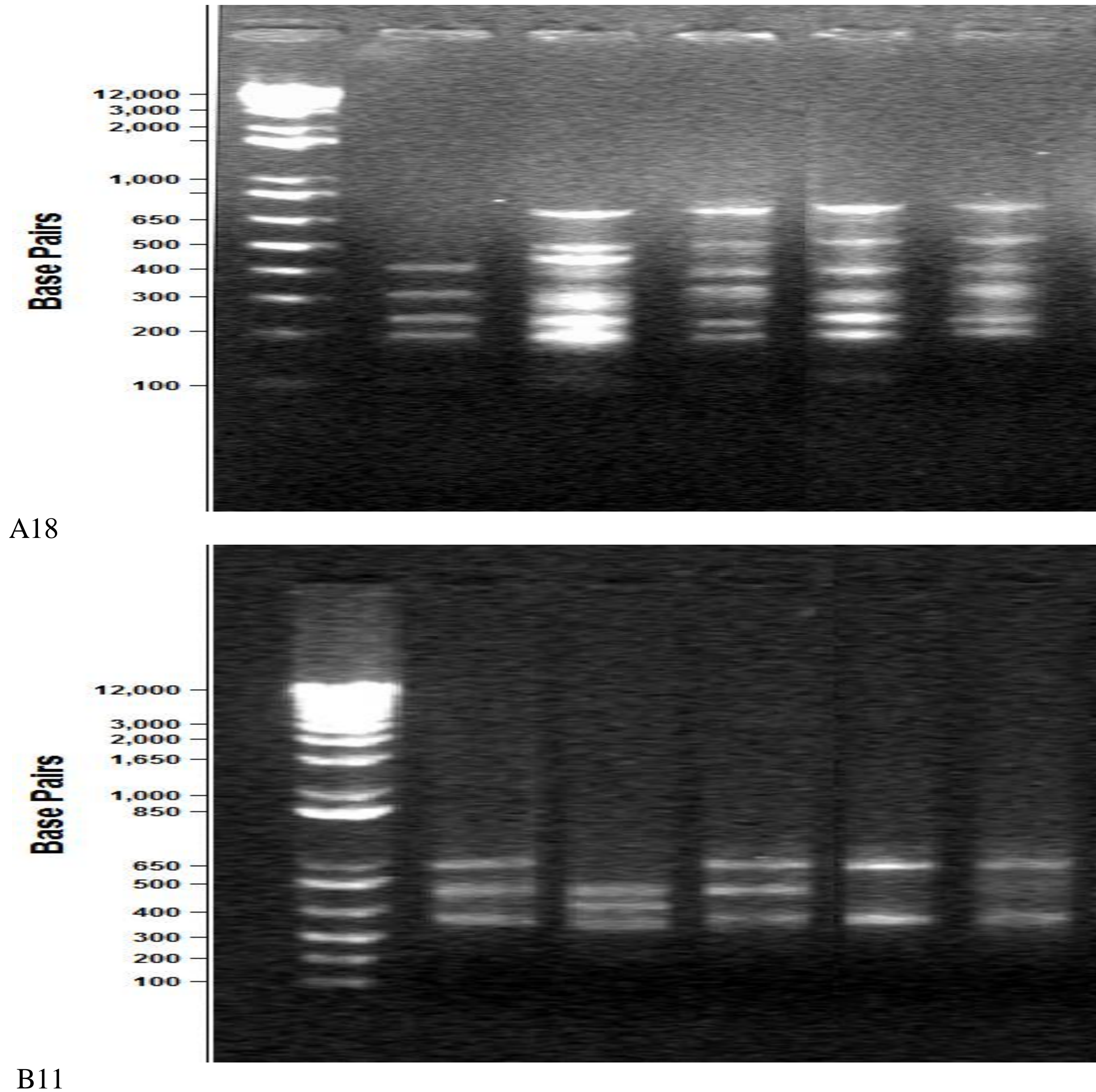


Figure 2. Comparison of RAPD fingerprinting profiles of different rats genomic DNA. Groups 1, 2, 3, 4 and 5 represent PCR products with primers A9-A12, A16, A18 and B11 respectively. M represents DNA marker.

soluble protein, DNA and RNA contents) were done to evaluate the alleviating influences of celery diet or extract on the toxicity of CCl_4 (Table). The results observed that CCl_4 ingestion significantly decreased the total soluble protein in liver and testes tissues to 66 and 72% at normal control respectively. The treatment with srlymarin as well as celery diet and celery extract alleviated the harmful effect of CCl_4 which readjusted the values to 98 and 103% respectively relative to normal health control. The same trend was observed in the treatment of celery diet and extract which improved the total soluble protein values around that of normal control. The same table showed also that DNA and RNA contents of liver or testes tissues were decreased by CCl_4 ingestion which was 74 and 58% for liver and 80 and 70% for testes respectively relative to those of the normal health control. These harmful effects of CCl_4 were reduced by celery diet or extract. The induction of celery increased both the DNA and RNA contents in liver and testes which readjusted to values ranged between 94 and 99% for DNA and from 87 to 95% for RNA. That showed insignificant differences at normal health control.

It means that celery as medicinal plant reduced the harmful toxicity of CCl_4 ingestion to around normal state.

Table 3. Frequencies of different sperm abnormalities in all experimental groups.

Groups	Total examined cells	Types of abnormal heads					Abnormal tails		Total abnormal sperms	Abnormal sperms	
		Amorphous	Big	Small	Without hook	Banana	Coiled	Divided		No.	%
Control	4000	$4.60^{d} \pm 0.68$	$2.60^{d} \pm 0.40$	$3.60^{d} \pm 0.81$	$3.60^{b} \pm 1.12$	$2.60^{c} \pm 0.68$	0.60 ± 0.25	0.00 ± 0.00	$17.60^{d} \pm 1.33$	88	2.20
CCl_4	4000	$79.20^{a} \pm 4.08$	$18.60^{a} \pm 1.29$	$20.00^{a} \pm 1.00$	$30.60^{a} \pm 4.41$	$14.60^{a} \pm 1.63$	2.20 ± 0.86	0.40 ± 0.25	$165.60^{a} \pm 8.29$	828	20.70
Silymarin	4000	$44.80^{c} \pm 5.49$	$10.00^{b} \pm 0.71$	$13.60^{b} \pm 0.87$	$22.20^{a} \pm 1.93$	$6.60^{b} \pm 0.87$	1.60 ± 0.68	0.40 ± 0.25	$99.20^{b} \pm 9.26$	496	12.40
CCl_4 + celery diet	4000	$56.00^{b} \pm 1.73$	$7.00^{c} \pm 1.30$	$12.60^{c} \pm 1.33$	$24.80^{a} \pm 3.38$	$3.00^{bc} \pm 0.95$	1.60 ± 0.68	0.00 ± 0.00	$105.00^{b} \pm 5.22$	469	11.73
CCl_4 + celery extract	4000	$34.60^{c} \pm 3.71$	$6.60^{c} \pm 0.75$	$8.00^{c} \pm 0.84$	$9.80^{b} \pm 0.97$	$4.80^{bc} \pm 0.97$	1.80 ± 0.37	0.00 ± 0.00	$65.60^{c} \pm 6.24$	352	8.80

Different letters (a, b, c, d) in the same column are significantly different ($p \leq 0.05$).

Table 4. Frequencies of different chromosomal aberrations induced in male bone marrow cells of all experimental groups.

Groups	Total examined cells	Structural chromosomal aberrations						Numerical chromosomal aberration			
			Gap	Break	Deletion	Fragment	Centromeric attinuation	hyperploidy	hypoploidy	polyploidy	Total
Control	300	M±SE	$1.00^{c}\pm0.32$	$0.0^{c}\pm0.0$	$0.40^{d}\pm0.25$	$0.0^{c}\pm0.0$	$0.60^{c}\pm0.25$	0.00±0.00	$0.80^{c}\pm0.37$	0.0±0.0	$2.80^{d}\pm0.37$
CCl_4	300	M±SE	$7.00^{a}\pm0.55$	$4.00^{a}\pm0.63$	$7.80^{a}\pm0.58$	$2.60^{a}\pm0.25$	$5.00^{a}\pm0.32$	0.20±0.20	$5.40^{a}\pm0.40$	0.0±0.0	$32.00^{a}\pm0.78$
Silymarin	300	M±SE	$2.40^{b}\pm0.51$	$2.20^{bc}\pm0.37$	$5.20^{b}\pm0.37$	$1.80^{ab}\pm0.37$	$3.40^{b}\pm0.51$	0.00±0.00	$3.40^{b}\pm0.40$	0.20±0.20	$18.60^{b}\pm1.08$
CCl_4 + celery diet	300	M±SE	$3.00^{b}\pm0.32$	$2.60^{b}\pm0.25$	$3.80^{c}\pm0.37$	$1.80^{a}b\pm0.49$	$2.60^{b}\pm0.51$	0.40±0.25	$2.20^{bc}\pm0.92$	0.40±0.25	$16.18^{b}\pm1.11$
CCl_4 + celery extract	300	M±SE	$2.20^{b}\pm0.20$	$1.20^{c}\pm0.37$	$3.40^{c}\pm0.40$	$1.20^{c}\pm0.58$	$3.20^{b}\pm0.377$	0.20±0.20	$0.80^{c}\pm0.20$	0.20±0.20	$12.40^{c}\pm0.68$

DISCUSSION

Administration of CCl_4 to rats caused severe liver damage; it is used as a hepatotoxin in the experimental study of liver diseases. The hepatotoxic effects of CCl_4 are due to its active metabolite, trichloromethyl radical (Johnson and Kroening, 1998). Trichloromethyl free radical when combined with cellular lipids and proteins in the presence of oxygen from trichloromethyl-peroxyl radical, which may attack lipids on the membrane of endoplasmic reticulum, leads finally to cell death (Mayuren et al., 2010). While evaluating the genotoxic effects of any agent in an organism, it is much relevant to study the genotoxic effect on chromosomal aberrations and on the germinal cells because this will give information on transmissible genetic damage from one generation to another (Au and Hsu, 1980). Chemicals that showed positive response in the sperm abnormality tests are also proved to be carcinogenic (Wyrobek et al., 1983). Sperm head abnormalities may be due to chromosomal aberration that occurs during the packaging of the genetic material in the sperm head. It may also be due to occurrence of point mutations during spermatogenesis. The implication of this is that sperm with abnormal shapes might contain abnormal genetic material (Bruce et al., 1974).

The present study showed that administrations of CCl_4 increased the frequencies of chromosomal aberrations in somatic cells as well as sperm abnormalities significantly ($P < 0.01$) which are evidence of its toxicity when compared to normal animals (Tables 3 and 4). These frequencies were brought back near to the normal in the case of celery treated animals. Celery extract produced more significant reduction in the frequencies of chromosomal aberrations and sperm abnormalities than its diet. It is important to note that compounds that interfere with DNA synthesis can cause chromosomal aberration and

Table 5. Detected polymorphism for ISSR marker in the tested groups 1, 2, 3, 4 and 5.

Marker no.	Marker	Monomorphic	Polymorphic	Total	Polymorphism (%)
1	HB 8	1	11	12	92
2	HB 10	2	11	13	85
3	HB11	4	8	12	67
4	HB12	3	9	12	75
5	HB14	2	10	12	83
Total		12	49	61	80.4

Table 6. Number of obtained bands using ISSR analysis in treated groups compared with control group.

Marker type	Analysis type	Marker name	Total band number	Mobility Range bp	Number of bands				
					Group 1	Group 2	Group 3	Group 4	Group 5
1	ISSR	HB 8	12	300-850	5	4	6	9	5
2		HB 10	13	220-1350	6	4	5	10	7
3		HB11	12	190-900	7	6	7	6	6
4		HB12	12	300-1000	4	3	3	3	3
5		HB14	13	200-2000	8	7	9	8	9
Total					30	24	30	36	30

Table 7. Number of obtained bands using RAPD analysis in treated mice compared with control group.

Marker type	Analysis type	Marker name	Total band number	Mobility Range bp	Number of bands				
					Group 1	Group 2	Group 3	Group 4	Group 5
1	RAPD	A9	12	200-1000	5	3	4	6	4
2		A12	12	350-1000	6	3	5	8	7
3		A16	12	200-1000	9	6	9	9	8
4	ISSR	A18	12	200-800	8	4	8	7	6
5		B11	12	350-650	4	3	3	3	3
Total					32	19	29	33	28

abnormal sperm chromatin structure (Wiger et al., 1995). Celery is an excellent source of Vitamin C, A, B1 and B2 (Mitra et al., 2001). It has been reported that supplementation of the diet with vitamin C results in a highly significant decrease in endogenous oxidative base damage in the DNA of patients lymphocytes and lymphocytes of antioxidant-supplemented subjects showed increase resistance to oxidative damage *in vitro* (Duthie et al., 1996). The results of the present study about the active effect of celery extract could be possibly due to the presence of sugar or amino acid side chains (S) compounds. Also, celery contains Vitamin C which is a known immune system booster and reduces the free radical in the body.

The DNA strand breaks can originate from the direct modification of DNA by chemical agents or their metabolites; from the processes of DNA excision repair, replication and recombination; or from the process of apoptosis (Eastman and Barry, 1992). Direct breakage of the DNA strands occurs when reactive oxygen species (ROS) interact with DNA (Moller and Wallin, 1998). When ROS interact with cells and exceed endogenous antioxidant systems, there is indiscriminate damage to biological macromolecules such as nucleic acids, proteins, and lipids (Offord et al., 2000).

Oxidative stress induced by oxygen-derived species can produce a multiplicity of modifications in DNA including base and sugar lesions, strand breaks, DNA-protein cross-links and base-free sites. If left un-repaired, oxidative DNA damage can lead to detrimental biological consequences in organisms, including cell death, mutations and transformation of cells to malignant cells.

The molecular studies results of cluster and genetic diversity analysis was performed based on the changes of the ISSR and RAPD bands in the pattern. The changes in ISSR and RAPD pattern between control and

Table 8. Effect of celery on total soluble protein, DNA and RNA contents of liver and testes tissues of the experimental rats.

Groups	Liver						Testes					
	Total soluble protein		DNA		RNA		Total soluble protein		DNA		RNA	
	Mg/g tissues	%	Mg/g tissues	%	Mg/g tissues	%	Mg/g tissues	%	Mg/g tissues	%	Mg/g tissues	%
Control	190.51±5.11	100	0.500 ±0.034	100	0.241±0.016	100	104.92±5.55	100	0.351±0.017	100	0.201±0.011	100
CCl_4	126.24±6.12	66	0.371±0.023	74	0.140±0.011	58	75.11±4.00	72	0.200±0.012	80	0.140±0.010	70
CCl_4+ Silymarin	187.36±10.12	98	0.471±0.021	94	0.211±0.010	88	108.21±5.12	103	0.341±0.016	97	0.191±0.013	95
CCl_4+ celery diet	189.61±9.37	100	0.496±0.032	99	0.209±0.011	87	110.07±6.10	105	0.334±0.017	95	0.187±0.010	
CCl_4+ celery extract	188.74±7.76	99	0.481±0.027	96	0.217±0.013	90	111.00±5.96	106	0.340±0.017	97	0.186±0.014	

% relative to control. Values (mean ± SD) are significantly different at normal control ($P < 0.05$).

treated groups were obvious, including the changes in the number, the position and the intensity of the bands (Figures 1 and 2 and Tables 6 and 7). The appearance of new fragments may attribute to some sites becoming accessible to the primer after point mutations and/or large rearrangements take place in genomic DNA (Williams et al., 1990), while the disappearance of bands possibly resulted from the presence of DNA photoproducts (pyrimidine dimmers), which can act to block or reduce DNA polymerization in the PCR reactions (Nelson et al., 1996). Band intensity decreasing was considered as the result of the loss of some alleles (Weinberg, 1991; Peinado et al., 1992). Previous studies proved that changes in DNA fingerprint could reflect DNA alteration in genome from single base changes (point mutations) to complex chromosomal rearrangements (Atienzar et al., 1999; 2002).

The present studies of total soluble protein, DNA and RNA contents of liver and testes showed that phenolic compounds of celery increased the three investigated parameters (protein, DNA and RNA) in both liver and testes of CCl_4 treated animals. The decreases in total soluble protein, DNA and RNA in liver and testes under the effects of CCl_4 ingestion may be because CCl_4 caused oxidative stress in rats which showed chromosomal aberrations and prevented the replication and translation process. The harmful effects of oxidative stress of CCl_4 which inhibited the antioxidative enzymes with alteration in the collagen and basement membranes reduced tissue soluble protein. Celery treatment reduced this oxidative stress in CCl_4 experimental animals (Czinner et al., 2000; Lotio and Frei, 2004).

The present studies confirmed each other in which the increase in chromosomal aberration sperm abnormalities, the changes in the number, position and intensity of DNA bands, the reduced content of total soluble protein of CCl_4 induction were paralleled with those of decreased DNA and RNA contents. The treatment of celery reduced and alleviated the toxic effects of CCl_4 which stimulated the protein biosynthesis to produce antioxidative enzymes which treat the oxidative stress of CCl_4 (Abdou et al., 2009; Salah et al., 2010).

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