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The Possible Protective Effect Of Saffron Against Acrylamide-Induced Neurotoxicity In Albino Rats (A Molecular Study)

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Abstract: Nowadays, there is a continuous and progressive global public exposure to acrylamide (ACR) due its presence in fried and backed food. Thermal preparation of sugar-rich food mostly initiates chemical reactions that yield dangerous chemicals. Of these chemicals arise acrylamide and its metabolite glycidamide. Those having a high affinity for binding to nervous tissues. The acrylamide-induced neurotoxicity involves reduction in proliferation of neuronal progenitor cells associated with apoptotic cell death. Concerning acrylamide-induced toxicity, preventive medicine research focused on natural antioxidants. Studies on active constituents of Saffron showed obvious antioxidant, and anti-toxic properties. Here, we designed this study to investigate the possible protective effect of saffron against acrylamide-induced neurotoxicity in hippocampal area of brain. Adult male albino rats were assigned equally into 4 groups (n = 8); control; saffron group; acrylamide (ACR)-administered group and combined ACR and saffron- treated group. Our results recorded molecular changes as a significant decrease on genomic DNA fragmentation in saffron co-administered group as compared to ACR-exposed group. The changes on DNA mutagenicity were studied by analyzing the random amplification of polymorphism of DNA that showed an elevated genomic integrity on rat co -administered saffron with ACR in contrast to those exposed to ACR only.

Keywords: Acrylamide, Neurotoxicity, Brain, Saffron, DNA, RAPD.

1. INTRODUCTION

Acrylamide, was recorded as a major pollutant to the man's environment due to progressive accumulation of this material during the process of cooking food.[1,2] Owing to its small size and high-water solubility, ACR has a rapid absorption and distribution throughout the body and reacts with hemoglobin, DNA, neurons, and essential enzymes and this, in its turn, increases the problem of toxicity as neurotoxicant, carcinogen, and mutagen.[3,4]

Chemically, ACR is a chemical compound that contains highly reactive α-β unsaturated carbonyl groups. It exists in 2 forms; monomeric form is toxic to the nervous tissues, and a carcinogen for humans and laboratory animals, and polymeric form is not known to be toxic.[5] ACR is used as an additive in chemical engineering, molecular laboratories, water purification, textiles, mining, paper manufacturing, cosmetics and in proteins electrophoresis.[6,7,8,9] ACR has been recorded to be toxic to nervous system ,[10,11] reproductive system[12,13] and precancerous to laboratory animals.[14]

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ACR is not genotoxic by itself, but it becomes an active glycidamide (GA) via epoxidation. Cytochrome P450 2E1 (CYP2E1) leads to the formation of GA-DNA and hemoglobin adducts.[15] It is found to have high tendency for binding to brain tissue.[16] Its neurotoxicity affects sensory and motor functions of both central and peripheral nervous system.[17] As regards acrylamide-induced toxicity, the alternative medicine focused on natural antioxidants.[18] Studies on active constituents of saffron or its extract showed anticonvulsant, antidepressant, anti-inflammatory and antitumor effects.[19] Therefore, this study was designed to investigate the neurotoxic effect of ACR in the brain tissue of albino rats at the molecular level and the possible protective effect of saffron.

2. MATERIALS AND METHODS

Animals

Thirty-two adult healthy male albino rats (200 ±10 g) strain were obtained from the Animal house of the Faculty of Veterinary Medicine, Zagazig University. Animals were housed in special plastic cages (4/cage) in animal house under standard conditions of light/dark cycle, temperature (25°C ±2) and humidity (54%). Rats were fed a standard pellet diet with free access to tap water at least one week before the experimental work for acclimatization.

Acrylamide:

Acrylamide crystals (anatase form, molecular formula: C3H5NO) was a Sigma chemical (St. Louis, MO, USA). It is an odorless and colorless reactive molecule.

Saffron extract

Saffron, the dried stigmas of *Crocus Sativus* flower were obtained from El-attar market, Taif, Saudi Arabia. One gram of saffron was soaked in 100 ml distilled water. After 2 hours it was homogenized in the same distilled water, stirred for 1 hour and filtered. This aqueous extract was lyophilized and stored at 4 °C until further use.[20]

Experimental design

Animals were divided randomly into four groups from G1 to G4 (8 rats each) and subjected to the following schedule of treatments for 8 weeks.

G1: Control (healthy animals); fed on basal diet during all the experimental period.

G2: Saffron group; fed on basal diet and supplemented with saffron (water extract, 80 mg/kg B. Wt.) via oro-gastric tube.

G3: ACR group, fed on basal diet but their animals were exposed to ACR solution (50 mg/kg B. Wt.) via oro-gastric tube.

G4: ACR+ saffron group; where saffron (80 mg/kg B.Wt.) was given three days prior to acrylamide exposure.

At the end of treatment schedule, rats were anesthetized using Na barbitone and serum samples were collected. Anesthetized animals were scarified by cervical dislocation and brains (hippocampal areas) were rapidly dissected out for molecular analysis.

Ethical consideration

This experimental protocol was reviewed and approved by The Institutional Animal Care and Use Committee (ZU-IACUC Committee), Zagazig University and the approval number is ZU-IACUC/1/F/108/2020.

Molecular analysis

The genomic DNA was extracted from dissected brain using Gene JET^{TM} genomic DNA purification kit (*Fermentas, Thermo Fisher Scientific, USA, K0721*) and following the manufacturer protocol at National Research Center.

Extraction of genomic DNA of brain

20 mg of brain tissue was mixed with 180µl of lysis buffer plus 20µl of proteinase K solution, incubated at 56°C until the tissue is completely lysed. The prepared lysate was transferred to Gene JET™ genomic DNA purification columns, mixed with 400µl of 50% ethanol and centrifuged for 1 min at 6000 xg, washed twice and centrifuged for 3 min at 12000 xg. 200 µl of elution buffer were added to the center of Gene JET™ genomic DNA purification columns membrane to elute genomic DNA, incubation for 2min at room temperature then centrifugation for 1min at 8000 xg was performed. DNA was estimated by the Nanodrop spectrophotometer (ND1000; Nano Drop Technologies, USA) and assessed on agarose gel (0.8%) electrophoresis.[21]

Random amplification of polymorphic DNA-PCR

The molecular genetic variability of the treated rat genomes and their control were assessed using four random primer kit (Operon Biotechnologies, Bio Campus Colonge Nattermannalle, Germany). According to **Williams** *et al.*[22] and **Plotsky** *et al.*[23]**;** amplification of DNA fragments was carried out using an I Cycler (Bio-Rad, Herts, UK) thermal cycler using four primers A06 5**'GGTCCCTGAC3',** C09 **5'CTCACCGTCC3',** D01 **5'ACCGCGAAGG3'** and D03 5**'GTCGCCGTCA3'.** PCR amplification was conducted in 25 µl reaction volumes containing 10 ng genomic DNA, 12.5 pmol/ l master mix (×2) (Thermo Fisher Scientific, Carlsbad, CA, USA). The PCR reactions were carried out in a thermocycler (Bio-Rad C1000, Bio-Rad, Hercules, CA, USA), programed with a first denaturation for 5 min at 95 °C, followed by 40 cycles for 30 s denaturation at 95 °C, annealing at 37 °C and 1 min extension at 72 °C. Final extension at 72 °C for 5 min was allowed before holding at 4 °C for 5 min. DNA was separated on electrophoresis of 1.5% agarose at 50V for 1.5 hour (Bio-Rad power space 300, USA). DNA was visualized using a 312 nm UV Gel Documentation System (Gensnap), software (Synegen, UK).

Band analysis

The bands for PCR products were analyzed by Total Lab Quant (V11.5: TL100-LX59-7YF4-EX). The fluorimetric profiles of each amplification reaction were studied both qualitatively and quantitatively by comparing profiles from control and treated DNA. Each change observed in random amplification of polymorphic DNA (RAPD) profiles of treated groups (disappearances and appearance of bands in comparison to the control RAPD profiles) was given the arbitrary score of +1. The obtained dendrogram for all used primers were produced using UPGM analysis.

Quantitative analysis of genomic DNA fragmentation

The fragmentation analysis of genomic DNA in hippocampal brain tissue was determined by homogenizing 20 mg of brain tissue with 400 µl hypotonic lysis buffer, centrifuged at 3,000 rpm for 15 min at 4°C. Samples were treated with equal volumes of absolute isopropyl alcohol and 0.5M NaCl to precipitate the DNA, centrifuged at 2,000 rpm for 15 min.[24]

Statistical analysis

In the present study, all results were expressed as mean ±standard error of the mean (SEM). Data from these studies were analyzed by Two-tailed ANOVA followed by Tukey's multiple comparisons test comparing all groups. Differences were considered statistically

significant for p values ≤ 0.05. Analysis was conducted with Graph Pad Prism Software V.7.00 (Inc., SanDiego, CA; USA).

3. RESULTS

Random amplification of polymorphic DNA-PCR analysis

In the present study, four primers were used to differentiate between experimental animals. The tested primers produced total bands 78 for all samples, 72 polymorphic bands and 6 monomorphic bands as shown in table 1. The highest number of bands produced by primers A06 (**GGTCCCTGAC)** and the lowest number of bands produced by primers D01 (**ACCGCGAAGG)**. The lowest percent of polymorphism was 77.7, produced by C09 (**CTCACCGTCC)** and highest percent of polymorphism was 100 at two primers, A06 (**GGTCCCTGAC),** D03 **(GTCGCCGTCA)** (table 1).

Primer A06:

The DNA fingerprinting using this primer is illustrated in figure (1), table (2). The total number of bands were 25 with molecular weights ranged from 165.358 to 2453.456 base pair (b.p.). There were no monomorphic bands, only 25 polymorphic bands.The fingerprinting profile of this primer includes 16 unique bands as shown in tables (1& 3) and figure (1).The UPGMA dendrogram revealed similarity between the different groups and gave 3 major clusters (RAPD genotypes: group1 and group2 clustered in the same genotype while the group3 clustered in separated genotype and group4 clustered in a separate genotype in between group3 genotype and control, group2 genotype (table 3 & figure 2).

Primer C09:

The DNA fingerprinting using this primer is illustrated in figure (3), table (5). The total number of bands were 18 with molecular weights ranged from 162.505 to 2742.885 bas b.p.. The fingerprinting profile of this primer includes 4 monomorphic bands and 14 polymorphic bands, 8 unique bands and 6 none-unique bands as shown in tables (1&5) and figure (4).The UPGMA dendrogram revealed similarity between the different groups and gave 3 major clusters (RAPD genotypes: group1 and group2 clustered in the same genotype while the group3 clustered in separated genotype and group4 clustered in a separate genotype in between group3 genotype and control, group2 genotype) (table 5 & figure 4).

Primer D01

The DNA fingerprinting using this primer is illustrated in figure (5), table (6). The total number of bands were 16 with molecular weights ranged from 241.571 to 1861.641 b.p..The fingerprinting profile of this primer include 2 monomorphic bands and 14 polymorphic bands, 11 unique bands and 3 none-unique bands as shown in tables (1& 7) and figure (5).The UPGMA dendrogram revealed similarity between the different groups and gave 3 major clusters (RAPD genotypes: group1 and group2 clustered in the same genotype while the group3 clustered in separated genotype and group4 clustered in a separate genotype in between group3 genotype and control, group2 genotype) (table 7 & figure 6).

Primer D03:

The DNA fingerprinting using this primer is illustrated in figure (7), table (8). The total number of bands were 19 with M. Wt. ranged from 209.079 to 2414.303 b.p.. There were no monomorphic bands, only 19 polymorphic band. The fingerprinting profile of this primer include 12 unique bands as shown in tables (1&8) and figure (7).The UPGMA dendrogram revealed similarity between the different groups and gave 3 major clusters (RAPD genotypes: group1 and group2 clustered in the same genotype while the group3 clustered in separated

genotype and group4 clustered in a separate genotype in between group3 genotype and control, group2 genotype (table 9 & figure 8).

Quantitative analysis of DNA Fragmentation

Isolated DNA from brain of ACR-treated rats showed complete degradation into oligonucleotide fragments forming a clear ladder of apoptosis when separated by agarose gel electrophoresis as shown in figure9. While, DNA of brain tissue in rats treated with saffron showed a similar normal pattern compared to control DNA. The genomic DNA of rats, treated with saffron co administrated with ACR, was highly recovered and similar to the normal pattern (figure9). Data recorded in table (10) and figure. (9&10), the percent of fragmented DNA released from brain tissue of ACR-exposed rats recorded a significant increase (21.5 ± 0.68^{a}) while, the rats administrated with saffron extract recorded a significant low change (7.8 ± 0.55^{c}) in the percent of fragmented DNA related to control. The rats treated with saffron co-administratedwith ACR showed significant reduction in the percent of fragmented DNA as compared to ACR-exposed rats.

4. DISCUSSION

The general population exposed extensively and accumulatively to ACR via their diets since ACR is formed during process of cooking particularly those food with rich carbohydrate content.[25] Hence, the present study was conducted to evaluate the repeated dose toxicity of acrylamide and its possible protection by a natural antioxidant, saffron, in albino rats.

The results of the current study recorded molecular disturbances presented by two main techniques first was the analysis of RAPD that revealed a significant mutagenesis in ACR-exposed rats as compared to unexposed control ones. Our results showed that exposure to ACR at 50 mg/kg b.wt. significantly increased DNA fragmented cells (marker of apoptosis) in comparison to control. The authors attributed this effect to the ability of ACR to generate reactive oxygen species (ROS). This in agreement with previous studies of **Mehri** *et al.*[26]

Several previous studies confirmed the ACR's ability in production of ROS. The latter are very active free radicals attacking cellular macromolecules such as DNA. The free radical-mediated DNA damage results in oxidative damage and this represents the first step in mutagenesis, carcinogenesis and ageing.[27,28] The recorded apoptosis in the liver of rats that received Monosodium glutamate (MSG) and/ or ACR was achieved by a significant down-regulation of the B-cell lymphoma 2 (Bcl-2) protein level as well as a significant increase in PD-1 level. Moreover, early and late apoptotic cells revealed the genotoxic effect of MSG and ACR.[29] **Goudarzi** *et al*.[30] **and Santhanasabapathy** *et al.*[31] attributed ACR-induced neurotoxicity to oxidative stress and enhanced lipid peroxidation. **Ibrahim and Ibrahim**[32] recorded significant elevation in percentage of DNA fragmentation in brain compared to the liver and kidneys. This increase was attributed to the ACR concentration to which the fish were exposed. ACR is a potent neurotoxin because it inhibits Acetylcholinesterase (Ach-E) action by phosphorylating hydroxyl group in the serine in the substrate binding domain, which leads to the accumulation of acetylcholine and subsequent neurotoxicity**.**

The treatment of the acrylamide-induced dietary genotoxicity was investigated in different directions. The most available and safe is the administration of natural herbs. Several reports on preventive medicine focused on common natural antioxidants.[18] Studies on active constituents of Saffron or its extracts showed anticonvulsant, antidepressant, anti-inflammatory and antitumor effects.[19] Saffron extract is also reported to be chemo-preventive and showed protective effects on genotoxins-induced oxidative stress in animals.[33] It was showed that saffron aqueous extract protects from genotoxicity as well as genotoxins-induced oxidative stress in mice**.**[34,20,35] Acrylamide was reported to reduce the proliferation of

neuronal progenitor cells and induce apoptotic cell death.[36,1] The mechanisms by which acrylamide or its metabolite, glycidamide exerts genotoxicity and DNA damage is thought to be via releasing intracellular reactive oxygen species and Glutathione (GSH) depletion.[37,38] ACR application significantly increases renal 8-hydroxy-2' deoxyguanosine (8-OHdG)[39] and Bax expression.[40] 8-OHdG is the most important indicator of DNA[41] and DNA damage was diminished following the administration of some antioxidants in organ toxicity models.[42]

The present study also recorded a significant decrease observed on percentage of DNA fragmentation on saffron-acrylamide co-administered rats and significantly suppressed DNA damage as compared to untreated control rats. Saffron extract was reported to be chemo-preventive and showed protective effects on genotoxin-induced oxidative stress in animals.[33] **Guo *et al.*[43] recorded** upregulated levels of Interleukin 1 beta (IL–1β) and tumor necrosis factor alpha (TNF-α), as inflammatory responses in the brain tissues due to ACR exposure for 4 weeks. However, curcumin improved this ACR-induced neuro-inflammation. **In conclusion**, these results here indicate that neurotoxicity of industrial or dietary ACR could be partially-protected or treated by saffron supplementation.

Abbreviations

ACR	Acrylamide
GA	Glycidamide
CYP2E1	Cytochrome P450 2E1
b.p.	Base pair
ROS	Reactive oxygen species
MSG	Monosodium glutamate
Bcl-2	B-cell lymphoma 2
Ach-E	Acetylcholinesterase
GSH	Glutathione
8-OHdG	8-hydroxy-2' deoxyguanosine
IL1-β	Interleukin 1 beta
TNF-α	tumor necrosis factor alpha

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Table 1: Number of polymorphic, monomorphic, unique, non-unique bands produced by using four RAPD primers in the study of different treated groups of male rats

Primer	Total no.	Monomorphism	Polymorphism			% polymorphism
			Polymorphic	Unique	Non-unique	
A06	25	Zero	25	16	9	100 %
C09	18	4	14	8	6	77.7 %
D01	16	2	14	11	3	87.5 %
D03	19	Zero	19	12	7	100 %
Total	78	6	72	47	25	-

Table 2: Size in base pair detected DNA from different genomic DNA groups from rats with using Primer A06

MW	Lane1	Lane2	Lane3	Lane4	Frequency	Polymorphism
2453.456	+	-	-	-	0.250	Unique
2326.208	-	+	+	+	0.750	Polymorphic
1802.837	-	+	-	+	0.500	Polymorphic
1775.612	+	-	-	-	0.250	Unique
1468.054	-	-	+	-	0.250	Unique
1451.395	-	+	-	+	0.500	Polymorphic
1289.941	+	-	-	-	0.250	Unique
1213.769	-	+	-	+	0.500	Polymorphic
1095.289	+	-	-	-	0.250	Unique
947.869	-	+	+	-	0.500	Polymorphic
944.270	+	-	-	+	0.500	Polymorphic
885.136	-	-	+	-	0.250	Unique
693.864	+	+	+	-	0.750	Polymorphic
607.366	-	+	-	-	0.250	Unique
586.923	+	-	+	-	0.500	Polymorphic
488.967	-	-	-	+	0.250	Unique
483.418	-	+	-	-	0.250	Unique
474.310	-	-	+	-	0.250	Unique
467.147	+	-	-	-	0.250	Unique
446.302	-	-	+	-	0.250	Unique
376.082	-	+	+	+	0.750	Polymorphic

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357.936	+	-	-	-	0.250	Unique
302.769	+	-	-	-	0.250	Unique
236.442	-	-	+	-	0.250	Unique
165.358	-	-	+	-	0.250	Unique

Table 3: Similarity matrix for brain tissues of male rats collected different experimental groups using primer A06

Proximity Matrix				
Groups	Jaccard Measure			
	G1	G2	G3	G4
G1	1.000			
G2	0.860	1.000		
G3	0.416	0.571	1.000	
G4	0.667	0.777	0.455	1.000

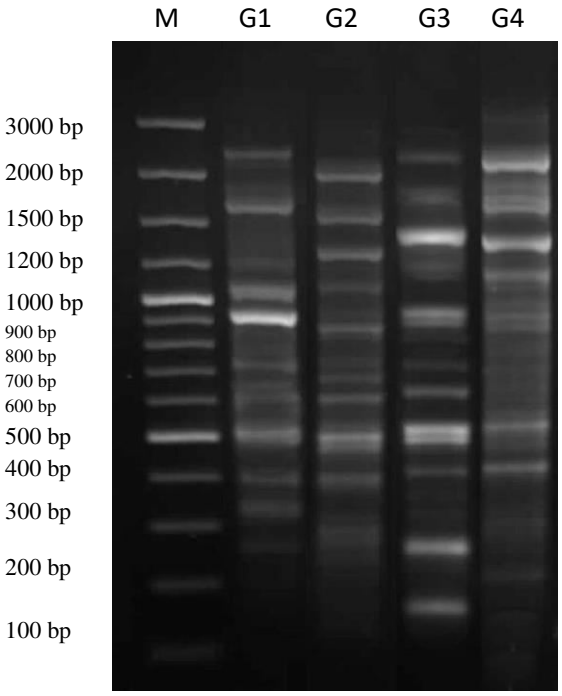

Figure 1: RAPD fingerprinting profiles of DNA from different groups of using primer A06.

Lane (M): DNA ladder.
Lane (1): control group.
Lane (**2**): treatment with saffron (80 mg/kg B. Wt.).
Lane (3): treatment with ACR (50 mg/kg B. Wt.).
Lane (4): treatment with saffron and ACR.

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Table 4: Size in base pair detected DNA from different genomic DNA groups from rats with using Primer C09

MW	Lane1	Lane2	Lane3	Lane4	Frequency	Polymorphism
2742.885	+	+	+	+	1.000	Monomorphic
2381.445	-	+	+	+	0.750	Polymorphic
2337.577	+	-	-	-	0.250	Unique
1955.464	-	+	+	+	0.750	Polymorphic
1905.221	+	-	-	-	0.250	Unique
1788.511	+	+	+	+	1.000	Monomorphic
1641.907	-	+	-	-	0.250	Unique
1430.857	+	+	+	+	1.000	Monomorphic
1192.519	-	+	-	+	0.500	Polymorphic
1153.270	+	-	-	-	0.250	Unique
905.651	+	+	+	+	1.000	Monomorphic
822.192	-	+	-	+	0.500	Polymorphic
807.046	+	-	-	-	0.250	Unique
692.926	-	+	-	+	0.500	Polymorphic
670.120	+	-	-	-	0.250	Unique
546.175	+	+	+	+	1.000	Monomorphic
326.943	-	-	+	-	0.250	Unique
162.505	-	-	+	-	0.250	Unique

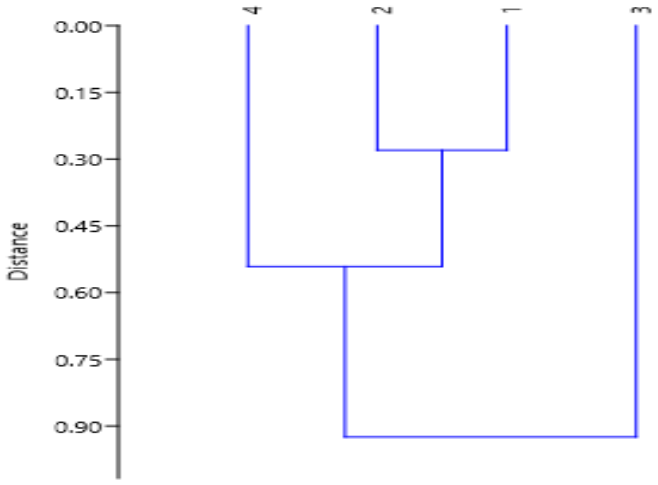

Figure 2: A dendrogram constructed on the basis of similarity index among brain samples using primer AO6. 1: group 1, 2: group 2, 3: group 3 and 4: group 4.

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Table 5: Similarity matrix for brain tissues of male rats collected different experimental groups using primer C09

Proximity Matrix				
Groups	Jaccard Measure			
	G1	G2	G3	G4
G1	1.000			
G2	0.910	1.000		
G3	0.416	0.410	1.000	
G4	0.880	0.800	0.583	1.000

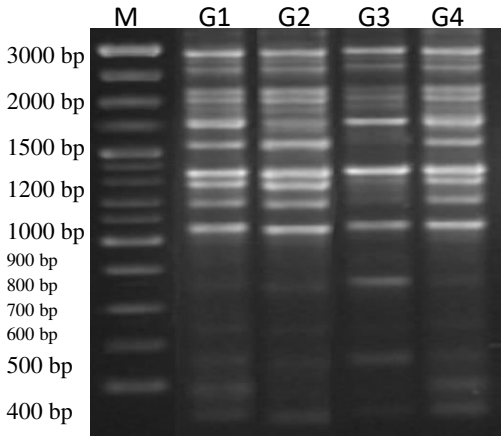

Figure 3: RAPD fingerprinting profiles of DNA from different groups of using primer C09.
Lane (M): DNA ladder.
Lane (1): control group.
Lane (**2**): treatment with saffron (80 mg/kg B. Wt.).
Lane (3): treatment with ACR (50 mg/kg B. Wt.).
Lane (4): treatment with saffron and ACR.

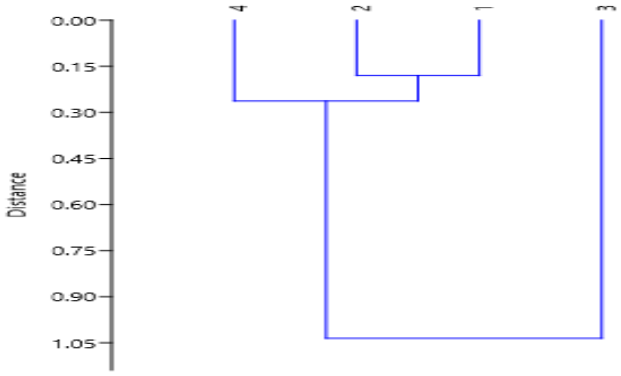

Figure 4: A dendrogram constructed on the basis of similarity index among brain samples using primer C09. 1: group 1, 2: group 2, 3: group 3 and 4: group 4.

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Table 6: Size in base pair detected DNA from different genomic DNA groups from rats with using Primer D01

MW	Lane1	Lane2	Lane3	Lane4	Frequency	Polymorphism
1861.641	+	-	-	-	0.250	Unique
1842.698	+	+	-	+	0.750	Polymorphic
1727.125	-	-	+	-	0.250	Unique
1219.853	+	+	+	+	1.000	Monomorphic
852.804	+	+	+	+	1.000	Monomorphic
791.183	-	-	+	-	0.250	Unique
721.610	-	+	-	+	0.500	Polymorphic
704.593	+	-	-	-	0.250	Unique
642.634	-	+	+	-	0.500	Polymorphic
623.216	-	-	-	+	0.250	Unique
602.328	+	-	-	-	0.250	Unique
506.203	-	-	-	+	0.250	Unique
458.552	-	+	-	-	0.250	Unique
438.674	+	-	-	-	0.250	Unique
362.435	-	-	+	-	0.250	Unique
241.571	-	-	+	-	0.250	Unique

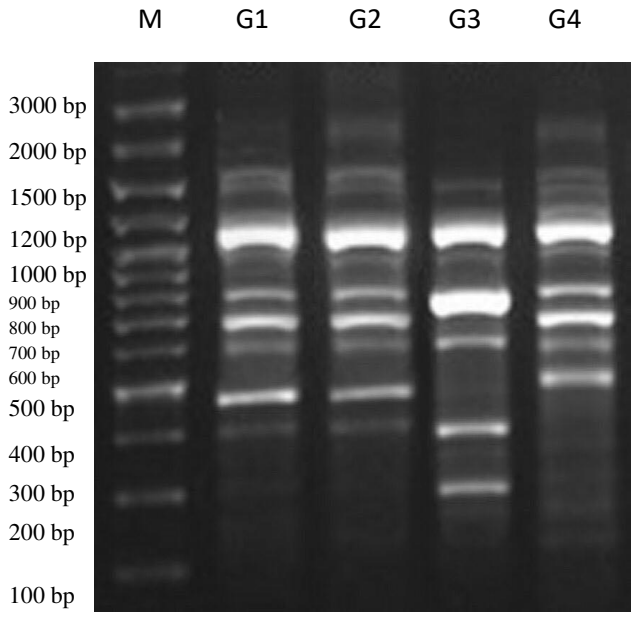

Figure 5: RAPD fingerprinting profiles of DNA from different groups of using primer D01.

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Lane (M): DNA ladder.
Lane (1): control group.
Lane (**2**): treatment with saffron (80 mg/kg B. Wt.).
Lane (3): treatment with ACR (50 mg/kg B. Wt.).
Lane (4): treatment with saffron and ACR.

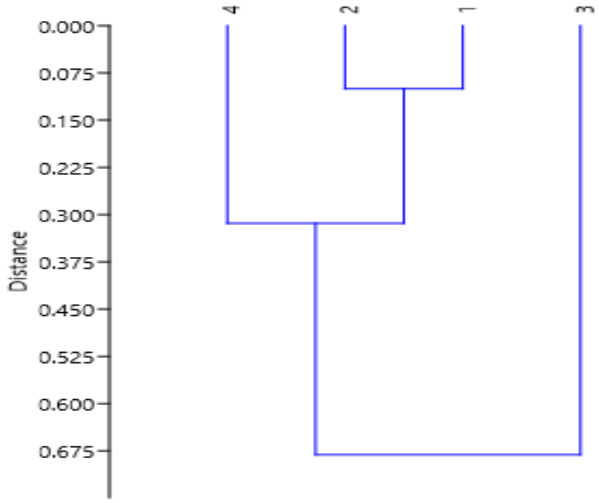

Figure 6: A dendrogram constructed on the basis of similarity index among brain samples using primer D01. 1: group 1, 2: group 2, 3: group 3 and 4: group 4.

Table 7: Similarity matrix for brain tissues of male rats collected different experimental groups using primer D01

MW	Lane1	Lane2	Lane3	Lane4	Frequency	Polymorphism
2414.303	-	-	-	+	0.250	Unique
1638.073	-	-	-	+	0.250	Unique
1347.227	-	+	-	+	0.500	Polymorphic
1302.717	+	-	-	-	0.250	Unique
948.200	-	+	-	+	0.500	Polymorphic
779.843	-	-	+	-	0.250	Unique
673.501	-	-	+	-	0.250	Unique
614.532	-	+	-	+	0.500	Polymorphic
521.095	+	-	-	-	0.250	Unique
517.921	-	+	-	+	0.500	Polymorphic

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427.265	-	+	-	+	0.500	Polymorphic
422.077	-	-	+	-	0.250	Unique
370.131	-	-	+	-	0.250	Unique
351.403	-	+	-	+	0.500	Polymorphic
328.567	+	-	-	-	0.250	Unique
286.374	-	+	-	+	0.500	Polymorphic
277.760	+	-	-	-	0.250	Unique
256.556	-	-	+	-	0.250	Unique
209.079	-	-	+	-	0.250	Unique

Proximity Matrix

Groups	Jaccard Measure			
	G1	G2	G3	G4
G1	1.000			
G2	0.950	1.000		
G3	0.706	0.553	1.000	
G4	0.900	0.702	0.412	1.000

Table 8: Size in base pair detected DNA from different genomic DNA groups from rats with using Primer D03.

Table 9: Similarity matrix for brain tissues of male rats collected different experimental groups using primer D03

Proximity Matrix

Groups	Jaccard Measure			
	G1	G2	G3	G4
G1	1.000			
G2	0.830	1.000		
G3	0.333	0.125	1.000	
G4	0.800	0.720	0.225	1.000

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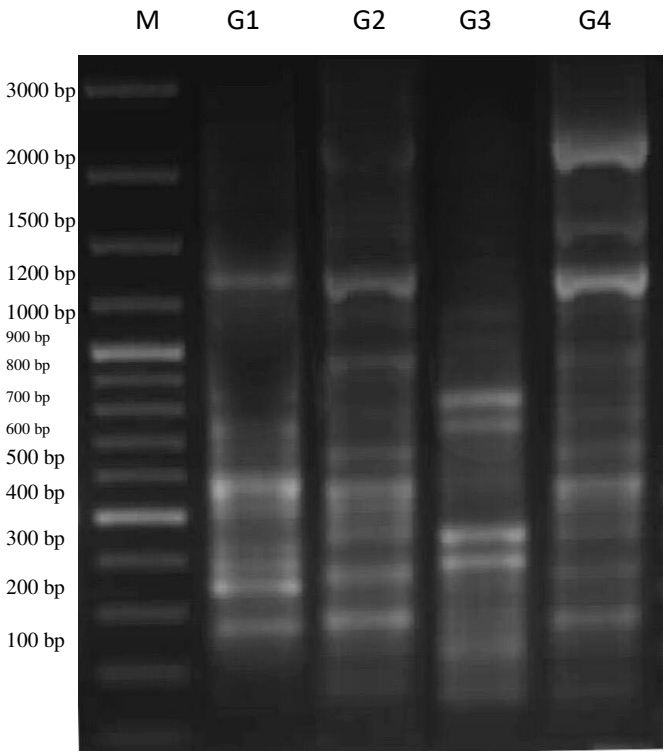

Figure 7: RAPD fingerprinting profiles of DNA from different groups of using primer D03.

Lane (M): DNA ladder.
Lane (1): control group.
Lane (**2**): treatment with saffron (80 mg/kg B. Wt.).
Lane (3): treatment with ACR (50 mg/kg B. Wt.).
Lane (4): treatment with saffron and ACR.

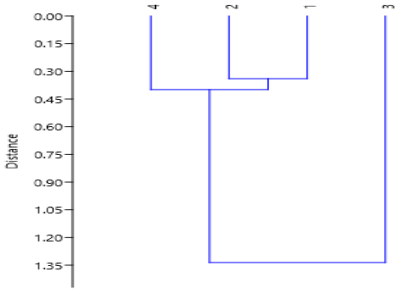

Figure 8: A dendrogram constructed on the basis of similarity index among brain samples using primer D03. 1: group 1, 2: group 2, 3: group 3 and 4: group 4.

Table 10: DNA fragmentation detected with agarose gel in brain DNA extracted from male rats exposed with ACR and treated with saffron.

Animal group	**DNA Fragmentation %M ± SEM**
G1	7.1 ± 0.64^{c}

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G2	7.8±0.55[c]
G3	21.5±0.68[a]
G4	14.9±0.71[b]

Means with different superscripts (a, b, and c) between locations in the same column are significantly different at P<0.05.

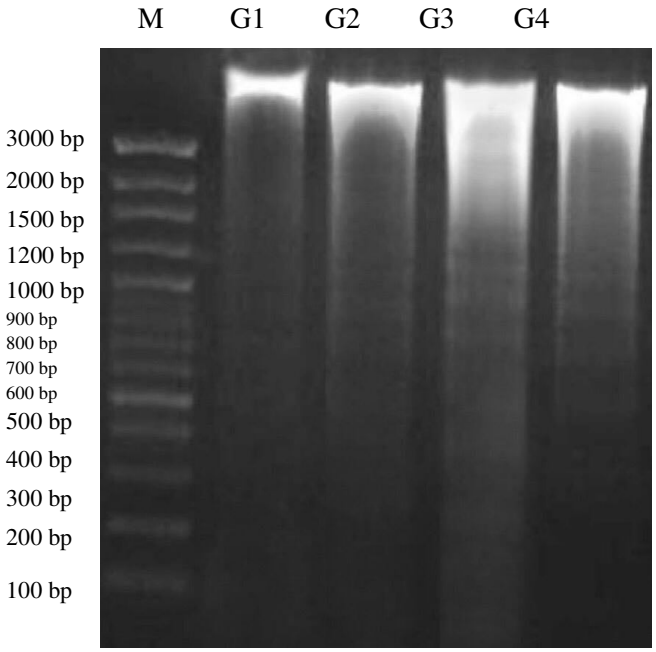

Figure 9: DNA fragmentation detected with agarose gel in brain DNA extracted from male rats exposed to ACR and treated with saffron. M: marker, G1; control, G2; saffron group, G3: ACR group and G4: saffron + ACR group.

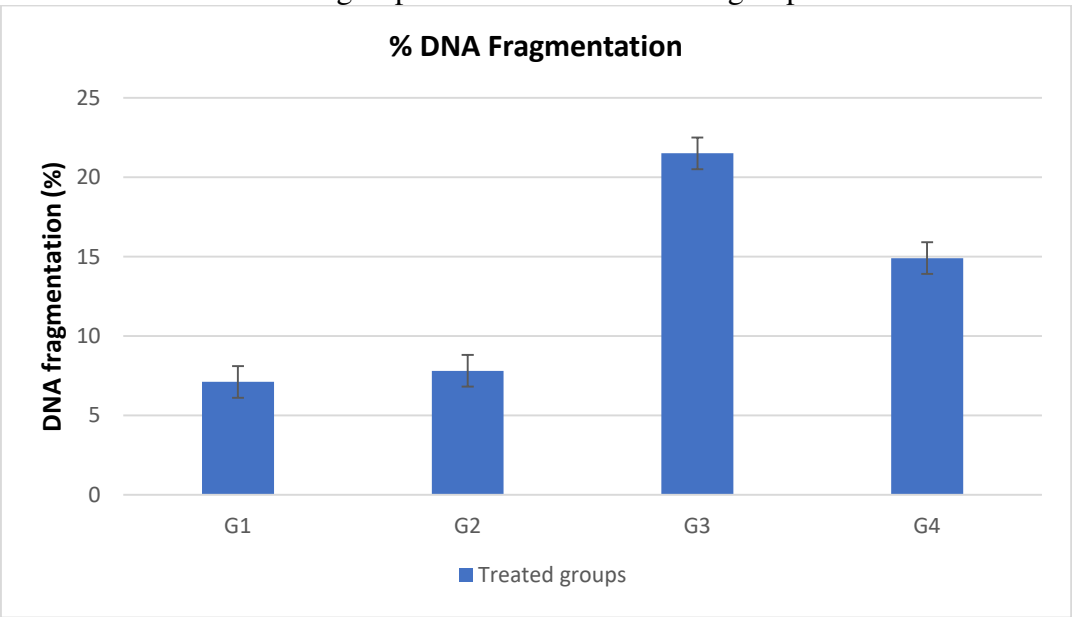

Figure 10: the percent of DNA fragmentation in different experimental groups.