EVALUATION OF MUTAGENIC POTENTIALITY OF SOME COOKED CHICKEN EXTRACTS USING THREE MUTAGENICITY TESTING ASSAYS

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Cooking of food is a process unique to humans. It enhances the taste and digestibility of food so much that its beneficial nature is taken for granted. However, it induces profound changes in all types of foods. Several lines of evidence indicate that diet and dietary behaviors can contribute to human cancer risk. One way that this occurs is through the ingestion of food mutagens (Goldman and Shields, 2003; Li *et al*., 2007; Kabat *et al.*, 2008).

Thirty years ago, the discovery that cooked fish and beef showed high mutagenic activity, as detected by the Ames/*Salmonella* test system (Commoner, *et al*., 1978), began an intensive search for the mutagens present in these foods. A number of studies showed that these mutagens were formed during the pyrolysis of amino acids and proteins and during the cooking of a variety of muscle meats (Wakabayashi, *et al*., 1993). The novel mutagens were identified as heterocyclic amines (HCAs). The major subclass of HCAs found in cooked meats was identified as the aminoimidazoazaarenes (AIAs), which includes those compounds with a quinoline, quinoxaline or pyridine moiety. Recent studies indicated that the AIAs are present at the p.p.b. (ng/g) range in meats cooked by ordinary household methods (Wakabayashi, *et al*., 1993; Ni *et al.,* 2008). The formation of these compounds in meats depends largely on cooking temperature and duration (Sinha, *et al*., 1995). However, chemical modeling has shown that several of the AIAs are produced by the reaction of creatinine and free amino acids at normal cooking temperatures (reviewed after Felton and Knize, 1990). These compounds are distinct from the polycyclic aromatic hydrocarbons derived from the pyrolysis of fat in meat, which occurs, for example, during barbecuing. In addition to their mutagenic activity in *in vitro* assays, 10 HCAs bioassayed for carcinogenicity to date have been shown to be carcinogenic in rats and/or mice (Ohgaki, *et al*., 1991; Kimura *et al*., 2003). One of the AIAs, IQ, has also been shown to be carcinogenic in monkeys (Adamson *et al*., 1990).

Since the identification of HCAs in cooked meats, considerable progress has

been made in characterizing HCA-DNA adducts and clarifying pathways of metabolism necessary for DNA adduct formation. In addition, many investigations have attempted to relate HCA–DNA adduct formation to mutagenic and carcinogenic activity. Cytogenetic assays, including chromosomal aberrations, micronucleated normochromatic erythrocytes and sister chromatid exchanges of bone marrow and peripheral blood of rodents dosed with HCAs, indicate that the HCAs and, by extension, AIA–DNA adducts, appear to be only weakly clastogenic *in vivo* (Tucker, *et al*., 1989; Pfau *et al*., 1999).

Many investigations demonstrated that HCAs have a potent mutagenic activity in *Drosophila* mutagenicity assays; sex-linked recessive lethal (SLRL) (Fujikawa *et al.*, 1983), somatic mutation and recombination test (SMART) either in wing spot assay (Graf *et al.,* 1992; Delgado-Rodriguez. *et al.,* 1995) or in eye spot assay (Fujikawa *et al*., 1983). Moreover, the results show structure-activity relationships among the heterocyclic aromatic amines and nitro compounds similar to those found in Salmonella, which is indicating that the Drosophila short-term test is promising for quantitative prescreening of potential carcinogens.

Nowadays, fast food series for fried and vertical broiled chicken become two of the most common meals over the world. In addition, microwave cooking becomes more easy way of modern cooking methods. Therefore, the aim of this study was to evaluate the mutagenic potentiality of food mutagens formed in chicken meat during frying, broiling and microwave cooked chickens on yeast, Drosophila and rat systems. The food mutagens, which may be formed in cooked chicken, were extracted using blue rayon method.

MATERIALS AND METHODS

1- Chicken meat

White broiler chickens were purchased from local market, Cairo, Egypt. Twenty chicken pairs (with an average weight of 1100 g were slaughtered, eviscerated and chilled at 4°C for 3 hrs. Chicken carcasses were transferred in iceboxes within one hour to the Lab.

2- Yeast strain

Diploid *Saccharomyces cerevisiae* (D_7) strain, which was described by Zimmermann *et al*. (1975), was kindly obtained from Professor F. K. Zimmermann: Darmstadt. Germany.

3- Drosophila stocks, medium and culture condition

For SMART wing spot assay, two *Drosophila* strains were kindly offered by Prof. Dr. R. Marcos, University of Autonoma de Madrid, Madrid, Spain. The first one, which carries homozygous sex-linked yellow body marker and multiple wing hair mutation on the left arm of the third chromosome (*y*; *mwh*), is used as female stock. The second one, flare strain

is used as male stock with a genetic structure of: *flr*^{3} /*In (3LR) TM*$_{3}$, *ri p*^{p} *sep bx*^{34e} *e*^{s} *Ser*. Detailed information on the genetic markers is given by Lindsley and Zimm (1992). All Drosophila experiments were made on a corn meal- sugar-agar Drosophila medium at 25°C.

4- White rat animals

Random breed mature male albino rats aged between four to six months (150-200 g body weight) were obtained from the animal house of National Research Center, Dokki, Egypt. They were housed in appropriate conditions under observation for acclimatization for two weeks before starting the experimental work. All rats were caged and allowed free access to food and water.

5- Marinade seasoning

The marinade mixture; composed of tomato juice (100 g), onion (100), green pepper (20 g), black pepper (0.25 g), sodium chloride (12 g), garlic (3 g), seasoning mixture (10 g), Vinegar (15 ml) and 250 ml of water (for mixing) was used to marinate chicken before cooking.

6- Positive mutagens

Sodium azide (NaN_3, Sigma, USA, Catalog # S8032), was used as a positive control mutagen in yeast experiments. The dysentery cure drug, Amrizole [2-(2 methyl-5 mitroimidazole-1 yl) ethanol; metronidazole, Amria Pharm Ind., Egypt.] was used as a positive control in Drosophila experiments. The chemotherapy drug Mitomycine C (MMC, Bristol-Myers Squibb Caribbean Company, Princeton, New Jersey, USA) was used as a positive clastogenic agent in chromosomal aberrations assay in rat bone marrow cells.

7- Extraction reagents and equipments

The reagents and equipments which were used for extraction of heterocyclic aromatic amines from cooked chicken were Blue Rayon (ICN Biomedical, Inc., Aurora, Ohio, USA, Catalog #808688), Methanol and Concentrated Ammonium Hydroxide solution (ADWIC Co. Egypt), Dimethyl sulfoxide (Fisons, Sci., equ. Bishop, Meadow USA), Fibber glass, Checker water path (GFL-1083, Germany) and Oven with Vacuum pump (Saritorus).

1- Cooking methods and conditions

Chilled carcasses were randomly divided into three groups (four pairs/ group). The following traditional and non-traditional cooking methods were carried out after marinating of chicken overnight with marinade seasoning mixture before cooking.

Vertical broiling chicken

Broiling with vertical flame broiling machine was used. The cooking temperature was reached to 200°C within 50 min for whole marinated ones.

Fried chicken

Marinated samples were immersed in a beaten egg; coated with spiced

coating mixture "VEGETAR" and fried in sun flower oil (160°C/15 min.) using a frying pan type Braun, Germany.

Microwave-cooked chicken

Microwave oven (type Genius, National Japan) was used to cook the chicken pieces for 25 min.

2- Extraction of food mutagens

The blue rayon method recommended by Hayatsu (1992) was used to extract the heterocyclic aromatic amines (HCA), which may be formed during cooking of chicken by one or more of the three cooking methods. The extracts were evaporated to dryness under reduced pressure. The residue dry matter were stored in (-20°C) until use. For the mutagenicity assays, the residue was dissolved into 2 ml of dimethyl sulfoxide.

3- Yeast point mutation, gene conversion and Mitotic crossing over assay

The mutagenic potentialities of sodium azide and cooked chicken extracts were evaluated using yeast D_7 assay. Cultures preparation, treatments, scoring the frequency of different mutation types and analysis of data were estimated as described by Parry and Parry (1984).

4- Somatic cell mutation and recombination test (SMART)

The used method for SMART assay, crosses, treatments and wing preparations were done according to Würgler and Vogel (1986). F_1 trans-heterozygous larvae of *mwh/flr*^{3} (72 hours after egg hatching) were treated with Amrizole as a positive control as well as LC_{50}% of each extracts (which was determined in preliminary tests according to Raymond (1985).

The number of mosaic spots as well as clone size on the dorsal and ventral surface of mounted wings were scored under a compound microscope at 400X for each treatment and statistically analyzed according to Frei and Würgler (1988) compared to the negative control.

5- Evaluation of chromosomal aberrations in rat bone marrow cells

Animals were orally treated with three different levels of each tested cooked chicken extract [High dose (H), medium dose (M) and low dose (L)], which were chosen after preliminary tests of cooked chicken extracts according to the hazardous genotoxic effects of different cooking methods.

Treated animals were given standard laboratory diet and watered adlibitum. Colchicine treatment, bone marrow cells isolation and cytological preparations were applied according to Brusick (1980). Fifty well spread metaphase cells per animal were assayed and scored for chromosomal aberrations. These results were statistically analyzed using the Student's *t-test* according to Snedecor (1980) to compare different treatments with the corresponding negative control result.

RESULTS AND DISCUSSION

1 - Mutagenic effects of tested cooked chickens extracts on S. cerevisiae strain D_7

The percentage of cell survival after exposure D_7 strain to different concentrations of HCA extracted from vertical gas broiled chicken (VGBC), fried chicken (FC), microwave chicken extracts (MWC), and dimethyl sulphoxide (DMSO) comparing with survival curve of sodium azide (SA) which was drastically declined with the increase of extracted HCA concentrations. This result indicated the toxic effects of the extracted HCA on yeast D_7 strain, where the order of toxicity for tested HCA extracted from different cooked chickens and compounds was AZ > VGBC > FC > MWC >DMSO.

The results of genotoxic potentialities of extracted HCA from vertical gas broiled chicken (VGBC), fried chicken extracts (FC), microwave chicken extracts (MWC), dimethyl sulphoxide (DMSO) and sodium azide (AZ) to induce reverse mutations, gene conversions and mitotic crossing over mutations in yeast D_7 assay, compared with the negative control are summarized in Table (1).

The spontaneous rates of revertants, crossing over and gene conversion in control experiment were carried out at accepted limits according to Parry and Parry (1984). DMSO gave only a slight positive effect on mitotic crossing over level; while, sodium azide treatment (5 ppm) increased the frequencies of all mutation types (Table 1). This result is in agreement with Silhankova *et al.,* (1979) who found that sodium azide is directly induced gene mutation in yeast.

All extracted HCA from vertical gas broiled chicken; fried chicken and microwave-cooked chicken at concentrations of (10, 10 and 20 µl/ml, respectively) significantly increased the frequencies of reverse, gene conversion and crossing over mutations comparing with the corresponding negative control. The frequencies of induced mutations were ranged from 2.37 to 4.2 folds of spontaneous mutation rate, as shown in Table (1). These results indicated that extracted HCAs were capable of inducing point mutations, gene conversion and mitotic crossing over mutations, which indicated the formation of one or more of HCA food mutagens in cocked chickens by any one of these methods. The obtained results are in agreement with those of Paladino *et al.* (1999)

2- Mutagenic effects of tested cooked chicken extracts on Drosophila somatic mutation and recombination test (SMART)

The results of induced mosaics in somatic mutation and recombination test using wing mosaic spot assay after feeding larvae heterozygous for multiple wing hair (*mwh*) and flare^3 (*flr*^{3}) mutations on standard Drosophila medium supplemented with LC_{50} concentration of extracted HCA of vertical gas broiled chicken (25 µl/ml), fried chicken (25 µl/ml) or microwave-cooked chicken (12

µl/ml) for 24 hours as well as spontaneous mutations (the concurrent negative control), DMSO (50 µl/ml) and Amrizole (2 mg/ml) are shown in Table (2).

In the negative control experiment, the frequencies of small single spots (SSS 1-2 cells/spot) and large single spots (LSS <2 cells/spot) were 0.58 and 0.9 per wing, respectively, with an average frequency of 1.48 spot/wing single spots, while no twin spot was observed (Table 2).

DMSO insignificantly increased the frequencies of SS spots and LS spots (0.97 and 1.225 spot/wing, respectively). The total average of mosaic spots was 2.2 spots/wing (88/40) as shown in Table (2).

The frequencies of mosaic small single spots and large single spot induction exhibited highly significant increases after feeding of Drosophila larvae with Amrizole (Metronidazole, 2 mg /ml), where the frequencies of 2.3 and 2.6 spots/wing, respectively, with an overall average of 4.9 spots/wing were scored (Table 2).

After treatment with extracted HCA from VGBC, the average of observed small single spots was similar to the spontaneous SS spot mosaic rate (0.58 spot/wing). In contrast, the average of LS spots (2.95 spot/wing) as well as the average of overall spot induction was highly significant comparing with the negative control (Table 2).

The averages of induced small single spots (1.68 spots/wing) and large single spots (2.3 spots/wing) after extracted HCA of fried chicken treatment were highly significant differed when compared with the negative control. In general, the overall spot induction was highly significant different from the control level (Table 2).

The frequency of induced small single spots was significantly high after treatment with extracted HCA of microwave-cooked chicken, where 49 spots among 40 examined wings with an average of 1.23 spots/wing were recorded. On the contrary, the frequency of large single spots (1.08 spots/wing) was insignificant after MCE treatment. However, the overall average of spot induction (2.3 spots/wing) was statistically highly significant different from the control level (Table 2).

According to Graf *et al.* (1992), the distribution of induced single spots according to their size can be used to discriminate between the mutagen and pro-mutagen compounds, where the direct mutagen induce large spots comparing to the pro-mutagen which induces high frequency of small single spots. The obtained results showed that extracted HCA of VGBC and FC were capable of inducing high frequencies of small single spots, indicating the presence of one or more of pro-mutagen substance in these extracts. However, the activated promutagen of extracted HCA from VGBC seemed to be detoxificated more rapidly, where the frequency of small single spots (1-2 cells) was slightly

decreased than the control level (Table 2). The obtained results of extracted HCA from MC showed that this extract had especial characteristics for its mutagenic activity, which affect the frequencies of different size single spots, which could interpret with the nature of the mutagen and/or the presence of highly toxic compound(s) for Drosophila in this extract. In general, these results are in agreement with the results of Graf *et al.* (1992) and Delgado-Rodriguez *et al.* (1995).

3- Clastogenic effects of tested cooked chicken extracts on chromosomes of rat bone marrow cells

Table (3) presents the obtained results of *in vivo* clastogenicity assay of tested extracted HCA from different cooked chickens using three doses; low (L), medium (M) and high (H) doses for each assay, to evaluate their capabilities to induce chromosomal aberration in white rat bone marrow cells comparing with the negative, solvent (DMSO) and the positive controls.

In the negative control, the frequencies of abnormal cells with chromosomal aberrations were the same (2.67%) with an average of 1.33 ± 0.33. The observed chromosomal aberrations were 2 gaps, one break and one dicentric chromosome.

DMSO at a dose of 6.25 ml/kg b.wt., insignificantly increased the frequency of abnormal cells by 4% as shown in Table (3). This result is in agreement with Kapp and Eventoff (1980) who found that DMSO in low concentrations couldn't increase the frequency of chromosomal aberrations in rat bone marrow cells, while the highest doses could effectively disrupt the integrity of rat chromosomal structure.

MMC induced highly significant increment of abnormal cells and the chromosomal aberrations (18.67% and 21.33 % with the average of 9.33 ±1.2 and 10.67 ± 1.86, respectively). MMC as a DNA cross-linkage agent induced many types of chromosomal aberrations (Table 3). This result confirmed the clastogenic potentiality of the anticancer drug MMC on chromosomes of rat bone marrow cells, sister chromatid exchanges and chromosomal aberrations in rat (Fischman and Kelly, 1999).

In extracted HCA of vertical gas broiled-chicken treatments, all tested doses gave clastogenic effects on treated animals as shown in Table (3), whereas at low dose (1.56 ml/kg) of treated group, the average of abnormal cells (6.67 ± 1.2) and the chromosomal aberrations average (11.33 ± 2.33) were significantly different from the corresponding average of the control group. The average of abnormal cells (9.33 ± 0.88) as well as the average of chromosomal aberrations (13.67 ± 1.2) in medium dose treatment were highly significant different from the corresponding averages of the negative control (Table 3). Similar results were obtained with high dose treatment, where the average of abnormal cells (9.33 ± 0.58) as well as the average of chromosomal aberrations (13.33 ± 2.19) were detected

in this experiment. With regard to the observed chromosomal aberration types, 22 gaps, 14 breaks and 4 fragments were scored (Table 3).

These results indicated that extracted HCA VGBC might contain one or more clastogenic compound(s), which was capable to induce chromosomal aberrations in rat bone marrow cells. It could be noticed that gaps, breaks and fragments were the most frequently induced aberrations of this extract (Table 3); therefore, the suspected HCA clastogen may exert its effects via DNA breaks.

Treatments of extracted HCA from fried chicken showed that the average of induced chromosomal aberrations (8.00 ± 2.65) in low dose treatment was non-significantly different from the averages of the corresponding negative control. While the difference between chromosomal aberrations average of treated group with the medium dose (11.33 ± 2.33) and the control group (1.33 ± 0.33) was significant. However, at the high dose treatment, extracted HCA of FC gave highly significant increase for averages of both abnormal cells and chromosomal aberrations (8.00 ± 0.58 and 15.00 ± 2.08, respectively) as shown in Table (3).

These results indicated that fried chicken extract might contain one or more of HCA weak clastogenic compound(s), which was capable of inducing chromosomal aberrations in rat bone marrow cells. It could be observed that gaps and breaks were the most frequently induced aberrations due to treatment with this extract (Table 3).

After treatment with different doses of extracted HCA from microwave-cooked chicken, the frequencies of aberrant cells as well as chromosomal aberrations increased. AT low dose, the average of abnormal cells was significantly different from the negative control (8.00 ± 1.53), while the average of chromosomal aberrations was highly significant differed from the corresponding averages of negative control (10.33 ± 1.86) as shown in Table (3). Moreover, the treatment with medium dose (1.56 ml/kg b. wt) showed that the average of induced aberrant cells (9 ± 1.53) was highly significantly different from the control. The frequency of chromosomal aberrations (11.00 ± 1.15) was also highly significant different from the control value. Meanwhile, at the high dose treatment, the averages of aberrant cells (12.33 ± 1.67) and chromosomal aberrations (16.00 ± 2.56) showed highly significant increases compared with the control values (Table 3).

These results indicated that microwave-cooked chicken extract might contain one or more clastogenic compound(s), was capable to induce chromosomal aberrations in rat bone marrow cells. It could be observed that the most induced aberrations of this extract were breaks and gaps which might reflect that mode of action of this mutagen(s) may be due to DNA strand breaks.

The results of mutagenic activity of extracted HCA of different cooked chick-

ens on the three mutagenicity assays in present study are summarized in Table (4). From this table, the cooked chicken extracts could be arranged according to their mutagenic potentiality to: fried chicken > microwave-cooked chicken > vertical gas broiled chicken.

To interpret the variation between different cooking methods, according to Knize *et al.* (1998) and Skog and Solyakov (2002) chemical analysis of foods showed that frying forms predominantly HCA ranged from 0.1 to 14 ng/g. The high levels of HCA, over 300 ng/g were detected in horizontal grilled chicken breast. Heterocyclic aromatic amines (HCAs) gain their biological activity upon metabolic conversion by phase I and phase II enzymes. Where, HCAs require metabolic activation by drug metabolizing-enzymes to exert their mutagenic and carcinogenic effects (Kato and Yamazoe, 1987). Therefore, understanding the processing conditions that form HCA can lead to methods that greatly reduce their occurrence in processed foods. However, Solyakov and Skog (2002) showed that the content of HCAs in chicken cooked by different ways is low if prepared at low temperatures, and increases with increasing cooking temperature. Chicken cooked at low temperatures contained low amounts of HCAs.

The obtained results indicated that the mutagenic activity of extracted HCA compound(s) from microwave-cooked chicken showed a special characteristic of mutagenic activity in *Drosophila* wing spots assay. Although, it gave a weak positive response to increase induced mutations rates of yeast D_7 assay, it gave high significant increase of chromosomal aberrations in rat bone marrow cells. These results indicated the presence of food mutagens in this extract but the expected compound and the mutagen mechanism induction is not clear. Scientists declare that the quantum energy of microwave radiation is some orders of magnitudes less than required to dissociate covalent bonds and to trigger chemical reactions. It is therefore assumed that proteins, fatty acids, vitamins, etc. were not changed and no chemical effects can be detectable in microwave-cooked nutrients. On the contrary, some studies revealed that microwave may even result in the development of new unknown substances, where Ji and Bernhard (1992) showed clearly that maillard reaction between aldoses and amino acids, which is the first step in HCA formation induced by microwave heating. There is little information about the effects of microwave-cooked food on human beings. Only one study by Blanc and Hertel (1992) was proved that food which had been cooked in a microwave oven caused significant changes in the blood immediately after incorporation by the test persons. The authors noted that some of these changes could be highly significant, indicated the beginning of a pathological process, e.g. the beginning of cancer. However, the present results indicated the hazardous of microwave-cooked food where, the extracted material induced remarkable mutagenic effects in the three test systems.

The vertical gas broiled was expected to reduce the chicken content of PAH, where, little of smoked fats produced due to the heat source beside cooked material. The obtained results indicated that method of cooking gained low level of mutagenic potentiality, but it was significant in yeast assay and highly significant in *Drosophila* and rat assays. The reduction of PAH contents interpret the decrease of mutagenic activity which is in partial agreement that of Solyakov and Skog (2002).

The three mutagenicity test systems, which were used in present study, revealed good indications for the degree of mutagenic activity and covered all the expected genetic end points which could be affected due to DNA damage. However, *Drosophila* assay gave the best sensitivity to the mutagenic activity of tested extracts and reflected the variation of different extract mixtures as revealed from studying of spot size distribution. The obtained results supported that somatic mutation and recombination test is more efficient as genotoxicity assay, which is able to detect a wide spectrum of genetic end points, such as point mutations, deletions, certain types of chromosomal aberrations as well as mitotic recombination and gene conversion as indicated by Graf *et al*. (1984) and Würgler and Vogel (1986). Chromosomal aberrations assay of rat bone marrow indicated good sensitivity to the clastogenic activity of tested compounds. However, weak positive activity of tested extracts that was obtained using yeast D_7 strain showed the marked variation between yeast and both Drosophila and rats. Although Bronzetti *et al.* (1992) reported that the yeast cells contain a metabolizing system similar to that of mammalian systems and provided a good biological model alternative to S9 fraction. The obtained results indicated that metabolizing system was not sufficient to catalyze the metabolic transformation of PAH and/or HCA to high potent mutagens. On the other hand, these results revealed that Drosophila possesses similar metabolizing activity of mammalian system as indicated by Würgler and Vogel (1986) and Vogel (1987) who demonstrated that Drosophila is capable to activate enzymatically pro-mutagens and pro-carcinogens *in vivo*.

SUMMARY

Several evidences indicated that diet and dietary behaviors can contribute to human cancer risk. One way is this occurs through the ingestion of food mutagens. Heterocyclic amines (HCAs) are important class of food mutagens and carcinogens, which can be found in cooked meat, chicken and fish. The presence of these mutagens and their types is dependant on cooking method, heating temperatures and time of cooking. In this study, the effect of cooking method (frying, broiling and microwave-cooking as conventional and unconventional cooking methods) on formation of food mutagen(s) in Egyptian cooked chicken was studied using three short-term mutagenicity assays; yeast D_7, somatic

mutation and recombination test in *Drosophila* and rat bone marrow chromosomal aberrations assay. Mutagenic/carcinogenic heterocyclic amines (HCAs) are formed at low levels (ng/g) during heat processing of protein-rich food; therefore blue rayon was used as effective extraction and purification method. The results of mutagenicity bioassays indicated that all used cooking methods in this study were capable of forming food mutagen(s) and gave positive significant mutagenic effects in all tested assays. The cooked chicken extracts could be arranged according to their mutagenic potentiality as: fried chicken > microwave-cooked chicken > vertical gas broiled chicken.

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Table (1): Response of *S. cerevisiae* strain (D_7) for treatment with Vertical gas broiled chicken extract (VGBCE), Fried chicken extract (FCE), microwaved chicken extract (MWCE), dimethyl sulfoxide (DMSO) and sodium azide comparing with the negative control.

Treatments	Concentration	Survival	Revertants			Convertants			Mitotic Crossing Over		
			Mutation frequency E-05	T/C	Degree of activity	Mutation frequency E-05	T/C	Degree of activity	Mutation frequency E-05	T/C	Degree of activity
Control	0.0	100.00	30.00	1.00	-	27.67	1.00	-	68.33	1.00	-
DMSO	20 µl/ml	19.62	35.67	1.19	-	45.33	1.64	-	186.67	2.73	+
Sodium azide	5ppm	9.80	750.00	25.00	++	72.00	2.60	+	820.00	12.00	++
Vertical gas broiled Chicken(VGBCE)	10 µl/ml	11.39	97.33	3.24	+	93.67	3.39	+	287.00	4.20	+
FCE	10 µl/ml	12.66	96.67	3.22	+	74.67	2.70	+	236.67	3.46	+
MWCE	20µl/ml	10.76	82.33	2.74	+	65.67	2.37	+	216.67	3.17	+

T/C = Frequency of treatment induced mutations/ frequency of spontaneous mutations in negative control.
+ and ++ significant and highly significant difference from negative control: + when T/C > 2 to 10 and ++ when T/C > 10 according to Parry and Parry (1984)

Table (2): Frequencies of wing spots after larvae exposure to Vertical gas broiled chicken extract (VGBCE), Fried chicken extract (FCE), microwaved chicken extract (MWCE), dimethyl sulfoxide, and metronidazole comparing with the negative control.

Compounds	Treatments[a]	No. of wings	Spots per wing (No. of spots) Statistical diagnosis			
			Small singles (1-2 cells)	Large singles (>2 cells)	Twin spots	Total
Control	0.0	40	0.580 (23)	0.90 (36)	0.00 (0)	1.48 (59)
Dimethyl sulfoxide	50 µl/ml LF	40	0.975 (39)	1.225 (49)	0.00 (0)	2.2 (88)
Metronidazole	2 mg/ml LF	40	2.300 (92)++	26.0 (104)++	0.00 (0)	4.9 (196)++
Vertical gas broiled chicken	25 µl/ml LF	40	0.580 (23)	2.95 (118)++	0.00 (0)	3.53 (141)++
Fried chicken	25 µl/ml LF	40	1.680 (67)++	2.30 (92)++	0.00 (0)	3.98 (159)++
Microwaved chicken	12 µl/ml LF	40	1.230 (49)+	1.08 (43)	0.00 (0)	2.3 (92)++

a: LF = Larvae feeding
b: Statistical diagnosis according to Frei and Würgler (1988): +, positive; i, inconclusive. Probability level $\alpha = \beta = 0.05$, one-sided statistical test.

Table (3): Number and percentages of total chromosomal aberration types in rat bone marrow cells after treatments with three different concentrations of Vertical gas broiled chicken extract (VGBCE), Fried chicken extract (FCE), Microwaved chicken extract (MWCE), comparing with the negative, Dimethyl sulfoxide (DMSO) and Mitomycine C (MMC) as a positive control.

Treatments	Dosage	No. of examined cells	Abnormal Cells			Chromosomal Aberrations			Type of Aberrations					
			No.	%	Mean ± SE	No.	%	Mean ± SE	Gap	Breaks	Fragments	Dicentric	Deletions	Ring chro-mosom
Negative control		150	4	2.67	1.33 ± 0.33	4	3.33	1.33 ± 0.33	2	1	0	1	0	0
MMC	3.5 mg/kg	150	28	18.67	9.33 ± 1.2**	32	21.33	10.67 ± 1.86**	12	6	7	2	5	0
Dimethyl sulfoxide	6.25 ml/kg b.wt.	150	6	4.00	2.00 ± 0.0	6	4.00	2.00 ± 0.0	4	0	2	0	0	0
Vertical gas broiled chicken	1.56 ml/kg b.wt.	150	20	13.33	6.67 ± 1.2*	34	22.67	11.33 ± 2.33*	10	20	3	1	0	0
	3.125 ml/kg b.wt.	150	28	18.67	9.33 ± 0.88**	38	25.33	12.67 ± 1.2**	13	22	3	0	0	0
	4.688 ml/kg b.wt.	150	28	18.67	9.33 ± 0.58**	40	26.67	13.33 ± 2.19**	22	14	4	0	0	0
Fried chicken	1.56 ml/kg b.wt.	150	14	9.33	4.67 ± 1.2	24	16.00	8.00 ± 2.65	6	17	0	1	0	0
	3.125 ml/kg b.wt.	150	17	11.33	5.67 ± 1.2*	35	23.3	11.67 ± 2.19*	10	25	0	0	0	0
	4.688 ml/kg b.wt.	150	24	16.00	8.00 ± 0.58**	45	30.00	15.00 ± 2.08**	24	19	2	0	0	0
Microwaved chicken	0.625 ml/kg	150	24	16.00	8.00 ± 1.53*	31	20.67	10.33 ± 1.86**	9	19	3	0	0	0
	1.56 ml/kg	150	27	18.00	9.00 ± 1.53**	33	22.00	11.00 ± 1.15**	5	25	3	0	0	0
	3.125 ml/kg	150	37	24.67	12.33 ± 1.76**	48	32.00	16.00 ± 2.65**	21	23	4	0	0	0

* and ** significant and highly significant difference from the negative control at $P<0.05$ and $P<0.01$ using Student's t test.

Table (4): The mutagenic activity of different cooked chicken extracts (Vertical gas broiled chicken extract (VGBCE), Fried chicken extract (FCE), and microwaved chicken extract (MWCE) in different mutagenicity test systems: Yeast D_7 assay, Drosophila wing spot assay and Rat bone marrow chromosomal aberration assay.

Cooking method	Yeast D_7 assay[a]				Drosophila wing spot assay[b]				Rat bone marrow chromosomal aberration assay[c]		
	Treatment	Rev.	Con.	Rec.	Treatment	S. S. Spots	L. S. Spots	Total Spots	Treatments	Ab. cells	Ch. Ab.
Vertical gas broiling	10 µl/ml	+	+	+	25 µl/ml	-	++	++	1.560 ml/kg	+	+
									3.125 ml/kg	++	++
									4.688 ml/kg	++	++
Frying	10 µl/ml	+	+	+	25 µl/ml	++	++	++	1.560 ml/kg	-	-
									3.125 ml/kg	+	+
									4.688 ml/kg	++	++
Microwave	20 µl/ml	+	+	+	12 µl/ml	+	-	++	0.625 ml/kg	+	++
									1.560 ml/kg	++	++
									3.125 ml/kg	++	++

a: Rev. = reverse mutations, Con.= gene conversion and Rec.= mitotic recombination.
b: S. S.= small single, L. S.= Large single.
c: Ab.= abnormal and ch. Ab.= chromosomal aberrations.

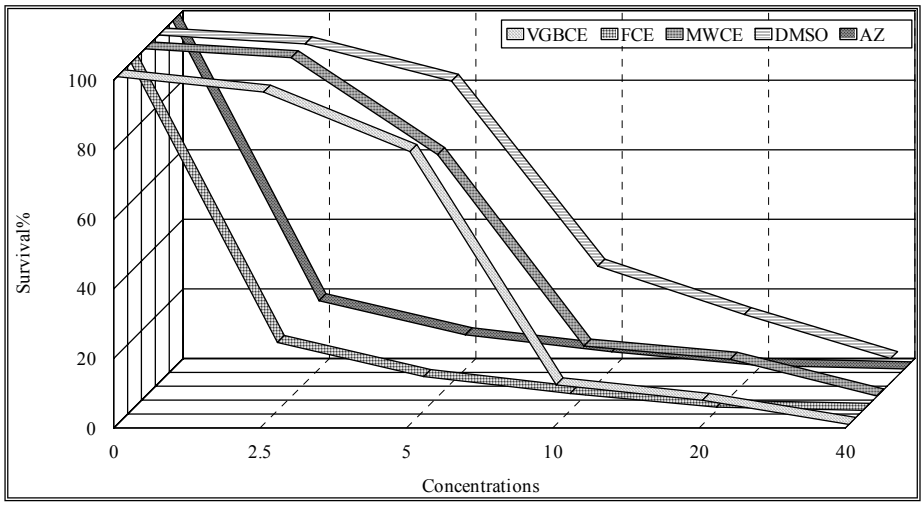

Fig. (1): Diagram represents the sensitivity of yeast D_7 strain for the toxic effects of different concentrations of vertical gas broiled chicken extract (VGBCE), fried chicken extract (FCE), microwave chicken extracts (MWCE), Dimethyl sulphoxide (DMSO) comparing with survival curve of Sodium Azide (AZ).

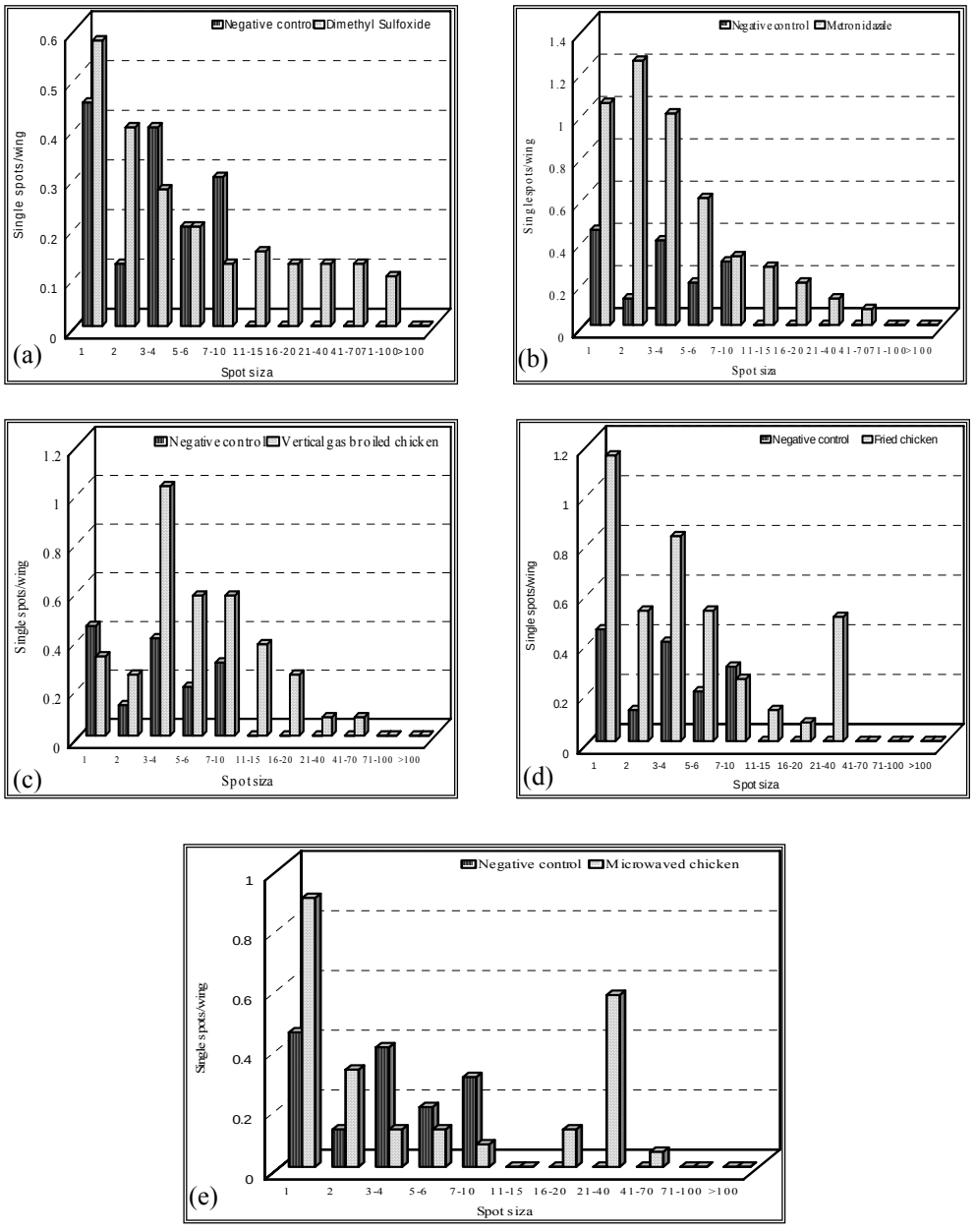

Fig. (2): Diagram represents induced wing spots distribution according to spot size for single spots after treatment with (a) dimethyl sulfoxide (b) Metronidazole (c) Vertical gas broiled chicken extract (VGBCE), (d) Fried chicken extract (FCE), and (e) Microwaved chicken extract (MWCE).

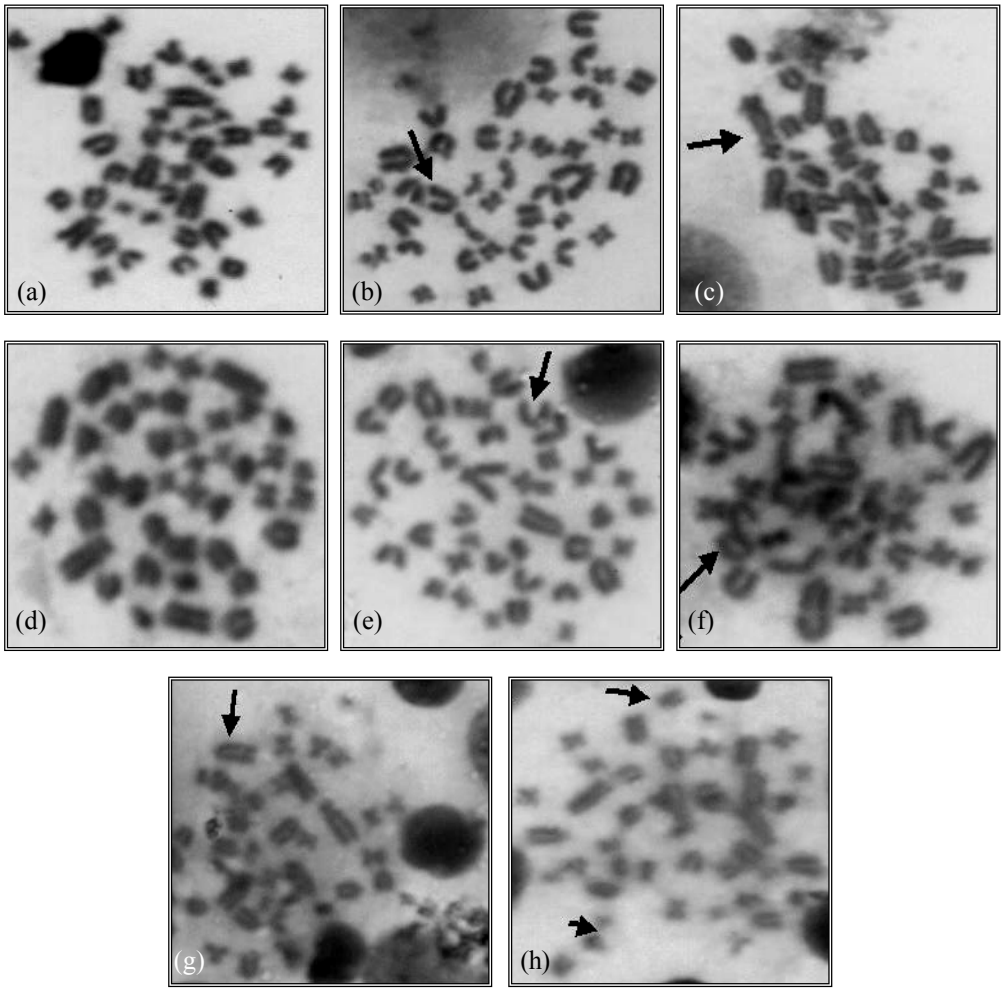

Fig. (3): Photograph represents typical rat bone marrow cells with either normal or aberrant metaphase plates: (a) normal chromosomes (b) deletion, (c) dicentric (d) Sticky, (e) break (f) ring chromosome (g) break and (h) gap and fragment.