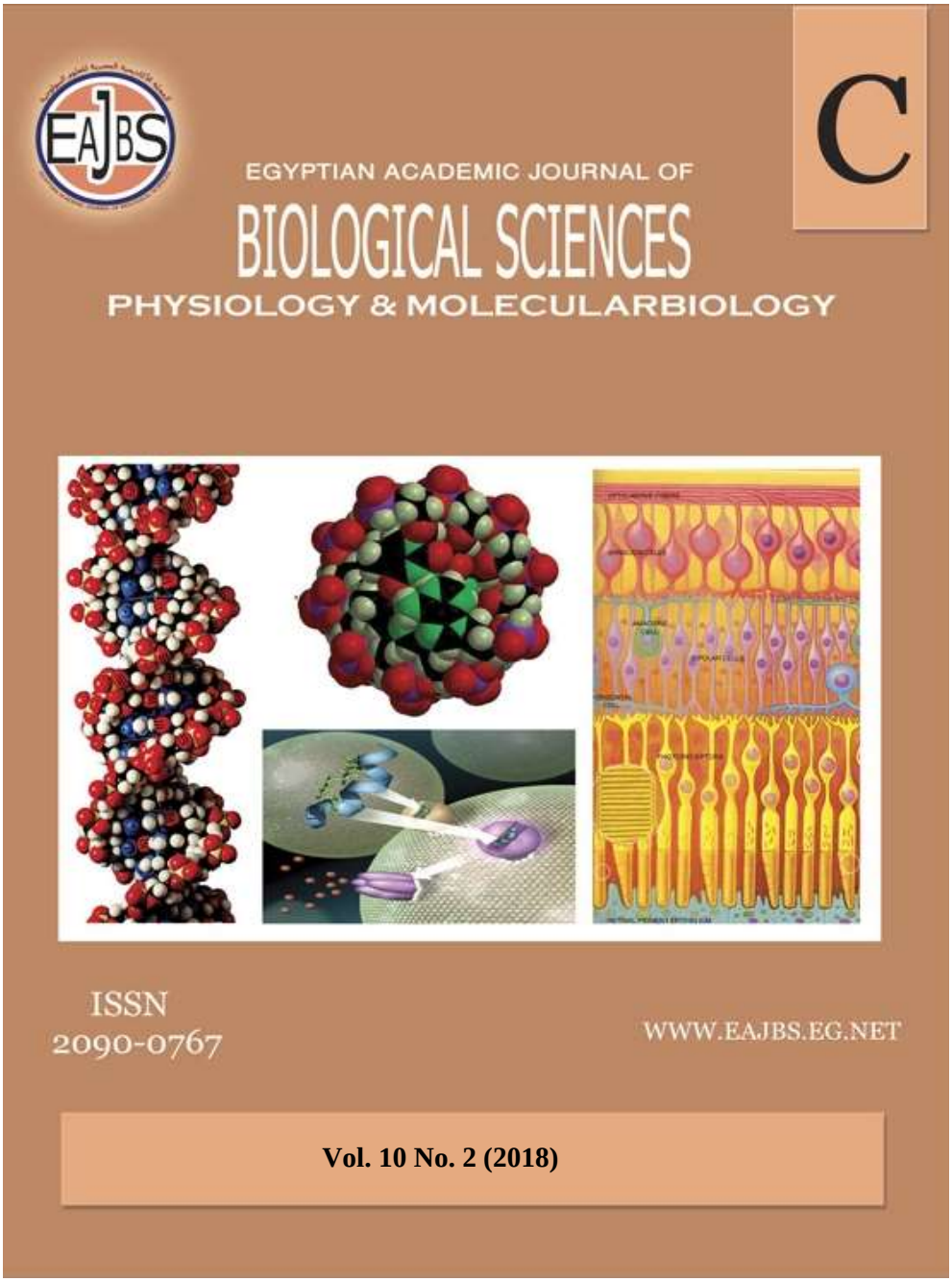

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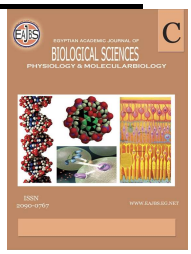
www.eajbs.eg.net

Egypt. Acad. J. Biolog. Sci., 10(2): 83- 97 (2018)

Egyptian Academic Journal of Biological Sciences
C. Physiology & Molecular Biology
ISSN 2090-0767
www.eajbs.eg.net



Assessment of Genotoxicity of Potassium Nitrate and Sodium Benzoate in *Drosophila melanogaster* Using Smart and Comet Assays

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ARTICLE INFO
Article History
Received:2/8/2018
Accepted: 10/9/2018

***Keywords*:**
Genotoxic
Comet
SMART
Drosophila
food additives

ABSTRACT

Modern food industry increasingly relies on food additives. The dramatic increase in the demand on preserved ready to eat food products making up to 75 percent of the diet of western society lead to neglectance of the harmful effects of the food additives on human health; among these are hypersensitivity, allergic reactions, genotoxicity, mutagenicity and more. This proposal investigates genotoxic effects of two commonly used food preservatives; sodium benzoate and potassium nitrate using the somatic mutation and recombination test (SMART) and comet assay. Two important end points of genotoxicity can be covered by *in vivo* assay systems; comet assay detects DNA damage, while the *Drosophila* transgenic animal model recognizes gene mutation and/or chromosomal aberrations. Both of the tested compounds showed significantly high levels of tumor induction and frequency compared to a negative control in SMART assay accompanied with a significant amount of DNA damage detected by the comet assay indicating their high potential of being genotoxic materials.

INTRODUCTION

Food additives are substances frequently added to processed food to serve several purposes including; extending shelf time by retarding or inhibiting the growth of microorganisms, colouring, sweetening, flavouring and thickening (Rekha and Dharman 2011). The food and drug administration FDA regulates the allowed amounts of these substances to reduce the possible overconsumption of food additives. However, long-time consumption of such substances even in small amounts may harm the consumer (Tuormaa 1994). It is well documented that certain types of foods and beverages allowed for human consumption may pose toxic, genotoxic or carcinogenic hazards (Aeschbacher 1990, Wakabayashi 1990). One of the sources of these hazards was attributed to food additives that may have an obvious harmful effect (IARC 1983).

The carcinogenic risk of food additives cannot be neglected and may be attributed to various factors that may include; interaction of additives with some food ingredients, food processing may change the chemical formula of food additives to a formula like carcinogenic compound, a negative synergistic effects when combined with other additives, unsuitable storage conditions and unknown carcinogenic by-products occurring during the food processing (Gülsoy et al. 2015).

Potassium nitrate (E252) is a preservative commonly used in meat products, certain kinds of cheese and for reducing discoloration of vegetables and fruits such as dehydrated potatoes and dried apples (Binkerd and Kolari 1975). The carcinogenic potential of nitrates and nitrites used as preservatives and color-enhancing agents in meats was confirmed by (Nujić and Habuda-Stanić 2017). *In vitro* treatment of human peripheral blood cells with potassium nitrate caused a decrease in mitotic index MI as compared to control (Mpountoukas et al. 2008). Gömürgen (2005) study on the effect of potassium nitrate on root tips of *Allium cepa* showed the reduction in the MI, indicating mitotic inhibition and increased frequency of abnormal mitosis. The types of abnormalities observed were chromosome stickiness, c-metaphase, disturbed chromosomes of anaphase and telophase stages, anaphase and telophase bridges, anaphase lagging and forward chromosomes at anaphase and telophase and micronuclei formation at interphase cells. The same results were observed in root tips of *Allium cepa* treated with sodium nitrate (Pandy et al. 2014).

Sodium benzoate is one of the synthetic additives that is widely used in the food industry and is generally recognized as safe (GRAS) (Hanes 2017). It is widely used as a preservative against fungi, yeast and bacteria in food and soft drinks industry (Hong et al. 2009). Maximum recommended a concentration of sodium benzoate as a preservative according to WHO (1997) was in the range of 2000 mg/kg. Genotoxicity tests for benzyl alcohol, benzoic acid and sodium benzoate have mostly reported negative results but some assays were positive in cell lines and plants (Turkoglu 2007). Ishidate and Odashima (1977) reported chromosomal aberrations *i*n Chinese hamster cell lines grown in culture with sodium benzoate. Onyemaobi et al. (2012) evaluated the effect of sodium benzoate and sodium metabisulphite with different concentrations on root length, chromosomal aberrations and MI of *Allium cepa* plant. The MI decreased with increasing concentration of both sodium benzoate and sodium metabisulphite. Clumping and fragmentation were the most common among the observed cytological aberrations. The percentage of chromosomal aberrations at mitosis increased as the concentration of the food preservatives increased. The irreversible cytotoxic effects induced by the two tested additives supports the call for banning these substances as food preservatives.

Currently, there are more than 100 genotoxicity assays published so far (Nohmi et al. 2012). The major end points of short-term genotoxicity assays include aneuploidy and chromosomal aberrations, DNA

damage, and point mutations (Fairbairn et al. 1995, Nohmi et al. 2012).

The use of alternative small organisms as models in toxicology has grown tremendously in the last decade. *Drosophila* has always been a premier model for both developmental biologists and geneticists, however, several recent toxicology studies have used this organism. Currently, *Drospohila* is being used in studies of a number of priority environmental contaminants and toxicants (Rand *et al.* 2015). Using *Drosophila* as a genetic model, there are certain mutagenicity test systems to detect aneuploidy and chromosomal aberrations in germ line cells and in somatic cells, dominant lethal mutations, sex-linked and autosomal recessive lethal mutations, and translocations (Würgler and Vogel 1986, Zimmering et al. 1990). One of the most widely accepted genotoxicity tests is the Somatic Mutations and Recombination Test (SMART) carried out in *Drosophila melanogaster* (Demir et al. 2013). This assay uses tumor suppressor gene *warts* which is a homolog to the mammalian tumor suppressor gene *LATS* (Nepomuceno 2015, Vasconcelos *et al*. 2017). Loss of the *wts* gene not only results in over proliferation but also in apical hypertrophy of epithelial cells, leading to abnormal deposition of extracellular matrix (cuticle) during adult development (Justice *et al*. 1995). The test for detection of epithelial tumor clones (*warts*) represents a rapid, very sensitive to different classes of agents and inexpensive assay to evaluate the carcinogenic activity of single compounds as well as of complex mixtures. Various protocols are available for the application of the test materials; single or combined as well as sequential treatments of larvae by feeding. Factors capable of inducing tumors in *Drosophila* instead of marker clones might directly adverse the risk of these factors for inducing cancer in humans (Sidorov et al. 2001). Genetic events that can lead to the tumor appearance in flies heterozygous for the *wts* gene and hence can be detected by SMART may include; gene mutations in the *wts* gene, multilocus-deletions (partial), chromosomal loss and somatic recombination collectively referred to as loss of heterozygosity (Eeken et al. 2002).

Comet assay is a microgel electrophoresis technique for DNA damage detection at the level of single cells. The most important advantage of this technique is that DNA lesions can be measured in any organ, regardless of the extent of mitotic activity (Sasaki et al. 2000). The DNA damage measured by DNA strand breakage in the form of single- or double-strand breaks performs as a reliable indicator of genotoxicity. A number of studies showed that comet assay is a rapid, sensitive and inexpensive test for detecting DNA damage which is widely used for detecting genotoxicity of chemical compounds under laboratory and field conditions in mice (Sasaki et al. 2000), *zebra fish* (Gülsoy et al. 2015), human germ cells (Pandir 2016), and *Drosophila melanogaster* (Eid et al. 2017). Comet assay has been described as one of the most promising methods for genotoxicity studies against environmental chemicals due to its rapidity and sensitivity. Moreover, the comet assay has been recently adapted to use *in vivo* in *Drosophila* to combine its advantages with those well-established of this fly (Mukhopadhyay et al. 2004, Shukla et

al. 2011). Alkaline comet assay (pH>13), the most commonly used version, is able to detect all possible kinds of DNA damage (Tice et al. 2000).The assay is clearly useful as a tool for the evaluation of local genotoxicity, particularly organs or cell types, which can hardly be evaluated with other standard tests (Brendler-Schwaab *et al.* 2005).

The objective of this study was to evaluate the carcinogenic and genotoxic effects of two food additives; potassium nitrate, and sodium benzoate using SMART and comet assays in *Drosophila melanogaster*.

MATERIALS AND METHODS

Somatic Mutation and Recombination Test (SMART) in *D. melanogaster*:

***Drosophila melanogaster* Strains**:

Two different *Drosophila* strains were used in this study; wild-type strain and a strain that carries *wtsMT*$^{4\text{-}1}$, a lethal warts allele balanced on TM3, characterized by multiple inversions and marked by the dominant mutation stubble according to Eeken *et al*. (2002) and Fly Base (2006). The genetic structure of this strain, which was abbreviated *wts/TM3*, is; *st* p in *ri wtsMT4-1/ TM3 Sb*. Details about the various markers and the balancer chromosome was described by Lindsley and Zimm (1992).

Crosses and Treatments:

The *wts*/TM3 females were crossed to wild-type males resulting in two genotypes offspring, *wts*/+ and TM3, Sb *wts*$^{+/+}$. After 2 days, the parental flies were removed and 56-68 hours old larvae were washed with 20% glycerol, collected using a fine mesh sieve and transferred to four different vials representing the four test groups. For food additives, treated groups (potassium nitrate and sodium benzoate); the flies were transferred to a standard *Drosophila* medium to which a 100 mM of each food additive powder was added and properly dissolved at 50^{0}C. The larvae were submitted to chronic treatment for approximately 24 hours, then they were transferred to standard *Drosophila* medium. The positive control group was transferred to a vial where 20 µg/ml of an appropriate mitomycin C (MMC) solution was mixed with a standard *Drosophila* medium, kept for 24 hours, then they were transferred to standard *Drosophila* medium. The negative control group was directly transferred to a standard *Drosophila* medium. Afterwards, larvae of all groups were left to feed on the medium until completion of their development when they leave the medium and pupate. All *Drosophila* stocks and crosses were maintained at 25^{0}C. Only adult flies, without the chromosome balancer (*TM3, Sb*) with no truncated bristles were analysed.

Scoring of Warts:

After metamorphosis, the adult flies were transferred to flasks containing 70% ethanol. Flies were analysed for tumor presence using a Leica stereomicroscope used at a standard magnification of 25 X and entomological tweezers. Only tumors that were large enough to be unequivocally classified are recorded (Eeken *et al*. 2002).

Statistical Analysis:

For the evaluation of the observed genotoxic effects, in the wing, eye and whole body spots, the frequencies of spots per individual of a treated series were compared to its concurrent negative control series using

χ^2-test. A multiple-decision procedure was used to decide whether a result is positive, weakly positive, inconclusive, or negative (Frei and Würgler 1996).

DNA Fragmentation by Comet Assay (Single Cell Gel Electrophoresis, SCGE): 2nd instar larvae of the isogenic strain w^{1118} of *Drosophila melano-gaster* were treated for 24 hours with the same concentration of tested compounds as before in the SMART assay. Both treated and untreated (control) adults were assessed using the alkaline comet assay to measure the extent of DNA strand breaks in all types of cells (Singh *et al*. 1988). Adult flies were frozen in liquid nitrogen, around 100 flies were gently homogenized into powder, and then an alkaline comet assay was performed as described by Tice *et al*. (2000). 1 g of crushed samples were transferred to 1 ml ice-cold PBS. This suspension was stirred for 5 min and filtered. Cell suspension (100 µ1) was mixed with 600 µ1 of low-melting agarose (0.8% in PBS). 100 µ1 of this mixture was spread on pre-coated slides. The coated slides were immersed in lysis buffer (0.045 M TBE, pH 8.4, containing 2.5% SDS) for 15 min. The slides were placed in the electrophoresis chamber containing 0.045 M TBE buffer. The run conditions were 2 V/cm for 2 min and 100 mA. Slides were then stained with ethidium bromide 20µg/m1 at 4ºC. The observation was carried out while the samples still humid, the DNA fragment migration patterns of 100 cells for each dose level were evaluated with a fluorescence microscope using 40X objective (With excitation filter 420-490nm [issue 510nm]). A Komet™ analysis system 5.0 developed by Kinetic Imaging, LTD (Liverpool, UK) linked to a CCD camera was used to measure the length of DNA migration (tail length, in µm) (TL) and the percentage of migrated DNA (DNA %). Finally, the program calculated tail moment. Fifty to one hundred randomly selected cells were analyzed per sample (at least 25 cells per slide and 3 slides per treatment were evaluated). Three different parameters were used as indicators of DNA damage: tail moment (TM) (arbitrary units), tail DNA (%), and tail length (mm).

RESULTS AND DISCUSSION

Detection of Mutagenic Agents Using Somatic Mutation and Recombination Test (SMART) in *D. melanogaster*:

The F1 generation of the crossed flies was divided into four treatment groups: a negative control group transferred to basic Drosophila medium, positive control group transferred to a medium containing 20µg/ml MMC) and two treatment groups each was transferred to a medium containing 100mM of one of the tested compounds; potassium nitrate and sodium benzoate.

The frequency of tumors which is calculated as the ratio of the number of scored tumors to a total number of the scanned flies was 0.07 in negative control flies. Tumor induction in the negative control samples, calculated as the ratio of the number of tumors scored to the number of flies bearing the tumors, was also low (1.03). In contrast, MMC treatment scored the highest frequency (0.81) accompanied with the highest tumor induction (1.7). These tumors were found in every part of the examined flies.

As observed from Table (1) the frequency was increased significantly by either potassium nitrate or sodium benzoate as compared with the negative control. Potassium nitrate treatment showed higher tumor frequency (0.65) than sodium benzoate (0.52) while the reverse was observed in tumor induction 1.36 and 1.53, respectively, as represented by Fig. (1).

Table 1: Frequencies of induced tumor in trans-heterozygous (*wts*/+) offspring after larvae feeding treatments with concentrations of Potassium Nitrate (P.N) and Sodium benzoate (S.B) compared to both the MMC as the positive control and the negative control.

Treatments	Total No. of fly scored	No. of fly scored with tumor	No. of tumor scored	Tumor induction	Frequency (No. of Tumor/fly)
Negative control	510	33	34	1.03	0.07
MMC 20µg/ml	421	262	340	1.7	0.81
P.N 100mM	316	151	205	1.36	0.65*
S.B 100mM	299	102	156	1.53	0.52*

*and ** significant, highly significant difference from the negative control at $P<0.05$.
Frequency (No. of Tumor/fly) = Number of tumors/Total number of tested flies.
Tumor induction = Number of tumors/ Number of tumor flies.

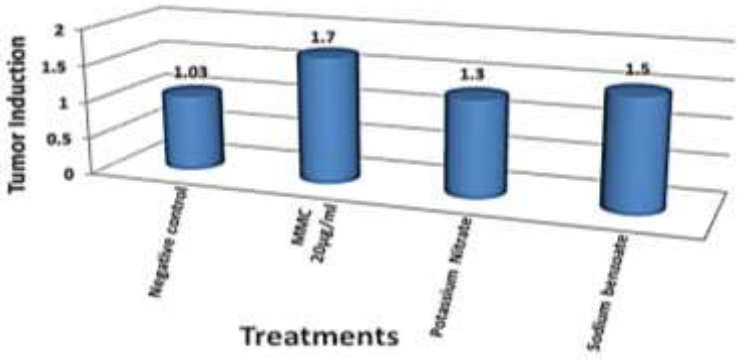

Fig. 1: Tumor induction of spontaneous and induced *warts* epithelial tumors in +/*wts* flies after treatments with mitomycin C (MMC), potassium nitrate and sodium benzoate.

DNA Fragmentation by Comet Assay (single cell gel electrophoresis, SCGE):

Comet assay (also called, single cell gel electrophoresis, SCGE) is used to detect any prospective damage for DNA after certain treatments. It detects DNA strand breaks and alkali-labile sites by measuring the migration of DNA from immobilized nuclear chromatin. Advantages of the comet assay for assessing DNA damage include: (1) damage to the DNA in individual cells is measured; (2) only small number of cells are needed to carry out the assay (<10,000); (3) the assay can be performed on virtually any eukaryotic cell type; (4) and it is faster and more sensitive than the alkaline elution method for detecting DNA damage DNA (Singh *et al*. 1988).

In the current study, DNA damage in a homogenous strain w^{1118} of adult *Drosophila* emerged from 2rd larval instar exposed to the potassium nitrate and sodium benzoate was assessed by comet assay. Data presented in Table (2) showed DNA damage parameters; tailed%, untailed %, tails length, tail DNA% and tail moment. According to the obtained data, both of the two tested salts caused significant DNA damage. Moreover, an increase in tail length was observed in Potassium nitrate and sodium benzoate treated groups as compared to control group (Fig. 2) as an indication of DNA degradation and strand breaks. Migration length is considered to be directly related to fragment size and proportional to the level of single-stranded breaks and alkali-labile sites (Tice *et al*. 2000).

Potassium nitrate exhibited a significantly higher deleterious effect on DNA of *D. melanogaster* (about 6 folds tails length as compared with control) than sodium benzoate (about 3.4 folds tails length as compared with control). Tail DNA percentage was 1.48% in control, 6.23% in potassium nitrate treatment and 3.36% in sodium benzoate treatment as recorded in Table (2).

Table 2: Detection of DNA damage by the comet assay, assessed as the tail moment (TM) in whole body cells of white eye adult *Drosophila* treated with the Potassium nitrate (P.N) and sodium benzoate (S.B).

Group	Tailed %	Untailed %	Tails length µm	Tail DNA%	Tail moment
control contr Control	1.5	98.5	1.32±0.12 [c]	1.48	1.95
P.N P.NpP P.N	22	78	7.89±0.11 [a]	6.23	49.15
S.B S.B	9	91	4.43±0.18 [b]	3.36	14.88

[a, b and c] Different superscript letters in the same column of tail length showed significant difference at $P< 0.05$.

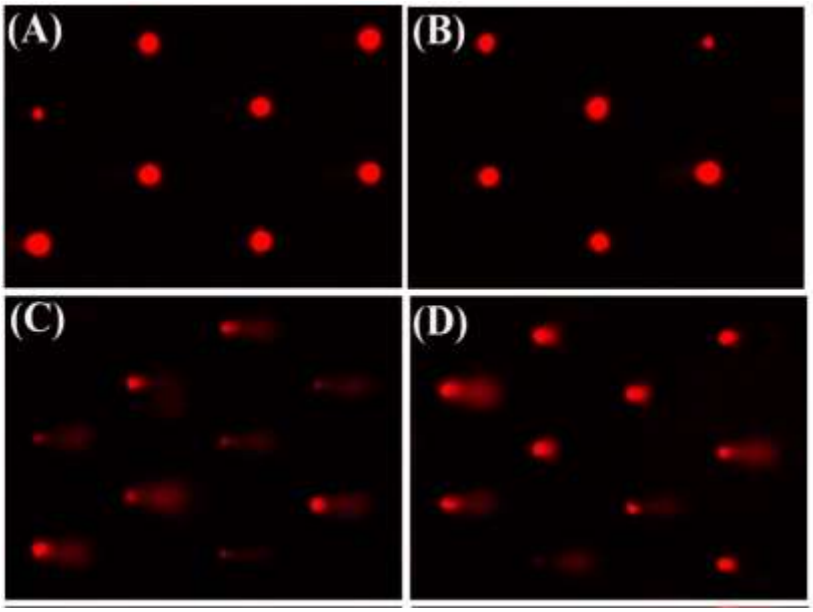

Fig. 2: Comet images representing DNA damage in adult *Drosophila* whole body cells. (A and B) represents DNA strand breaks of control, while (C) and (D) represents those after the exposure to Potassium nitrate and sodium benzoate, respectively.

This study evaluated the potential genotoxicity and carcinogenicity of two food additives commonly used in the food industry using SMART and comet assays

on *Drosophila melanogaster* system. The obtained results clearly imply the genotoxic potential of both of the tested materials. High frequency of tumor formation in *Drosophila* SMART assay strongly indicates Loss of heterozygosity (LOH) in somatic cells. Further studies will be carried out to investigate the mechanism of LOH that might involve mutation, chromosome loss or somatic recombination. The use of *Drosophila* model for understanding the human condition under stress of toxicants has been widely accepted on the basis of the presence of numerous highly conserved genes and pathways controlling the development of stress response across these two divergent species (Mackay and Anholt 2006, Misra et al. 2011, Sykiotis and Bohmann 2010). The evolutionary conservation of tumor suppressor genes among Drosophila and mammals has prompted studies of tumor induction in *Drosophila*, such studies have contributed to the understanding of cancer in human (Potter et al. 2000, Eeken et al. 2002).This striking resemblance implies that the potential risk of these tested compounds on human health cannot be ignored.

The current results are consistent with the findings of Sarikaya and Cakir (2005) who evaluated the genotoxicity of four food preservatives; sodium nitrite, sodium nitrate, potassium nitrite and potassium nitrate in SMART wing spot test. Exposure to 50, 75 and 100 mM of each tested substance increased the frequency of small single, large single and total spots. The genotoxic effect increased as concentration used increased. The four tested chemicals were ranked according to their genotoxic and toxic effects as sodium nitrite, potassium nitrite, sodium nitrate and potassium nitrate.

Nitrite or nitrate in food may react with endogenous amines, forming mutagenic and carcinogenic compounds (Ertuğrul 1998). Nitrates can be converted into nitrites, which can in turn react with secondary amines or amides to produce mutagenic and carcinogenic compounds i.e. nitrosoamines (Loon *et al*. 1998, Ertuğrul 1998). The genotoxicity of N-nitrosamines like N-nitrosodimethyl amine, N-nitrosodiethyl amine, N-nitrosodi-*n*-butyl amine, N-nitrosopiperidine and N-nitrosopyrrolidine in *Drosophila* somatic cells was detected by (Negishi *et al*. 1991). The genotoxic effects arising from the *in vivo* exposure to nitrosation precursor in *D. melanogaster* could be retarded by co-treatment with catechin (nitrosation inhibitor) (Rincon *et al*. 1998). Gömürgen (2005) found potassium nitrate to be mitotoxic, and that at higher doses it was able to induce chromosomal aberrations.

Benli and Turkoglu (2017) found that sodium benzoate among ten other tested preservatives had a significant effect on decreasing survival and longevity in *D. melanogaster* using 5, 10, 15 and 20 ppm concentrations. Sarikaya and Solak (2003) declared that 50, 75 and 100 mM concentrations of benzoic acid induced mutations and decreased the life period in *D. melanogaster*. Deepa *et al.* (2012) reported that benzaldehyde which is used to give aroma to foods have mutagenic and genotoxic effects on *D. melanogaster* via SMART assay. Saatci *et al.* (2016) evaluated the effect of sodium benzoate on lymphocytes of pregnant rats and their fetuses. They declared that Sodium benzoate usage increased micronuclei formation. Sodium benzoate was also found to inhibit DNA synthesis and induce the anaphase bridges, chromosomal condensation in root meristems of *Vicia faba* (Njagi and Gopalan 1982). Enzymes activity in the mitochondria and cytosol of rat liver hepatocytes were suppressed at doses ≥ 500 µg/ml of Sodium benzoate and DNA synthesis was suppressed at 100 µg/ml of sodium benzoate (Oyanagi et al. 1987).

The single cell gel electrophoresis test, or comet assay, was originally developed by Östling and Johanson (1984) as a microelectrophoretic technique to

visualize DNA damage in single cells. Subsequently it was improved by Singh et al. (1988), and since then it was so extensively used that some working-groups were created to standardize its application to mammal and human cells studies (Burlinson et al. 2007, Karlsson 2010, Azqueta and Collins 2013, Ersson et al. 2013, Godschalk et al. 2013, Collins et al. 2014). Its usefulness and easy performance lead to its rapid application to several fields including genotoxicity analyses (Tice et al. 2000, Hartmann et al. 2003, Collins 2004). Surprisingly, its application to *Drosophila melanogaster* was rather late, despite the fact that this organism is one of the most valuable higher eukaryotic model organism, for all kind of processes and situations related to human health (Reiter et al. 2001, Koh et al. 2006, Wolf et al. 2006, Khurana et al. 2006, Rand 2010), including the *in vivo* DNA damage response processes (Søndergaard 1993, Sekelsky et al. 2000, Vecchio 2014). In the first published work, the comet assay was performed with brain ganglia cells from third instar larvae (Bilbao et al. 2002).

As with other organisms, several cell types, apart from the brain cells, have been used to carry out this assay in *Drosophila in vivo*, such as midgut cells (Mukhopadhyay et al. 2004, Siddique et al. 2005, Sharma et al. 2011), hemocytes (Carmona et al. 2011), and imaginal disk cells (Verma et al. 2012). Most of these authors used the comet assay for its original purpose, the *in vivo* analyses of genotoxicity and DNA repair.

Suzuki and inukai (2006) reported that nitrite and nitrate may play a role in enhancing the genotoxic effects and DNA damage of UV light in humans. Saatci *et al.* (2016) found that sodium benzoate usage may cause DNA damage in liver cells of pregnant rats and their fetuses. They declared that and increase micronuclei formation. Ishidate *et al*. (1984) observed inhibition of DNA synthesis in rat liver cells following application of 100 mg/mL of Sodium benzoate. Zengin *et al*. (2011) investigated the *in vitro* effects of Sodium benzoate and potassium benzoate on cultured human peripheral lymphocytes. They found a significant DNA damage in Sodium benzoate treated groups, providing further evidence for a cytotoxic, mutagenic and clastogenic activity of sodium benzoate.

Sasaki *et al*. (2000) determined the genotoxicity of 39 chemicals used as food additives, most of them induced DNA damage in gastrointestinal organs.

CONCLUSIONS

It can be safely concluded from the present and previous work that both of the tested compounds has a noticeable genotoxic and cytotoxic potential that cannot be ignored while using such chemicals in the food industry. Further thorough investigations are recommended before continuing using these substances in food and cosmetics as additives.

REFERENCES

Aeschbacher, H.U. (1990) Genetic toxicology of food products. In: mendelsohn, M.L., Albertini, R.J. (Eds.), In Mutation and the Environment. Part E: Environmental Genotoxicity, Risk and modulation. Wiley-Liss, New York, pp. 117–126.

Azqueta, A. and Collins, A. R. (2013) The essential comet assay: a comprehensive guide to measuring DNA damage and repair. *Arch. Toxicol.* 87, 949–968. doi: 10.1007/s00204-013-1070-0

Benli D. and Turkoglu Ş. (2017) The Effect of Some Food Preservatives on Percentage of Survival and Longevity in *Drosophila melanogaster*. Cumhuriyet Sci. J., Vol., 38(3): 461-472.

Bilbao, C.; Ferreiro, J. A.; Comendador, M. A. and Sierra, L. M. (2002) Influence of mus201 and mus308 mutations of *Drosophila melanogaster* on the genotoxicity of model chemicals in somatic cells in vivo measured with the comet assay. *Mutat.Res.* 503, 11–19. doi: 10.1016/S0027-5107(02)00070-2

Binkerd, E. F. and Kolari, O. E. (1975) The history and use of nitrate and nitrite in the curing of meat. Food Cosmet Toxicol13, 655 – 661.

Brendler-Schwaab, S.; Andreas, H.; Stefan, P. and Speit, G. (2005) The *in vivo* comet assay: use and status in genotoxicity testing. Mutagenesis vol. 20 no. 4 pp. 245-254.

Burlinson, B.; Tice, R. R.; Speit, G.; Agurell, E.; Brendler-Schwaab, S. Y. AND Collins, A. R. (2007) *In vivo* comet assay workgroup, part of the fourth international workgroup on genotoxicity testing: results of the *in vivo* comet assay workgroup. *Mutat. Res.,* 627:31–35.

Carmona, E. R.; Guecheva, T. N.; Creus, A. and Marcos, R. (2011) Proposal of an in vivo comet assay using hemocytes of *Drosophila melanogaster*. *Environ. Mol. Mutagen.* 52, 165–169.

Collins, A. R. (2004) The Comet assay for DNA damage and repair. principles, applications, and limitations. *Mol. Biotechnol.* 26, 249–261.

Collins, A.; Koppen, G.; Valdiglesias, V.; Dusinska, M.; Kruszewski, M. and Møller, P. (2014) The comet assay as a tool for human biomonitoring studies: the ComNet project. *Mutat. Res. Rev. Mutat. Res.* 759, 27–39.

Deepa, P.V.; Priyanka, V.; Swarna, R. and Akshaya, S. (2012) genotoxicity of benzaldehyde in drosophila melanogaster using the wing somatic mutation and recombination test (SMART) and protein profiling. International Journal of Medical and Clinical Research. 3; 6, 195-198.

Demir, E.; Kaya, B.; Marcos, R.; Kocaoğlu, C. S. and Cetin, H. (2013) Investigation of the genotoxic and antigenotoxic properties of essential oils obtained from two *Origanum* species by *Drosophila* wing SMART assay. Turk J Biol 37: 129–138.

Eeken, J. C.J.; Klink, I.; Bert, L.; Veen, V.; Pastink, A. and Ferro W. (2002). Induction of Epithelial Tumors in *Drosophila melanogaster* Heterozygous for the Tumor Suppressor Gene *wts*. Environmental and Molecular Mutagenesis 40:277–282.

Eid J. I.; Abdel-Fattah A. A.; Wesam T. B. and Akmal, A. E. (2017) Evaluation of Genotoxicity of Lufenuron and Chlorfluazuron Insecticides in Drosophila melanogaster Using a Germ-Line Cell Aneuploidy and Chromosomal Aberrations Test. Int'l Journal of Advances in Agricultural & Environmental Engg. (IJAAEE) Vol. 4, Issue 1. 93-101.

Ertuğrul, N. M.S. (1998) Thesis, in: Food additives regulations and health problems about upper limit of some food additives, Istanbul University.

Ersson, C.; Møller, P.; Forchhammer, L.; Loft, S.; Azqueta, A. and

Godschalk, R. W. (2013) An ECVAG inter-laboratory validation study of the comet assay: inter-laboratory and intra-laboratory variations of DNA strand breaks and FPG-sensitive sites in human mononuclear cells. *Mutagenesis* 28, 279–286.

Fairbairn, D.W.; Olive, P.L.; and O'Neill, K. L. (1995) The comet assay: a comprehensive review. Mutat. Res. 339(1):37-59.

Fly Base (2006) The FlyBase database of the *Drosophila* Genome Projects and community literature. Nucleic Acids Res. 27:85-88. http://flybase.bio.indiana.edu/.

Frei, H. and Würgler, F. E. (1996) Induction of somatic mutation and recombination by four inhibitors of eukaryotic topoisomerases assayed in the wing spot test of *Drosophila melanogaster*. Mutagenesis; 11(4): 315-25.

Godschalk, R. W.; Ersson, C.; Riso, P.; Porrini, M.; Langie, S. A. and van-Schooten, F. J. (2013) DNA-repair measurements by use of the modified comet assay: an inter-laboratory comparison within the European Comet Assay Validation Group (ECVAG). *Mutat. Res.* 757, 60–67.

Gömürgen, A. N. (2005) Cytological Effect of the Potassium Metabisulphite and Potassium Nitrate Food Preservative on Root Tips of *Allium cepa* L. Cytologia 70 (2): 119–128.

Gülsoy, N.; Yavas, C. and Mutlu, Ö. (2015) Genotoxic effects of boric acid and borax in zebrafish, *Danio rerio* using alkaline comet assay. EXCLI J; 14:890-9.

Hartmann, A.; Agurell, E.; Beevers, C.; Brendler-Schwaab, S.; Burlinson, B. and Clay, P. (2003) Recommendations for conducting the in vivo alkaline comet assay. *Mutagenesis* 18, 45–51. doi: 10.1093/mutage/18.1.45

Hanes, T. (2017) Facts on Sodium Benzoate. https://www.livestrong.com/article/444638-sodium-benzoate-side-effects/.

Hong, H.; Liang, X. and Liu, D. (2009) Assessment of benzoic acid levels in milk in china. Food Control.; 20(4):414–8.

IARC, (1983) Some food additives, feed additives and naturally occurring substances. In: Monographs on the Evaluation of Carcinogenic Risk to Humans, vol. 31. International Agency for Research on Cancer, Lyon, p. 314.

Ishidate, J. R. M.; Sofuni, T.; Yoshikawa, K.; Hayashi, M.; Nohmi, T.; Sawada, M. and Matsuoka, A. (1984) Primary mutagenicity screening of food additives currently used in Japan. Food Chem. Toxicol. 22(8):623-636.

Ishidate, J. R. M. and Odashima, S. (1977) Chromosome tests with 134 compounds on Chinese hamster cells *in vitro*. A screening for chemical carcinogens. Mutation Research/Fundamental and molecular mechanisms of mutagenesis 48(3-4): 337- 353.

Justice, R. W.; Zilian, O.; Woods, D. F.; Noll, M.; and Peter, J. B. (1995) The *Drosophila* tumor suppressor gene *warts* encodes a homolog of human myotonic dystrophy kinase and is required for the control of cell shape and proliferation. Genes Dev., 9: 534-546.

Karlsson, H. L. (2010) The comet assay in nanotoxicology research. *Anal. Bioanal.Chem.* 398, 651–666. doi: 10.1007/s00216-010-3977-0

Khurana, V.; Lu, Y.; Steinhilb, M. L.; Oldham, S.; Shulman, J. M. and Feany, M. B. (2006) TOR-mediated cell-cycle activation causes neurodegeneration in a *Drosophila* tauopathy model. *Curr. Biol.* 16, 230–241.

Koh, K.; Evans, J. M.; Hendricks, J. C.; and Sehagal, A. (2006) A *Drosophila* model for age associated changes in sleep: wake cycles. *Proc. Natl. Acad. Sci. U.S.A.* 103, 13843–13847.

Lindsley, D.L. and Zimm, G.G. (eds) (1992) The genome of *Drosophila melanogaster*. San Diego: Academic Press.

Loon, A. J.; Botterweck, A. A.; Goldbohm, R. A.; Brants, H. A.; Klaveren, J. D. and van, P. A. (1998) Intake of nitrate and nitrite and the risk of gastric cancer: a prospective cohort study. Bnbsh Journal of Cancer 78(1). 129-135.

Mackay, T. F.; Anholt, R. R.; Of flies and man (2006) *Drosophila* as a model for human complex traits. Annu Rev Genomics Hum Genet. 7, 339–367.

Misra, J. R.; Horner, M. A.; Lam, G.; Thumme, C.S. (2011) Transcriptional regulation of xenobiotic detoxification in Drosophila. Genes & development. 25, 1796–1806.

Mpountoukas, P. A.; Vantarakis, B.; Sivridis; E. C. and Lialiaris, T. (2008) Cytogenetic study in cultured human lymphocytes treated with three commonly used preservatives. Food and Chemical Toxicology. 46; 2390–2393.

Mukhopadhyay, I.; Chowdhuri, D. K.; Bajpayee, M. and Dhawan, A. (2004) Evaluation of *in vivo* genotoxicity of cypermethrin in *Drosophila melanogaster* using the alkaline Comet assay. Mutagenesis, 19(2):85-90.

Negishi, T.; Shiotani, T.; Fujikawa, K. and Hayatsu, H. (1991) The genotoxicities of N-nitrosamines in *Drosophila melanogaster in vivo*. Mutat. Res. 252 (2), 19–28.

Nepomuceno, J. C. (2015) Using the *Drosophila melanogaster* to Assessment Carcinogenic Agents through the Test for Detection of Epithelial Tumor Clones (*Warts*). Adv Tech Biol Med; 3:3, 149.

Njagi, G. D. E. and Gopalan, H. N. B. (1982) Cytogenetic effects of the food Preservatives—Sodium benzoate and sodium sulphite on *Vicia faba* root meristems. Mutation Research/Genetic Toxicology 102(3): 213–219.

Nohmi, T.; Yamada, M. and Masumura, K. (2012) *In vivo* approaches to identify mutations and *in vitro* research to reveal underlying mechanisms of genotoxic thresholds. Gen. Environ., 34(4): 146-152.

Nujić, M. and Habuda-Stanić, M. (2017) Nitrates and Nitrites, Metabolism And Toxicity. Food in Health and Disease, scientific-professional journal of nutrition and dietetics; 6 (2) 48-89.

Onyemaobi, O. I; Williams, G. O. and Adekoya, K. O. (2012) Cytogenetic Effects of two food preservatives, sodium metabis-ulphite and sodium benzoate on

the root tips of Allium cepa Linn. Ife J. Sci. 14(1):155.

Östling, O. and Johanson, K. J. (1984). Microelectrophoretic study of radiationinduced DNA damages in individual mammalian cells. *Biochem. Biophys. Res.Commun.* 123, 291–298.

Oyanagi, K.; Kuniya, Y.; Nagao, M.; Tsuchiyama, A. and Nakao, T. (1987) Cytotoxicities of sodium benzoate in primary culture of hepatocytes from adult rat liver. The Tohoku Journal of Experimental Medicine 152: 47–51.

Pandey, H.; Vikas, K. and Roy, B. K. (2014) Assessment of genotoxicity of some common food preservatives using Allium cepa L. as a test plant. Toxicology Reports; 1, 300–308.

Pandir, D. (2016) DNA damage in human germ cell exposed to the some food additives in vitro. Cytotechnology. 68:725–733.

Potter, C.J.; Turenchalk, G.S. and Xu, T. (2000) *Drosophila* in cancer research, anexpanding role. Trends Genet. 16, 33–39.

Rand, M. D. (2010) Drosophotoxicology: the growing potential for *Drosophila* in neurotoxicology. *Neurotoxicol. Teratol.* 32, 74–83.

Rand, M. D.; Sara, L.; Montgomery, L. P. and Vorojeikina, D. (2015) Developmental Toxicity Assays Using the *Drosophila* Model. Curr Protoc Toxicol. ; 59: 1.12.1–1.12.20.

Reiter, L. T.; Potocki, L.; Chien, S.; Gribskov, M. and Bier, E. (2001) A systematic analysis of human disease-associated gene sequences in *Drosophila melanogaster*. *Genome Res.* 11, 1114–1125.

Rekha, K. and Dharman, A. K. (2011) Mitotic aberrations induced by sodium benzoate: A food additive in *Allium cepa L*. Plant Archives; 11 No.2.945-947.

Rincón, G. J.; Espinosa, J. and Graf, U. (1998) Analysis of the *in vivo* nitrosation capacity of the larvae used in the wing somatic mutation and recombination test of *Drosophila melanogaster*. Mutat Res. 13; 412(1):69-81.

Saatci, C.; Yagut, E.; Ruslan, B.; Hilal, A.; Nazife, T. and Yusuf, O. (2016) Effect of sodium benzoate on DNA breakage, micronucleus formation and mitotic index in peripheral blood of pregnant rats and their newborns. Biotechnology & Biotechnological Equipment, VOL. 30, NO. 6, 1179–1183.

Sarikaya, R. and Cakir, S. (2005) Genotoxicity testing of four food preservatives and their combinations in the *Drosophila* wing spot test. Environmental Toxicology and Pharmacology, 20; 424–430.

Sarikaya, R. and Solak K., (2003) Genotoxicity of Benzoik Asit Studued in the *Drosophila melanogaster* Somatic Mutation and Recombination Test (SMART). GÜ, Gazi Eğitim Fakültesi, Dergisi Cilt; 23, Say 3, 19-32.

Sasaki, Y. F.; Sekihashi, K.; Izumiyama, F.; Nishidate. E.; Saga, A.; Ishida, K. and Tsuda, S. (2000) The comet assay with multiple mouse organs: comparison of comet assay results and carcinogenicity with 208 chemicals selected from the IARC

monographs and U.S. NTP Carcinogenicity Database. Crit Rev Toxicol. Nov; 30(6):629-799.

Sekelsky, J. J.; Brodsky, M. H. and Burtis, K. C. (2000) DNA repair in *Drosophila*: insights from the *Drosophila* genome sequence. *J. Cell. Biol.* 150, F31–F36.

Sharma, A.; Shukla, A. K.; Mishra, M. and Chowdhuri, D. K. (2011) Validation and application of *Drosophila melanogaster* as an *in vivo* model for the detection of double strand breaks by neutral comet assay. *Mutat. Res.* 721, 142–146.

Shukla, A. K.; Pragya, P. and Chowdhuri, D. K. (2011) A modified alkaline comet assay for in vivo detection of oxidative DNA damage in *Drosophila melanogaster*. *Mutat. Res.* 726, 222–226.

Siddique, H. R.; Chowdhuri, D. K.; Saxena, D. K. and Dhawan, A. (2005) Validation of *Drosophila melanogaster* as an in vivo model for genotoxicity assessment using modified alkaline comet assay. *Mutagenesis* 20, 285–290. doi: 10.1093/mutage/gei032

Sidorov, R. A.; Ugnivenko, E.G.; Khovanova, E. M. and Belitsky, G. A. (2001) Induction of tumor clones in *D. melanogaster wts*/+ heterozygotes with chemical carcinogens. Mutation Research; 498: 181–191.

Singh, N. P.; McCopy, M.T.; Tice, R. R. and Schneider, E. L. (1988) A simple technique for quantitation of low levels of DNA damage in individual cells. Exp. Cell Res. 175; 184-191.

Søndergaard, L. (1993) Homology between the mammalian liver and the *Drosophila* fat body. *Trends Genet.* 9, 193.

Suzuki, T. and Inukai, M. (2006) Effects of nitrite and nitrate on DNA damage induced by ultraviolet light. Chem Res Toxicol. 19(3):457-62.

Sykiotis, G. P. and Bohmann, D. (2010) Stress-activated cap'n'collar transcription factors in aging and human disease. Science signaling, 3:re3.

Tice, R. R.; Agurell, E.; Anderson, D.; Burlinson, B.; Hartmann, A. and Kobayashi, H. (2000) Single cell gel/comet assay: guidelines for *in vitro* and *in vivo* genetic toxicology testing. Environmental and molecular mutagenesis. 35(3):206-21.

Tuormaa, T. E. (1994) The adverse effects of food additives on health: a review of the literature with a special emphasis on childhood hyperactivity. J Orthomol Med.; 9: 225–243.

Turkoglu, S. (2007) Genotoxicity of five food preservatives tested on root tips of *Allium cepa* L. Mutation Research 626:4-14.

Vasconcelos, M. A.; Orsolin, P.C.; Silva-Oliveira, R.G.; Nepomuceno, J. C. and Spanó, M. A. (2017) Assessment of the carcinogenic potential of high intense-sweeteners through the test for detection of epithelial tumor clones (*warts*) in *Drosophila melanogaster*, Food and Chemical Toxicology; 101, 1-7.

Vecchio, G. (2014) A fruit fly in the nanoworld: once again *Drosophila* contributes to environment and human health. *Nanotoxicology* doi: 10.3109/17435390.2014.911985 [Epub ahead of print].

Verma, A.; Sengupta, S. and Lakhotia, S. C. (2012). DNApol-(gene is indispensable for the survival and growth of *Drosophila melanogaster*. *Genesis* 50, 86–101.

Wakabayashi, K. (1990) Identification of food mutagens. In: Mendelsohn, M.L., Albertini, R.J. (Eds.), In Mutation and the Environment. Part E: Environmental Genotoxicity, Risk and modulation. Wiley-Liss, New York, p. 107116.

WHO (1997) The world health report 1997- conquering suffering, enriching humanity.

Wolf, M. J.; Amrein, H.; Izatt, J. A.; Choma, M. A.; Reedy, M. C. and Rockman, H. A. (2006) *Drosophila* as a model for the identification of genes causing adult human heart disease. *Proc. Natl. Acad. Sci. U.S.A.* 103, 1394–1399.

Würgler, F. E. and Vogel, E.W. (1986) *In vivo* mutagenicity testing using somatic cells of *Drosophila melanogaster*. *In*: *Chemical mutagens*: *principles and methods for their detection* (de Serres, F.J., Ed.). Vol. 10. New York: Plenum Press. p 1–72.

Zengin, N.; Yüzbasıoğlu, D.; Ünal, F.; Yılmaz, S. and Aksoy, H. (2011) The evaluation of the genotoxicity of two food preservatives: Sodium benzoate and potassium benzoate. Food and Chemical Toxicology. 49; 763 –769.

Zimmering, S.; Osgood, C.; and Mason, J. M. (1990) Aneuploidy in *Drosophila*, I. Genetic test systems in the female *Drosophila melanogaster* for the rapid detection of chemically induced chromosome gain and chromosome loss. Mutat. Res., 234(5):319-326.