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Effects of two plant growth regulators, indole-3-acetic acid and β-naphthoxyacetic acid, on genotoxicity in *Drosophila* SMART assay and on proliferation and viability of HEK293 cells from the perspective of carcinogenesis

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Abstract

In this study, the mutagenic and recombinogenic effects of indole-3-acetic acid (IAA), a plant growth regulator naturally synthesized in plants but produced synthetically, and β-naphthoxyacetic acid (BNOA), a synthetic plant growth regulator widely used in agricultural regions, were investigated using the somatic mutation and recombination test (SMART) in *Drosophila* wings. The effect of the same plant growth regulators against the proliferation and viability of a human immortalized embryonic kidney HEK293 cells which is at the early stage of carcinogenesis were also examined with MTT and trypan-blue exclusion assays. For the SMART assay, two different crosses were used: a standard and a high-bioactivation (HB) cross, involving the flare-3 and the multiple wing hairs markers. The HB cross involved flies characterized by an increased cytochrome P-450-dependent bioactivation capacity, which permits the more efficient biotransformation of promutagens and procarcinogens. In both crosses, the wings of the two types of progeny, inversion-free marker heterozygotes and balancer heterozygotes, were analyzed. The results show that IAA and BNOA are not mutagenic or recombinogenic in the wing cells of *Drosophila*. Furthermore, neither plant growth regulator affected the proliferation rate of HEK293 cells; however, both of them induced cell death at high concentrations.

Keywords

Drosophila melanogaster, wing spot test, IAA, BNOA, genotoxic effect, cancer proliferation

Introduction

Various plant growth regulators are widely used to increase crop yields in agricultural regions. Plant growth regulators can be categorized as auxins, gibberellins, cytokinins, and abscisic acid. These compounds affect numerous changes in plant metabolism, such as flowering, aging, root growth, fruit enlargement, and the senescence of leaves, stems, and other parts of the plant. Auxins are the major group of plant growth regulators, and promote plant growth and development. Indole-3-acetic acid (IAA) is a natural plant hormone belonging to the auxins (Davies, 1995). Synthetic indole derivatives such as β-naphthoxyacetic acid (BNOA), naphthalene acetic acid, or indole butyric acid, which are more effective than IAA, are used in agricultural areas. BNOA is registered as a plant growth regulator in Turkey to remove the poor

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pollination, which can be set by the application of BNOA (Gökmen and Acar, 1998). However, these substances also have deleterious effects, such as mutagenesis and cancer (Çelik and Tülüce, 2007; John et al., 1979). Furthermore, the chemical pollutants released into the environment are continuously increasing. Among the undesirable side effects of chemical pollutants, carcinogenesis and mutagenesis are of special concern because of the irreversible nature of these processes and the long latency associated with their manifestation (Osaba et al., 1999).

Until now, contradictory findings on the toxicity of plant growth regulators and their metabolites have been reported (Kaya et al., 1999). Studies have demonstrated that some of these substances have various harmful effects, such as toxicity, teratogenicity, mutagenicity, and carcinogenicity, depending on the dose. For example, John et al. (1979) reported that IAA had teratogenic effects on gestation in mice and rats at 500 mg/kg per day. Although there are some data on the toxic effects of several other plant growth regulators in the auxin group, there is no convincing evidence in the literature of the mutagenicity or carcinogenicity of BNOA. Furthermore, no investigation of the genotoxic effects of IAA and BNOA has been undertaken with the *Drosophila* wing spot somatic mutation and recombination test (SMART). Similarly, the cancer-related effects of IAA and BNOA have not previously been investigated by MTT or trypan-blue exclusion assays based on the viability and proliferation rates of early carcinogenic (immortal but not tumorigenic) kidney cells.

Conversely, many investigations have focused on IAA and its metabolites as prodrugs for targeted cancer therapy (Chen et al., 2001; Dashwood, 1998; Edwards et al., 1999; Folkes and Wardman, 2001; Greco et al., 2002; Hong et al., 2002; Leong et al., 2001; Rossiter, 2002; Stresser et al., 1995; Wardman, 2002). It is also argued that IAA can act as a bioantimutagen (Yeşilada, 2000).

The *Drosophila* wing SMART assay is fast, reliable, and easy to perform and first described by Graf et al. (1984). This in vivo test can detect a wide spectrum of changes, including point mutations, deletions, mitotic recombinations, chromosomal loss, and nondisjunction. SMART also detects the activity of promutagens, using strains with a high capacity to transform some carcinogens to their active metabolites (Graf and Schaik, 1992). The activation of procarcinogen and promutagen is primarily performed by the cytochrome P-450 enzyme system that consists of several isozymes and has the capacity of metabolizing a variety of substances (Kaya et al., 2000). Investigating the cancer-promoting effects of these plant growth regulators may augment the results of SMART. The MTT assay, which is simple, accurate, and yields reproducible results in determining the cell viability and proliferation rates, can be used for this purpose. This method was originally developed by Mossman (1983). The key component is 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT). The mitochondrial dehydrogenases of viable cells cleave the tetrazolium ring, causing the formation of green crystals that are insoluble in aqueous solution. The crystals are redissolved in an organic solvent and the resulting purple solution is measured spectrophotometrically, from which the viability and proliferation rate are calculated. The trypan-blue exclusion assay is another test that determines cell viability and the proliferation rate. In this test, living (nonstained) and dead cells (stained) are identified microscopically. The proportion of living cells, their viability, and proliferation rate are calculated.

This study was undertaken to determine the genotoxicity and cancer-related proliferative effects of two plant growth regulators, IAA (naturally synthesized in plants but produced synthetically) and BNOA (a synthetic plant growth regulator).

Material and methods

Somatic mutation and recombination test

Chemicals. IAA (Sigma I-2886, USA) and BNOA (Sigma, CAS No: 120-23-0), less soluble in water, were dissolved in a mixture of 10% ethyl alcohol + 3% Triton X-100. Six different concentrations of each one of the plant growth regulators were prepared just before use and were administrated to 72-hour-old larvae. Concentrations used in the SMART assay has been determined as 0, 5–20 mM for IAA, and 0, 1–10 mM for BNOA. These concentration values include a wide range which also contains used concentrations. Distilled water and 10% ethyl alcohol + 3% Triton X-100 mixture were used as negative controls; 1 mM of ethyl methane sulphonate (EMS), an alkylating agent, was used as a positive control.

Strains. The genotoxicity of IAA and BNOA were assessed using two versions of SMART: For the standard cross (ST), flare-3 (*flr*^{3})/*TM3*, *BdS* virgin females (*flr*^{3} (*flr*^{3}/*In(3LR)TM3, ri pp sep l(3)89Aa*

bx34e e BdS) were mated to multiple wing hairs (*mwh*) males and for the high bioactivation cross (HB), *NORR/NORR*; *flr*^{3}*/TM3*, *BdS (flr*^{3}*/In(3LR)TM3, ri pp sep l(3)89Aa bx34e e BdS*) virgin females were mated to *NORR/NORR*; *mwh* males (Graf et al., 1984; Graf et al., 1989; Graf and Schaik, 1992). *NORR* stocks were constructed by Pacella (1992) similarly as described in Frölich and Würgler (1989). For detailed information on the genetic markers see Lindsley and Zimm (1992).

Experimental procedure. The wing spot test was performed according to Graf et al. (1989; ST cross) and Graf and Schaik (1992; HB cross). Eggs were collected for 8 h, and 72 $\pm$ 4 h later, the larvae were floated off with tap water. They were then transferred to plastic vials (2.6 cm diameter and 12 cm high) containing 4.5 g *Drosophila* Instant Medium (Formula 4–24, Carolina Biological Supply, Burlington NC, USA) rehydrated with 9 ml freshly prepared test compounds. The larvae were allowed to feed on this medium for 48 hour. All the experiments were performed at 25 $\pm$ 1°C and at a 60% relative humidity. The surviving adult flies from both the ST and HB crosses were collected from the treatment vials and stored in 70% ethanol. Their wings were mounted in Faure's solution and inspected under $\times$400 magnification for the presence of clones of cells showing malformed wing hairs. On marker-heterozygous wings, two types of spots could be observed: (i) single spots, either *mwh* and *flr*^{3}, which can be produced by somatic point mutation, chromosome aberration as well as mitotic recombination, and (ii) twin spots, manifest *flr* and *mwh* phenotypes in the same clone, which are originated exclusively from mitotic recombination. Three categories of spots were recorded: small single spots (1–2 cells), large single spots (>2 cells) and twin spots (Graf et al., 1984). On the inversion-heterozygous *mwh/TM3* wings, it was only possible to find *mwh* single spots, as the multiple inverted *TM3* balancer chromosome does not carry any other suitable marker mutation.

Statistical analysis. Evaluation of the genotoxicity of IAA and BNOA were based on the comparison of the frequencies of the different categories of spots per wing in the treatment groups to the concurrent negative control. The statistical significance of the results was determined with a multiple decision procedure that is based on two alternative hypotheses: (1) the mutation frequency in the treated group is not higher than the mutation frequency in the appropriate control, and (2) the induced mutation frequency in the treated group is no less than *m* times as high as the observed spontaneous mutation frequency in the control group. Frei and Würgler (1988) give details on the statistical procedure. For the statistical calculations, the conditional binomial test according to Kastenbaum and Bowman (1970) was used, with significance levels $\alpha = \beta = 0.05$.

Effects of IAA and BNOA on proliferation and viability of HEK293 cells

Cell line and culture conditions. Human embryonic kidney (HEK293) cells, which were at the early stage of the carcinogenesis (Berjukow et al., 1996), were grown in a 37°C, in 5% CO_2 atmosphere, and were routinely maintained in Dulbecco's Modified Eagle Medium (DMEM; ingredients: 1g/l D-glucose, l-glutamin, sodium pyruvate) supplemented with 10% heat inactivated foetal bovine serum (Sigma F9665), 10 units/ml piperacillin (Tazocin, Wyeth, Serial No: 92841, USA), and 25 µg/ml Amphotericin B (Sigma A2942, USA).

Preparation of plant growth regulators. IAA and BNOA concentrations were predetermined and calculated as 0.05 mg/m, according to a dose of BNOA, which a tomato seedling can be influenced and aggressive tomato consumer might be exposed (amount of tomatoes yielded from a tomato seedling and a person who can consume one kilogram tomato per day). IAA and BNOA were dissolved in 96% ethanol; diluted in DMEM, and finally applied to HEK293 cells. Additionally, HEK293 cells treated with DMEM and DMEM + ethanol mixture were used as a control.

Cell proliferation assay. Trypan blue exclusion assays were used to evaluate the effects of IAA and BNOA on cell proliferation (Mosmann, 1983).

We used 25,000 cells per well and 200 µl medium per well. HEK293 cells were seeded and treated with different concentrations (0.005, 0.05 and 0.5 mg/ml) of IAA and BNOA for 8 days. After the incubation period, 5 mg/ml MTT in 10 µL amounts were added to the treated wells. Then, this multiwell plate was incubated in 37°C CO_2 incubator for 5 hours. After the incubation period, 130 µL of medium were removed from all the treatment wells. Consequently, wells were treated with 250 µL DMSO for dissolving blue formazan crystals. After 24 hours, absorbance at

570 nm test wavelength and 630 nm reference wavelengths were measured and used for the proportion of surviving cells. For the trypan blue exclusion assay, non-dyed cells were considered alive. Dead and alive cells were counted under the light microscope and calculated for viability rate (Savas et al., 2006).

Statistical analysis

Differences between treatment groups were analyzed for variance analyze and concentrations were compared by Duncan Multiple Range Test. For MTT assay viability, range of DMEM control group was regarded as 100%.

Results and discussion

The data from the wing spot analysis of the two plant growth regulators are shown in Tables 1 and 2. The MTT and trypan-blue exclusion assay results are shown in Figures 1 and 2, respectively. Tables 1 and 2 present the data obtained for each compound in both the standard (ST) and high-bioactivation (HB) crosses. For each cross, the wings of the two types of offspring were scored: those with the inversion-free marker-heterozygous genotype, and those with balancer heterozygous genotype. Eighty wings were analyzed for each of six concentrations of IAA and BNOA.

In both the ST and HB crosses used in the SMART, all the results for IAA and BNOA in the MH individuals were negative. Therefore, we concluded that these plant growth regulators are non-mutagenic or non-recombinogenic in the wing spot test.

It has been thought that there was a reasonably large intersection database between carcinogenicity studies and genotoxicity studies and also thought that carcinogenic potency of genotoxic carcinogens were about 50 times higher than carcinogenic potency of nongenotoxic carcinogens (Parodi et al., 1991). HEK293 cells are immortal, but not tumorigenic cells. These types of cells are considered to be in the early stages of the carcinogenic process, and therefore more susceptible to carcinogenic and tumor-promoting agents. The increase in the rate of proliferation indirectly increases the chance of having new mutations and consequently progressing in the process of carcinogenesis, especially in the immortalized cells. This is well observed in the increased Burkitt Lymphoma incidence in malaria-infested areas of Africa (Cardy et al., 2001, Bosch, 2004), or increased hepatocellular carcinoma incidence during the exposure of Hepatitis B Virus-genome infected hepatocytes to aflatoxin (Mohandas, 2004; Yeh et al., 1989). We believe that this is also particularly meaningful for HEK293-like cells, which are already having had one or two steps in multistep process of carcinogenesis.

Moreover, IAA and BNOA were not found to affect the proliferation rate of HEK293 cells in our study. However, these hormones induced cell death in high concentrations (Figures 1 and 2). Several studies have shown the mutagenicity and recombinogenicity of the plant growth regulator, IAA. For example, Sasagawa and Matsushima (1991) investigated the mutagenicity of eight different indole compounds derived from indole glucosinolates (indole-3-acetonitryl, indole-3-carbinol, indole-3-acetamide, IAA, 3-methylindole, indole-3-aldehyde, indole-3-carboxylic acid, and indole) in different media, containing or not containing S9 mixture, in mutation tests using *Salmonella typhimurium* TA98 and TA100 or *Escherichia coli* WP2 uvrA/pKM101. It was found that, although none of these compounds were mutagenic in medium without nitrite, they were mutagenic in medium with nitrite at pH 3. These compounds have also demonstrated mutagenic effects in medium containing nitrite and S9 mixture. It has been reported that the addition of S9 mixture decreased their mutagenicity. In another study using *Aspergillus nidulans* in a plate test, Kappas (1983) tested different concentrations of the plant growth regulators IAA, indole-3-butyric acid (IBA), and kinetin for genetic activity. IAA and IBA greatly increased somatic segregation in the fungus, whereas kinetin was ineffective. In that study, the metabolic activation technique also showed that when S9 mixture was added to IAA or IBA, a further 3- to 5-fold increase was observed in the number of segregants.

As well as this evidence of the mutagenicity of IAA, there have been several demonstrations that IAA can act as a bioantimutagen. Yesilada (2000) investigated the antimutagenic effects of kinetin, GA_3, and IAA on EMS-induced mutations in *Drosophila*, and it has been showed that all tested plant growth regulators had antimutagenic effect. Besides the mutagenic or antimutagenic effects of IAA, there is evidence that IAA and its metabolites could be prodrugs for targeted cancer therapy (Chen et al., 2001; Dashwood, 1998; Edwards et al., 1999; Folkes and Wardman, 2001; Greco et al., 2002; Hong et al., 2002; Leong et al., 2001; Rossiter, 2002; Stresser et al., 1995; Wardman, 2002).

BNOA, one of the most common plant growth regulators used especially in greenhouse plants, was non-genotoxic in the wing spot test in our study. A study supporting the result above was performed by

Table 1. Results obtained with indole-3-acetic acid (IAA), in the Drosophila wing spot test

Concentration (mM)	No. of wings (N)	No. of spots (frequencies) / statistical diagnosis[a]					Freq. of clone formation per 10^5 cells[b]
		Small single spots (1–2 cells; m = 2)	Large single spots (>2 cells; m = 5)	Twin spots (m = 5)	Total *mwh* spots (m = 2)	Total spots (m = 2)	
ST Cross							
Marker heterozygous wings							
Distilled water	78	24(0.31)	6(0.08)	0(0.00)	26(0.33)	30(0.37)	1.36
1 mM EMS	80	163(2.04)+	89(1.11) −	32(0.40)+	273(3.41)+	284(3.55)+	13.98
10% ethanol + 3% Triton X-100	72	23(0.31)−	7(0.09) −	0(0.00) i	29(0.40) −	30(0.41)−	1.65
0.5	80	24(0.30)−	4(0.05)−	0(0.00) i	28(0.35)−	28(0.35)−	1.43
1	80	19(0.25)−	6(0.07)−	2(0.02) i	27 (0.34)−	27(0.34)−	1.38
2	80	24(0.30)−	3(0.04)−	0(0.00) i	27(0.34)−	27(0.34)−	1.38
5	80	28(0.35)−	4(0.05)−	1(0.01) i	33(0.41)−	33(0.41)−	1.69
10	80	25(0.31)−	6(0.07)−	0(0.00) i	30(0.37)−	31(0.39)−	1.53
20	80	19(0.25)−	6(0.07)−	1(0.01) i	25(0.31)−	26(0.31)−	1.28
HB Cross							
Marker heterozygous wings							
Distilled water	80	39(0.49)	12(0.15)	8(0.10)	58(0.72)	59(0.72)	2.97
1 mM EMS	80	81(1.01)+	30(0.37)+	8(0.10)−	112(1.40)+	119(1.49)+	5.73
10% ethanol + 3% Triton X-100	80	51(0.64)−	12(0.15) −	3(0.04)−	65(0.81)−	66(0.82)−	3.32
0.5	80	39(0.49)−	10(0.12) −	1(0.01)−	49(0.61)−	50(0.62)−	2.51
1	80	42(0.52)−	8(0.10)−	1(0.01)−	48(0.60)−	51(0.64)−	2.45
2	80	53(0.66)−	8(0.10)−	2 (0.02)−	61(0.76)−	63(0.79)−	3.12
5	80	27(0.34)−	8(0.10)−	3(0.04)−	36(0.45)−	38(0.47)−	1.84
10	80	38(0.47)−	6(0.07)−	2(0.02)−	43(0.54)−	46(0.57)−	2.20
20	80	47(0.59)−	10(0.12)−	0(0.00)−	57(0.71)−	57(0.71)−	2.92

+: positive, i: inconclusive, −: negative, m: multiplication factor, probability levels: $\alpha = \beta = 0.05$.

[a]Statistical diagnoses according to Frei and Würgler (1988).

[b]Clone frequencies/fly divided by the number of cell examined/fly (24.400) gives an estimate of formation frequency per cell and per cell division in chronic exposure experiment (Frei and Würgler, 1988)

Rashid and Mumma (1986). They evaluated the mutagenicity of BNOA in four strains of *Salmonella typhimurium* (TA97, TA98, TA100, and TA1535) in the presence and absence of liver microsomal and cytosolic enzymes derived from rats induced with the pesticide Aroclor. BNOA produced no significant increase ($p < 0.05$) in the reversion of any of the four test strains in the standard plate incorporation assay. The results of an agar overlay toxicity test indicated that the chemical had toxic effects at concentrations above 500 μg/plate. The researchers concluded that under the conditions of those tests, BNOA exhibited no mutagenic activity.

SMART assay, which has investigated genotoxic effects of IAA and BNOA, also presents the chronic effects of these substances. In this test, *Drosophila* larvae were allowed to feed on the medium containing chemicals until pupation. Hence, we concluded that neither IAA nor BNOA demonstrated genotoxic effects during long-term exposure.

Furthermore, both short- and long-term toxicities of these plant growth substances, especially IAA, have been revealed with miscellaneous studies. For example, IAA promotes chronically phagocytic capacity of neutrophils and decrease antioxidant enzyme activities of the neutrophil and lymph node (Lins et al., 2006). It also causes growth retardation, microencephaly and cleft palate in the embryos/fetuses and decrease bodyweight of the dam's (Furukawa et al., 2007). Furthermore, it stimulates $O^{\cdot -}_2$ and H_2O_2 production in cells with peroxidase activity, such as neutrophils (De Melo et al., 1998). IAA is converted in to its cytotoxic metabolites by peroxidase enzymes. Therefore, IAA and its metabolites

Table 2. Results obtained with β-naphthoxyacetic acid (BNOA), in the Drosophila wing spot test

Concentration (mM)	No. of wings (N)	No. of spots (frequencies) / statistical diagnosis[a]					Freq. of clone formation per 10^5 cells[b]
		Small single spots (1–2 cells) (m = 2)	Large single spots (> 2 cells; m = 5)	Twin spots (m = 5)	Total *mwh* spots (m = 2)	Total spots (m = 2)	
ST Cross							
Marker heterozygous wings							
Distilled water	78	24 (0.31)	6 (0.08)	0 (0.00)	26 (0.33)	30(0.37)	0.87
1 mM EMS	80	163 (2.04)+	89 (1.11)−	32 (0.40)+	273 (3.41)+	284(3.55)+	13.98
10% ethanol + 3% Triton X-100	72	23 (0.31) −	7 (0.09)−	0 (0.00) i	29 (0.40)−	30(0.41) −	1.65
0.1	80	14 (0.17) −	4 (0.05)−	0 (0.00) i	18 (0.23)−	18 (0.23) −	0.92
0.5	80	19 (0.24) −	4 (0.05)−	0 (0.00) i	23 (0.29)−	23 (0.29) −	1.17
1	80	28 (0.35) −	1 (0.01)−	0 (0.00) i	29 (0.36)−	29 (0.36) −	1.48
2	80	19 (0.24) −	1 (0.01)−	1 (0.01) i	21 (0.26)−	21 (0.26) −	1.07
5	80	23 (0.29) −	2 (0.02)−	1 (0.01) i	26 (0.32)−	26 (0.32) −	1.33
10	80	24 (0.30) −	5 (0.06)−	0 (0.00) i	29 (0.36)−	29 (0.36) −	1.48
HB Cross							
Marker heterozygous wings							
Distilled water	80	39 (0.49)	12 (0.15)	8 (0.10)	58 (0.72)	59 (0.74)	2.97
1 mM EMS	80	81 (1.01)+	30 (0.37)+	8 (0.10)−	112 (1.40)+	119 (1.49)+	5.73
10% ethanol + 3% Triton X-100	80	51(0.64)−	12 (0.15)−	3 (0.04)−	65 (0.81)−	66 (0.82)−	3.32
0.1	80	50 (0.62)−	8 (0.10)−	2 (0.02)−	59 (0.74)−	60 (0.75)−	3.02
0.5	80	37 (0.50)−	8 (0.10)−	3 (0.04)−	48 (0.60)−	48 (0.60)−	2.45
1	80	56 (0.70) i	9 (0.11)−	2 (0.02)−	67 (0.84)−	67 (0.84)−	3.43
2	80	51 (0.64)−	7 (0.09)−	2 (0.02)−	60 (0.75)−	60 (0.75)−	3.07
5	80	49 (0.61)−	10 (0.12)−	1 (0.01)−	58 (0.72)−	60 (0.75)−	2.97
10	80	39 (0.49)−	12 (0.15)−	2 (0.02)−	53 (0.66)−	53 (0.66)−	2.71

+: positive, i: inconclusive, -: negative, m: multiplication factor, probability levels: $\alpha = \beta = 0.05$.

[a]Statistical diagnoses according to Frei and Würgler (1988).

[b]Clone frequencies/fly divided by the number of cell examined/fly (24, 400) gives an estimate of formation frequency per cell and per cell division in chronic exposure experiment (Frei and Würgler, 1988).

could be prodrugs for cancer therapy (Chen et al., 2001; Dashwood, 1998; Edwards et al., 1999; Folkes and Wardman, 2001; Greco et al., 2002; Hong et al., 2002; Leong et al., 2001; Rossiter, 2002; Stresser et al., 1995; Wardman, 2002).

BNOA has been categorised in the chemical group that demonstrated slightly acute toxicity by the Pesticide Action Network (PAN, 2009) but there are no data on its long-term effects in this site. However, IAA was stated to have no toxicity in the same web site (PAN, 2009). Further investigations are necessary to clarify the long- and short-term effects of both IAA and BNOA.

On the basis of our preliminary studies with various cell numbers per well and with different incubation time with plant growth regulators, we decided that short period of time is not sufficient to observe their efforts on HEK293 cell line. Because HEK293 cells are immortal but not carcinogenic cells, they did not proliferate rapidly as observed in many cell lines that are developed from tumors which have a high grade. This is probably the main reason why the medium did not run out in 8 days. Furthermore, the effects of plant growth regulators, which are claimed to develop as not toxic to human cells, demonstrate their effects on human cells not in a short time. We think that investigators should be careful in conducting experiments for the examination of agents that are harmful to human cells in the long run.

In conclusion, our study demonstrates that neither IAA nor BNOA have genotoxic effects on either ST or HB crosses in the in vivo *Drosophila* wing SMART. Furthermore, these hormones did not affect the proliferation rate of human embryonic kidney

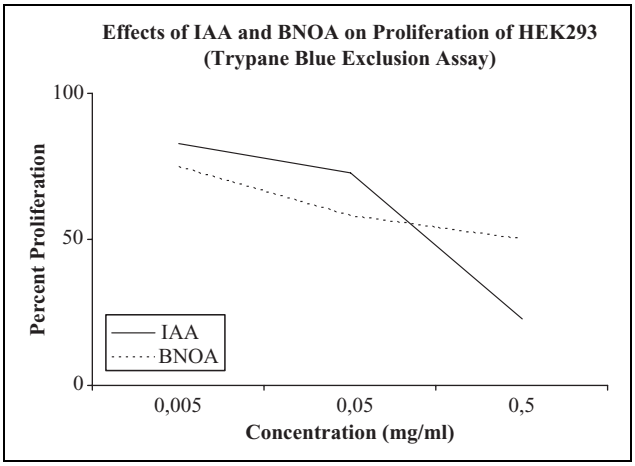

Figure 1. Proliferative susceptibility of human embryonic kidney cells (HEK293) to plant growth regulators: Indole-3-acetic acid (IAA) and b-naphthoxyacetic acid (BNOA) are determined by Trypan Blue Exclusion Assay. Plant growth regulator concentrations mentioned in X axis of the graphics were administered to 1–2 $\times$ 10^4 cells in 50 microlitre in a 200 microlitre DMEM medium. Percent effect was calculated negative control HEK293 cells which was incubated in DMEM medium without plant growth regulators. Ethyl alcohol was used as a positive control and one thousandth of the amount of plant growth regulators produced comparable effects on HEK293 cells (microgram scale for ethyl alcohol and milligram scale for plant growth regulators). The exposure time to the chemicals was 8 days at 37°C, in 5% CO_2.

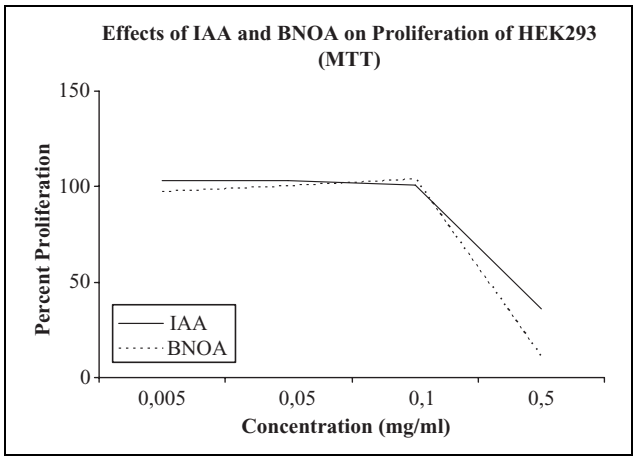

Figure 2. Effects of plant growth regulators (indole-3-acetic acid [IAA] and b-naphthoxyacetic acid [BNOA]) on Viability of human embryonic kidney cells (HEK293) as determined by MTT Assay. Plant growth regulator concentrations mentioned in X axis of the graphics were administered to 1–2 $\times$ 10^4 cells in 50 microlitre in a 200 microlitre DMEM medium. Percent effect was calculated by negative control HEK293 cells which was incubated in DMEM medium without plant growth regulators. Ethyl alcohol was used as a positive control and one thousandth of the amount of plant growth regulators produced comparable effects on HEK293 cells (microgram scale for ethyl alcohol and milligram scale for plant growth regulators). The exposure time to the chemicals were 8 days at 37°C, in 5% CO_2.

HEK293 cells. However, these hormones did induce cell death at high concentrations. Increased proliferation rate has greatly increased their chance of having additional mutations leading to move into further progress in the process towards carcinogenesis. Nevertheless, further studies are required to support our findings on the proliferative effects of IAA and BNOA by using some other early cell lines derived from different tissue types.

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