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Grafical Abstract

Abstract: Aspartame is commonly used as a sweetener in prepared foods, beverages and recipes. In the present study, the possible toxic effects of aspartame were studied in third instar larvae of transgenic Drosophila melanogaster (hsp70-lacZ) Bg9. The third instar larvae were allowed to feed on the diet having different doses of aspartame. The results obtained in the present study suggest that the exposure of third instar larvae to the diet having 20, 40, 80, 160 and 320 mM of aspartame did not show any significant toxic effects after 24 hours of the exposure compared to control (p<0.05). The results for pupae formation and the emergence of flies also did not show any significant difference compared to control (p<0.05).

Keywords: Aspartame; Drosophila melanogaster; toxicity

Introduction

Aspartame is widely used as an artificial sweetener in many soft beverages, cakes and variety of other foods (Simintzi et al. 2007). It is a low-calorie artificial sweetener consumed by 200 million people worldwide. It was discovered by James Schlatter in 1965 of G.D. Searle company and was approved for the use by FDA in the 1970s (Ashok and Sheeladevi 2014). It is a dipeptide, composed of the amino acids phenylalanine and aspartic acid plus a small quantity of...
methanol (Butchko et al. 2002). The acute toxicity of aspartame, tested in rats, mice, rabbits and dogs was very low (Marinovich et al. 2013). For more than 30 years aspartame has been widely used as a food additive because of its very strong, sweet taste (Soffritti et al. 2006). It has 200 times more sweet taste than sucrose (Mazur 1984). Although FDA has approved it after a series of tests, after an experimental demonstration of it as a multipotential carcinogenic agent at a dose of 20mg/kg body weight, has raised the issue regarding its use in more than 6000 products worldwide (Soffritti et al. 2006). The use of animals in toxicological research and testing has become an issue of concern for both science and ethics. As a result, the emphasis has been given on the use of an alternative to mammals in research and testing (Mukhopadhyay et al. 2003). The fruit fly (Drosophila) has been developed as an invivo model organism for the toxicological evaluations due to short life cycle with distinct developmental stages (Ging et al. 2014; Ong et al. 2015). In conserved domains, the overall identity between fly and mammal is about 80-90% (Celotto and Palladino 2005). Heat shock proteins (Hsps) were initially reported to be expressed in response to heat, but their expression is also triggered in response to adverse range of stress (Gupta et al. 2005). In the recent years, hsp70 has been considered to be one of the candidate genes for predicting cytotoxicity against environmental chemicals (Mukhopadhyay et al. 2003). Since there are contrary reports on the toxic potential of aspartame and most of them are reported at very high doses (Saleh 2014; Reshman et al. 2015; Martins et al. 2007; Holder and Yirmiya 1989; Tilson et al. 1991; Fisher 1989). Hence, we decided to investigate the toxic potential of aspartame on the third instar larvae of transgenic Drosophila melanogaster (hsp70-lacZ) Bg9.

Materials and Methods

Fly strain. A transgenic D. melanogaster line expressing bacterial β-galactosidase in response to stress was used in the present study (Lis et al. 1983). In the present strain, the transformation vector is inserted with a P-element i.e. the line contains wild-type hsp70 sequence up to lacZ fusion point. The flies and larvae were cultured on food (without sugar) containing agar, corn, yeast and propionic acid at 24 ± 1°C (Nazir et al. 2003; Siddique 2012).

Experimental design. The aspartame was dissolved in distilled water, and the final concentrations of 20, 40, 80, 160, and 320 mM were established in the diet. The third instar larvae were allowed to feed for 24 hours. To study the effect of aspartame at same selected doses on pupation and emergence of flies separate experiments were set. The larvae are voracious feeders and are routinely used to study developmental and physiological processes. The future adult structures of the fly are contained within the larvae, and for the primary high throughput screening, third instar larvae are widely accepted. Hence, we also decided to study the effect of aspartame on the pupation and emergence of flies.

Soluble O-nitrophenyl-β-D-galactopyranoside (ONPG assay). The expression of hsp70 was quantified by performing soluble O-nitrophenyl-β-D-galactopyranoside (ONPG) assay as described by Nazir et al. (2003). After giving a wash of phosphate buffer, the larvae were placed in microcentrifuge tubes (30 larvae/tube, five replicates/group), permeabilized for 10 min by acetone and incubated overnight at 37°C in 600µl of ONPG buffer. After incubation for the desired duration, the reaction was stopped by adding 300µl of Na2CO3, and the extent of the reaction was quantified by measuring absorbance at 420 nm (Chowdhuri et al. 1999; 2001).

In situ histochemical β-galactosidase activity. The larvae (30 larvae/treatment; 5 replicates/group) were dissected out in Pole’s salt solution (PSS), and X-gal staining was performed using the method as described by Chowdhuri et al. (1999). The larvae explants were fixed in 2.5% glutaraldehyde, washed in 50mM sodium phosphate buffer (pH 8.0) and stained overnight in X-gal staining solution at 37°C in the dark.

Trypan blue exclusion test. For studying the extent of tissue damage in larvae caused by the exposure to different dosages of aspartame dye exclusion test was performed (Nazir et al. 2003; Krebs and Feder 1997). Briefly, the internal tissues of larvae were explanted in a drop of PSS, washed in phosphate buffer saline (PBS), stained with trypan blue (0.2 mg/ml in PBS) for 30 min, washed thoroughly with PBS and scored immediately for dark blue staining. About 30 larvae per treatment (10 larvae per dose; 5 replicates/group) were scored for the trypan blue staining on an average composite index per larvae: no colour = 0; any blue = 1; darkly stained = 2; large patches of darkly stained cells = 3; or complete staining of most cells in the tissue = 4 (Krebs and Feder 1997).

Preparation of larval homogenate. The larvae (30 larvae/dose; 5 replicates/group) were homogenised in 1ml of cold homogenising buffer (0.1M Phosphate buffer containing 0.15M KCl; pH 7.4). After centrifugation at 9000g, the supernatant was used for estimating lipid peroxidation, glutathione content, glutathione-S-transferase activity and protein carbonyl content.

Lipid peroxidation assay. Lipid peroxidation was measured according to the method described by Ohkawa et al. (1978). The reaction mixture consisted of 5µl of 10mM butyl-hydroxytoluene (BHT), 200µl of 0.67% thiobarbituric acid, 600µl of 1% O-phosphoric acid, 105µl of distilled water and 90µl of the supernatant. The resultant mixture was incubated at 90°C for 45 min, and the OD was measured at 535nm. The results were expressed as µmol of TBARS formed/h/g tissue.

Estimation of glutathione (GSH) content. The glutathione (GSH) content was estimated colorimetrically using Ellman’s reagent (DTNB) according to the procedure described by (Jollow et al. 1974). The supernatant was precipitated with 4% sulphosalicyclic acid (4%) in the ratio of 1:1. The samples were kept at 4°C for 1 hour and then subjected to centrifugation at 5000 rpm for 10 min at 4°C. The assay mixture consisted of 550 µl of 0.1M phosphate buffer, 100 µl of supernatant and 100 µl of DTNB. The OD was read at 412
nm, and the results were expressed as µ moles of GSH/gramme tissue.

**Estimation of glutathione-S-transferase (GST) activity.** The glutathione-S-transferase activity was determined by the method of Habig et al. (1974). The reaction mixture consists of 500 µl of 0.1 M phosphate buffer, 150 µl of 10mM CDNB, 200 µl of 10mM reduced glutathione and 50 µl of supernatant. The OD was taken at 340 nm, and the enzyme activity was expressed as µ moles of CDNB conjugates/min/mg protein.

**Estimation of Protein Carbonyl content.** The protein carbonyl content was estimated according to the protocol described by Hawkins et al. (2009). The larvae homogenate was diluted to a protein concentration of approx 1mg/ml. About 250µl of each diluted homogenate was taken in eppendorf centrifuge tubes separately. To it, 250µl of 10mM 2,4-dinitrophenyl hydrazine (dissolved in 2.5M HCl) was added, vortexed and kept in the dark for 20 min. About 125µl of 50% (w/v) trichloroacetic acid (TCA) was added, mixed thoroughly and incubated at -20°C for 15min. The tubes were then centrifuged at 4°C for 10 min at 9000 rpm. The supernatant was discarded and the pellet obtained was washed twice with cold ethanol: ethyl acetate (1:1). Finally, the pellets were re-dissolved in 1ml of 6M guanidine hydrochloride, and the absorbance was read at 370nm.

**Effect of pupation and emergence of flies.** To study the effect of aspartame on the pupation and emergence of flies the aspartame was mixed in the diet at a final concentration of 20, 40, 80, 160 and 320mM. To these doses, 50 first instar larvae were introduced. Triplicate sets of each treatment group (including the untreated and positive control) were employed in the study. Each day the numbers of larvae pupate followed by flies emergence were recorded separately, and the data was expressed as the mean of three replicates (Podder and Roy 2015).

**Statistical analysis.** The statistical analysis was done using Statica Soft Inc. The student t-test was applied to observe the significant difference between treated and untreated groups.

**Results**

The results obtained for β-galactosidase activity are presented in Fig. 1. The exposure of larvae to 20, 40, 80, 160 and 320mM of aspartame did not show any significant increase in the activity of β-galactosidase (Fig. 1). However, the exposure of MMS (as a positive control) showed a significant 1.71 fold increase in the β-galactosidase activity compared to the control (p<0.05; Fig.1). The results obtained for X-gal staining are shown in Figs. 2 (A-G). The exposure of larvae to 20, 40, 80, 160 and 320mM of aspartame did not show any β-galactosidase expression (Figs. 2 A-E). However, the exposure of MMS to third instar larvae showed an intense staining in the midgut (Fig. 2G) compared to control (Fig. 2F). The results obtained for trypan blue staining are shown in Figs. 3 (A-G). The exposure of larvae to 20, 40, 80, 160 and 320mM of aspartame did not show any tissue damage compared to control (Figs. 3A-F). The results obtained for lipid peroxidation are shown in Fig. 4. The exposure of larvae to 20, 40, 80, 160 and 320mM of aspartame did not show any significant increase in the lipid peroxidation compared to control (p<0.05; Fig. 4). However, the exposure of larvae to MMS showed a 2.56 fold significant increase in the lipid peroxidation compared to control (p<0.05; Fig. 4).

**Figure 1** β-galactosidase activity measured in the transgenic Drosophila melanogaster (hsp70-lacZ)Bg9 third instar larvae exposed to different doses of aspartame for 24 hrs of duration. A1-20mM aspartame; A2-40 mM aspartame; A3-80 mM aspartame; A4-160 mM aspartame; A5-320 mM aspartame; C-Control; P-Positive control; P-MMS(0.5µl/ml methylmethane sulphonate)*significant at p<0.05 with respect to control.

**Figure 2(A-G) X-gal staining in the third instar larvae of transgenic Drosophila melanogaster (hsp70-lac Z) Bg9 after the exposed to
different doses of aspartame for 24 hrs of duration. {A1-20 µM aspartame; A2-40 µM aspartame; A3-80 µM aspartame; A4-160 mM aspartame; A5-320 mM aspartame; C-Control; P-Positive control; P-MMS(0.5 µl/ml methylmethane sulphonate). [BG- Brain ganglia, SG- Salivary gland, PV- Proventriculus, FG- Foregut, MG- Midgut, HG- Hindgut, MT- Malpighian tubule, GC- Gastric caeca].

The exposure of MMS to larvae showed the tissue damage in the brain ganglia, salivary gland, proventriculus, foregut, midgut, hindgut, malpighian tubule, gastric caeca. The results obtained for the GSH content is shown in Fig. 5. The exposure of larvae to 20, 40, 80, 160 and 320 mM of aspartame did not show any significant increase or decrease compared to control (Fig. 5). However, the exposure of MMS to larvae showed a 1.51 fold decrease compared to control (p<0.05; Fig. 5).

Figure 5. Glutathione content measured in the transgenic Drosophila melanogaster (hsp70-lacZ) Bg9 third instar larvae exposed to different doses of aspartame for 24 hrs of duration. {A1-20 µM aspartame; A2-40 µM aspartame; A3-80 µM aspartame; A4-160 µM aspartame; A5-320 µM aspartame; C-Control; P-Positive control; P-MMS(0.5 µl/ml methylmethane sulphonate)}*significant at p<0.05 with respect to control (p<0.05).

The results obtained for the GST activity are shown in Fig. 6. The exposure of larvae to 20, 40, 80, 160 and 320 mM of aspartame did not show any significant increase in GST activity compared to control (Fig. 6). The exposure of MMS to larvae showed significant 1.55 fold increase in GST activity compared to control (p<0.05; Fig. 6).

Figure 6. Glutathione-S-transferase activity measured in the transgenic Drosophila melanogaster (hsp70-lacZ) Bg9 third instar larvae exposed to different doses of aspartame for 24 hrs of duration. {A1-20 µM aspartame; A2-40 µM aspartame; A3-80 µM aspartame; A4-160 µM aspartame; A5-320 µM aspartame; C-Control; P-Positive control; P-MMS(0.5 µl/ml methylmethane sulphonate)}*significant at p<0.05 with respect to control.
The results obtained for protein carbonyl content are shown in Fig. 7. The exposure of larvae to 20, 40, 80, 160 and 320 mM of aspartame did not show a significant increase in the PC content compared to control (p<0.05; Fig. 7). However, the exposure of MMS to larvae showed a 2.66 fold significant increase in the PC content compared to control (p<0.05; Fig. 7).

The results obtained for pupae formation for the larvae exposed to different doses of aspartame are shown in Fig. 8. Almost similar number of larvae pupates in each of the exposed groups, and complete pupation was observed for eight (8) days in aspartame as well as control groups. No significant difference in the number of pupation/day was observed in aspartame exposed as well as a control group (p<0.05; Fig. 8). However, the larvae exposed to MMS showed a significant difference in the number of pupae formation for 5th, 6th, 7th and 8th day compared to control as well as aspartame exposed groups. The process of pupation was observed till 12th day for the larvae exposed to MMS (p<0.05; Fig. 8).

The results obtained for the emergence of flies are shown in Fig. 9. In each group, the number of flies emerged was counted each day. The first emergence was observed at the 12th day and no significant difference in the mean number of flies emergence at 12th, 13th, 14th, 15th and 16th day was observed in the aspartame-treated as well as control groups (p<0.05; Fig. 9). However, in MMS exposed group a significant decrease in the mean number of flies emerged at 13th, 14th, 15th and 16th day was observed compared to control as well as aspartame treated groups (Fig. 9; p<0.05). The emergence was observed till the 19th day in the MMS-treated group (Fig. 9; p<0.05).

Figure 7. Protein carbonyl content measured in the transgenic Drosophila melanogaster (hsp70-1acZ)BG9 third instar larvae exposed to different doses of aspartame for 24 hrs of duration. {A1-20 µM aspartame; A2-40 µM aspartame; A3-80 µM aspartame; A4-160 µM aspartame; A5-320 µM aspartame; C-Control; PC-Positive control; P-MMS(0.5 µl/ml methylmethane sulphonate)}* significant at p<0.05 with respect to control.

Figure 8. Pupation of larvae exposed to different doses of aspartame for 24 hrs of duration. {A1-20 µM aspartame; A2-40 µM aspartame; A3-80 µM aspartame; A4-160 µM aspartame; A5-320 µM aspartame; C-Control; PC-Positive control; P-MMS(0.5 µl/ml methylmethane sulphonate)}* significant at p<0.05 with respect to control.

Figure 9. Emergence of flies from the larvae exposed to different doses of aspartame for 24 hrs of duration. {A1-20 µM aspartame; A2-40 µM aspartame; A3-80 µM aspartame; A4-160 µM aspartame; A5-320 µM aspartame; C-Control; PC-Positive control; P-MMS(0.5 µl/ml methylmethane sulphonate)}* significant at p<0.05 with respect to control.

Discussion

The results obtained in the present study suggest that the aspartame is not toxic at 20, 40, 80, 160 and 320 mM. The selected doses in our present study are very high, and the plasma concentration of the aspartame in humans hardly reaches 320 mM (Ranney and Oppermann 1978). In humans and other mammalian species, aspartame is metabolised in the gastrointestinal tract by esterases and peptidases into three components i.e. aspartic acid, phenylalanine and methanol (Stegink 1987; Butchko et al. 2002). It may completely hydrolyze in the gastrointestinal line and absorbed or may be hydrolyzed to methanol and aspartylphenylalanine dipeptide. The dipeptide may be absorbed into the gastrointestinal mucosa cells and then cleaved into amino acids (Stegink 1987). In some cases, aspartame may be absorbed into mucosal cells before hydrolysis, and be cleaved within the cell into three components (Matthews 1984). There is very much similarity in both structure and function of Drosophila gut and human gastrointestinal tract. The midgut is the main site of digestion and nutrient absorption (Buchon et al. 2013). Drosophila genome encodes a vast array of putative digestive enzymes (probably 349) involved in the processing of carbohydrate, proteins and lipids (Lemaitre and Miguel-Aliaga 2013). Esterases and peptidases are predominantly present in the fly/larval midgut hence the possibility of the metabolism of...
aspartame cannot be ruled out in the larval tissue (Lemaître and Miguel-Aliaga 2013). Although the global organisation of mammalian and insect guts is not conserved, similar functional sequences can be clearly observed (Buchon et al. 2013). Drugs are chemical substances that exhibit some side effect (toxicity) in the organism due to overdose, accumulation or inability to eliminate it. The expression of hsp70 has been correlated with early cytotoxic events and cellular damage due to its conservation and inducibility by a wide variety of environmental agents and also being a part of the cellular defence (Steinmetz and Resing 1997; Ait-Aissa et al. 2000). In our present study, the β-galactosidase activity was not significantly higher as compared to control at all of the selected doses. But in MMS exposed group the activity was significantly higher as compared to untreated, and aspartame exposed groups. This demonstrates the sensitivity of larvae against the chemical agents. The β-galactosidase activity as an indicator of hsp70 expression has been used to monitor the environmental stress, heat and cadmium toxicity in transgenic Caenorhabditis elegans (Guven et al. 1999; Stringham and Candido 1994). Trypan blue staining performed on the larvae exposed to various doses of aspartame as well as control larvae showed no tissue damage compared to the larvae exposed to MMS. The trypan blue staining is widely accepted and used to evaluate the damaging potential of a particular compound on a specific organ or tissue (Mukhopadhyay et al. 2003; Siddique et al. 2013). The midguts of insects have been reported to have high microsomal and cytochrome activity (Wilkinson and Brattsten 1972). The results obtained for the GST activity showed no increase or decrease, compared to the control. The midguts of the insects express glutathione-S-transferase (GSTs) homologous to mammals and are associated with oxidative stress (Perrimon and Owusu-Ansah 2014). It has been suggested that a vast majority of harmful chemicals are taken care off with the help of P450s present in the midgut. The larvae exposed to MMS showed an increase in the activity of GST. The results obtained for the GSH content also show no increase or decrease in the level compared to the control. The larvae exposed to MMS showed a significant decrease in the GSH content compared to control as well as aspartame exposed groups. The results obtained for lipid peroxidation and protein carbonyl content also did not show any significant increase compared to control groups. Lipid peroxidation is a measure of oxidative stress resulting from the production of reactive oxygen species that damage the cell membranes, proteins and DNA (Ryter et al. 2007). However, there are reports of high TBARS and low GSH content in the kidney tissues of the rats exposed to 500mg/kg body weight for 42 days (Saleh 2014). The protective effect of pomegranate juice against the aspartame induce an oxidative injury i.e. reduction in superoxide dismutase, catalase and reduced glutathione has also been reported (Darwish et al. 2009). Here, the rats were given 250mg/kg/day by gavage for 28 days (Darwish et al. 2009). In another study, the Rosemary extract was reported to attenuate the increase in lipid peroxidation and enhanced the levels of reduced glutathione and antioxidant enzymes activities in kidney and testis in male rats exposed to 1000mg/kg body weight of aspartame (Hozayen et al. 2014). The low concentrations of aspartame metabolites did not affect the membrane enzyme activity, but the higher concentrations partially decreased the membrane acetylcholinesterase activity in human erythrocytes (Tsakiris et al. 2006). The long term consumptions of aspartame lead to the imbalance in the antioxidant/prooxidant status in the brain of rats exposed to 1000mg/kg body weight (Abhilash et al. 2013). The chronic ingestion of abuse doses of aspartame produced no significant chemical changes in brain capable of altering behavioural parameters believed to be controlled by monoamines in rats (Torii et al. 1986). The exposure of aspartame to 1000mg/kg body weight has been reported not only a hepatotoxic but also a potent carcinogenic agent (Alkafafy et al. 2015). Aspartame has been reported to increase protein carbonyl, lipid peroxidation levels, superoxide dismutase, glutathione-S-transferase, glutathione peroxidase and catalase activity in the rats exposed to 40mg/kg body weight of aspartame along with 0.2mg/kg/day of methotrexate (Ashok and Sheeladevi 2014). These results were obtained when methotrexate was also given with aspartame. But in normal humans or rats, the aspartame is quickly metabolised in the gastrointestinal tract (Magnuson et al. 2007). The inhibition of acetylcholinesterase activity in vivo conditions was reported by the metabolites of aspartame in the frontal cortex (Sinnintzi et al. 2007). The researchers have also focussed on the toxicity of its metabolites such as methanol, formaldehyde and formate (Oyama et al. 2002). Mean peak blood methanol concentration exceed 2mg/dl in subjects administered abuse doses of aspartame (Steingk 1987). But these methanol concentrations are still lower than those reported in methanol intoxication (Nolla-Salas et al. 1995; Meyer et al. 2000). In our study on the third instar larvae, no tissue damage was observed even at 360mM of aspartame. The direct and indirect cellular effects of aspartame on the brain have also been reported as the aspartic acid is also thought to play a role as an excitatory neurotransmitter in central nervous system (Humphries et al. 2008). According to the epidemiologic data intake of aspartame is not associated with hematopoietic neoplasms, brain cancer, digestive sites, breast, prostate and several other neoplasms (Marinovich et al. 2013). However, there are reports on the vestibular toxicity in a pair of siblings, suggesting a genetic susceptibility to aspartame toxicity (Pisarik and Kai 2009). Aspartame has also been reported to cause neurobehavioral changes and activation of neurodegenerative apoptosis following the long-term consumption of it in folate deficient rat brain (Ashok and Sheeladevi 2015). To study the effect on development by aspartame, the third instar larvae were also allowed to pupate, and the emergence of the flies was also followed. The durations were noted, and there was no significant difference in the number of larvae pupated and the emergence of flies in control and larvae exposed to various doses of aspartame. However, the delay and significant difference in the number of larvae pupated and the emergence of flies was observed in the MMS exposed group. Hence it is concluded that if aspartame possesses any toxic effect, it would have also induced the delay in the pupation or emergence of flies. In our study, no effect of aspartame was observed in the pupation and emergence of flies. Further, the emerged flies were also observed for any morphological differences; they exhibit no morphological differences compared to control.
group. However, one study has reported the toxic effect of aspartame in Drosophila melanogaster and Danio rerio. In this study, the same concentration was used for both invertebrate as well as vertebrate model, and the concentrations were exceptionally high (highest tested dose 500 mg/ml)(Reshman et al. 2015). The group has reported marked phenotypic changes and DNA damage in both flies and fishes at the same doses(Reshman et al. 2015). The genotoxicity studies carried out on aspartame in vivo as well as in vitro have been extensively reviewed by Magnuson et al.(2007). There are no reports of its genotoxicity in vitro as well as in vivo models. It did not induce micronucleus and chromatid exchanges in cultured human peripheral blood lymphocytes(Rencuzogullari et al. 2004). There was also no evidence of DNA damaging activity by aspartame in primary rat hepatocyte culture at 5 and10 mM of aspartame(Jeffrey and Williams 2000). The in vivo cytogenetic assay performed on rats for aspartame at a dose of 500, 1000, 2000, and 4000 mg/kg/body weight/day for 5 days did not induce chromosomal aberrations in bone marrow cells from all groups(Magnuson et al. 2007). The contradictory reports on the aspartame are mainly due to the dose differences, the route of administration and the duration of exposure. Here in our present study, we have studied various doses of aspartame on larvae for 24 hrs of duration and also the larvae were allowed to pupate on the same doses of exposure and the emergence of the flies were also noted. It is concluded from our study that aspartame does not exhibit any toxic effects at the doses selected in our study, and these doses are higher than the plasma concentrations in human even after a high dose intake (Stegink et al. 1983; Finkelstein et al. 1983). Aspartame is completely digested by the gastrointestinal tract, into amino acids and methanol, which is subsequently metabolised into carbon dioxide and water (Magnuson et al. 2007).

Conclusions

The contradictory reports on the aspartame are mainly due to the dose difference, the route of administration and the duration of exposure. Here in our present study, we have studied various doses of aspartame on larvae for 24 hrs of duration and also the larvae were allowed to pupate on the same doses of exposure and the emergence of the flies were also noted. It is concluded from our study that aspartame does not exhibit any toxic effects at the doses selected in our study, and these doses are still higher than the plasma concentrations in human even after a high dose intake (Stegink et al. 1983). Aspartame is completely digested by the gastrointestinal tract, into amino acids and methanol, which is subsequently metabolised into carbon dioxide and water (Oyama et al. 2002).

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