

Antimutagenic potentials of *L*-tyrosine and its metabolites on the genotoxic activity induced by methyl methanesulfonate



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ABSTRACT

L-Tyrosine an aromatic amino acid can be a potential chemical agent in treating Parkinson's disease. In this study, the potential of *L*-Tyrosine and its metabolites to inhibit the genotoxicity of methyl methanesulfonate (MMS) was assessed using *in vivo* and *in vitro* assays. *In vivo* test peripheral blood and bone marrow micronucleus assays, the antimutagenic potential of *L*-Tyrosine and its metabolites, *L*-DOPA, dopamine and epinephrine, to combat the breaking chromosome potential of MMS were evaluated using albino mice, in which the test compounds were administered orally through gavage with a double dosage regimen while *in vitro* assay Ames test, *Salmonella typhimurium* standard tester strains TA98 and TA104 were utilized as its test microorganisms. Based on the results using One-way ANOVA with square root transformation of raw data and Duncan's Multiple Range Test, it shows that the difference in the treatment means between *L*-Tyrosine and its metabolites are much lower and significantly different (P value < 0.05) from MMS which suggests that treatment samples lack the capacity of forming micronucleated polychromatic erythrocytes (MPCEs). It is also evident that the occurrences of MPCEs were observed clearly in MMS and was the highest. Generally, results suggest that *L*-Tyrosine and its metabolites lower the genotoxic activity of MMS and hence, it can be considered to have an antimutagenic potential against chemically induced mutation. The number of revertants induced by MMS were significantly reduced, and epinephrine has the lowest activity in reducing and inhibiting the number of revertants induced by MMS while both *L*-Tyrosine and *L*-DOPA showed the highest activity.

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1. Introduction

In genetic toxicology, studying of the extent of DNA damage at the chromosome level is very important because it is an important phase in carcinogenesis. Micronucleus assay is an important tool in assessing the chromosome damage inasmuch as it reliably measure both the chromosome loss and chromosome damage (Fenech, 2000).

Alkylating agents can cause cell death, mutation and cancer, are substances that alter the structure of DNA and classified as genotoxic to both somatic cells and germ cells. The biological properties and mechanism of action of alkylating agents have been extensively explored in cells and animal model

systems are categorized as mutagenic, toxic, clastogenic and teratogenic (Margison et al., 2002; Magee et al. 1976). Therefore, genotoxic and mutagenic substances in the environment must be evaluated and monitored as they often induce genetic toxicities and abnormalities.

Antimutagens are substances which can trap genotoxins and mutagens through molecular interaction which eventually inhibit the metabolism of promutagens (Sylianco, 1998). Amino acids are essential for the protein synthesis which is required for enzymatic catalysis, control growth and differentiation, transport, storage, protection, hormones, and regulation. Studies done by Bajo (1994) shows that *L*-Tyrosine and its metabolic product dopamine did not possess direct DNA damaging capacity on H₁₇Rec⁺ and M₄₅Rec⁺ strains of *Bacillus subtilis*. However, they were able to reduce the genotoxicity of quinoline. It has been shown from the studies of Sylianco (Sylianco and Guevara, 1985; Sylianco and Guevara, 1989; Sylianco, 1991) that

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amino acids, such as glutamic acid, aspartic acid, lysine, histidine, arginine, and cysteine; mineral ions such as calcium, magnesium, zinc, copper, iron, and manganese inhibit and reduce the activity of some mutagens and carcinogens such as dimethylhydrazine, benzo (a) pyrene, dimethylnitrosamine, azaserine and methyl methanesulfonate. In this study, the mutagenic and antimutagenic potential of *L*-tyrosine and its metabolites were evaluated using peripheral blood and bone micronucleus assays, and Ames test.

2. Materials and methods

Methyl methanesulfonate (MMS) (CAS No. 66-27-3), 3-4-Dihydroxy-*L*-phenylalanine (*L*-DOPA) (CAS No. 59-92-7), 3-Hydroxytyramine hydrochloride (dopamine hydrochloride) (CAS No. 62-31-7), Giemsa (CAS 51811-82-6) and fetal bovine serum are analytical grade reagents and were purchased from Sigma-Aldrich Co., Singapore. The epinephrine used in the experiment was bought from a local hospital in Iligan City, Philippines.

2.1. Dosage

In the micronucleus test, the experimental dosage of the genotoxic substance performed in the antimutagenicity study of Bajo (1994) were used for *L*-Tyrosine (30) and dopamine (300); for epinephrine (0.1), and *L*-DOPA (150) mg per kilogram body weight. For Ames test, *L*-Tyrosine (106.60 ppm), *L*-DOPA (509.43 ppm), dopamine (1,132.08 ppm), epinephrine (18.87 ppm) and MMS (37.74 ppm). There were two sets of control: Methyl methanesulfonate (10 mg/kg) as the positive control and distilled water as the negative control.

2.2. Test animals/microorganisms

Albino mice were purchased from a local petshop in Iligan City. They were fed with commercial pellets and distilled water throughout the acclimatization and experimental periods and were subjected to a regular light/dark cycle. The drinking bottles and cages were cleaned regularly. The animals were placed in a room at ambient temperature and caged in small groups according to feeding conditions. The test microorganisms *Salmonella* tester strains TA98 and TA104 were obtained from the Philippine National Collection of Microorganisms (PNCM), University of the Philippines-Los Baños, Laguna, Philippines with a permission from Dr. Bruce N. Ames.

2.3. The micronucleus test

Mice, both female and male, aged 8-12 weeks were randomly chosen. Each experimental group contained 5 mice. Prior to drug administration, mice were fasted from food and water for 16 hours and two hours after administration, food and water *ad*

libitum were given to the experimental mice. The micronucleus assay was performed using peripheral blood and bone marrow simultaneously from the same animal with a double dosing regimen. *L*-Tyrosine, *L*-DOPA, dopamine and epinephrine; *L*-Tyrosine, *L*-DOPA, dopamine and epinephrine plus methyl methanesulfonate respectively were administered orally twice, each with 0.20 mL/20 gram body weight using a 500 μ L microsyringe as an improvised gavage, 24 h apart, to five mice per dosage group. Both peripheral blood and bone marrow were collected 24 h after the second treatment.

2.4. Peripheral blood micronucleus assay

The method outlined in Aida et al. (1995), CSGMT (1990), Hayashi et al. (1983) and Heddle (1973) was followed with minor modifications. Prior to the collection of the blood, the mice were collapsed using chloroform and killed through cervical dislocation. Blood was collected by cutting the tail of the mouse and a drop of blood was placed on the pre-cleaned and coded glass slides, and smeared using a cover slip then air dried. The prepared blood films were fixed in methanol then air dried again. The smeared preparations were stained with acridine orange (AO).

2.5. Bone marrow micronucleus assay

The method outlined in Schmid (1976) was followed with modifications. The mice were sacrificed by cervical dislocation. The femora were excised and cleaned of muscle tissues present then the bone marrow was flushed with 0.20 mL fetal calf serum in a micro test tube. The suspensions were centrifuged at 1000 rpm for 5 minutes to sediment the cells; supernatants was discarded and each of the sediments were homogeneously mixed using pasteur pipette then smeared on the pre-cleaned and coded glass slides, and smeared using a cover slip. The prepared bone marrow films were air-dried and fixed in methanol then air dried overnight and was stained the following morning. Staining of slides were done in a undiluted May-Gruenwald stain for 5 minutes, then were transferred to a 50% May-Gruenwald stain for 2 minutes, after which, the slides were immediately transferred to a 15% aqueous Giemsa stain solution for 10 minutes then the back was wiped with a tissue paper.

Screening of the slides for micronucleated polychromatic erythrocytes in the peripheral blood and bone marrow cells followed using 1000x magnification. Five slides per mouse were prepared and scoring was done from 1000 cells per slide or 5000 per mouse.

2.6. Ames test

The method outlined in the studies conducted by Mortelmans and Zeiger (2000), Ames et al. (1973),

and Maron and Ames (1983) was followed minor modification. To the sterile 20 mL test tube the following were added as follows: 0.5 mL of phosphate buffer, 0.1mL of bacterial culture, and 0.05 mL or less of test solution. The mixture was mildly mixed using vortex. The mixture was incubated for 20 minutes at 37°C. After the incubation, 2 mL of top agar (with 0.5mM histidine/biotin solution) maintained at 43-48°C was added. The contents of test tubes are then mixed using vortex at moderate speed for 3 seconds and poured onto the surface of minimal-glucose agar plates then tilted and rotated for even distribution of the mixture. The top agar was set aside to harden for an hour. When the top agar has hardened, the plates are inverted and placed in a 37°C incubator. After 48-72 hours, the plates were removed from the incubator and number of visible colonies per plate was counted. Three trials per test sample were done with three plates per trial. Controls were done side by side with the test samples with three plates per trial. Methyl methanesulfonate and sterilized distilled water was used as the positive control and negative control, respectively.

2.7. Statistical evaluation

Statistical analyses of the results were undertaken using the One-way Analysis of Variance

(ANOVA) with square root transformation of raw data and Duncan's Multiple Range Test (DMRT).

3. Results and discussion

3.1. In vivo effects

Micronucleus assays using peripheral blood reticulocytes and bone marrow cells were used to evaluate the antimutagenic effects of the test compounds on the known alkylating and carcinogen, methyl methanesulfonate.

Results on Table 1 show that *L*-Tyrosine, *L*-DOPA, dopamine, and epinephrine lack the capacity of forming micronucleated polychromatic erythrocytes (MPCEs) which suggests a lack of chromosome breaking effects on the peripheral blood reticulocytes and bone marrow cells. These results indicate that the test samples do not alter the structure of DNA. The number of micronucleated erythrocytes found on these substances is mainly due to the spontaneous fragmentation of the chromatin material at the level of the negative control. The positive control, methyl methanesulfonate (MMS), showed a very significant number of micronucleated polychromatic erythrocytes in comparison with the negative control, and *L*-Tyrosine and its metabolites.

Table 1: The chromosome breaking potential of *L*-Tyrosine, *L*-DOPA, dopamine, and epinephrine

Test Compounds	Number of Micronucleated Polychromatic Erythrocytes			
	Peripheral Blood		Bone Marrow	
	Mean Raw Data	Transformed Data	Mean Raw Data	Transformed Data
Distilled water	0.200	3.814 ^b ± 0.401	0.0667	3.639 ^b ± 0.231
MMS	51.067	27.780 ^a ± 0.153	24.733	19.471 ^a ± 0.676
<i>L</i> -Tyrosine	0.133	3.743 ^b ± 0.284	0.133	3.743 ^c ± 0.284
<i>L</i> -DOPA	0.000	3.536 ^b ± 0.000	0.133	3.743 ^c ± 0.284
Dopamine	0.0667	3.639 ^b ± 0.231	0.933	4.810 ^b ± 0.635
Epinephrine	0.0667	3.639 ^b ± 0.231	0.133	3.743 ^c ± 0.284

Note: Values are based on the average of readings of 5 slides per mouse per 5 trials
Means having the same letter are not significantly different at $\alpha = 0.05$ DMRT

Results on Table 2 show that *L*-Tyrosine has the highest activity and epinephrine has the lowest activity in reducing the number of number of MPCEs in the peripheral blood micronucleus assay and in the bone marrow micronucleus assay, epinephrine and *L*-DOPA has the lowest activity in reducing the

number of MPCE's and the activity of *L*-Tyrosine, *L*-DOPA, and dopamine reducing the number of MPCE's were insignificantly different. Generally, these results mean that *L*-Tyrosine and its metabolites can inhibit the chromosome breaking potential of MMS.

Table 2: Effects of *L*-Tyrosine and its metabolites on the chromosome breaking effects of methyl methanesulfonate (CH₃⁺)

Test Compounds	Number of Micronucleated Polychromatic Erythrocytes			
	Peripheral Blood		Bone Marrow	
	Mean Raw Data	Transformed Data	Mean Raw Data	Transformed Data
Distilled water	0.200	3.814 ^f ± 0.401	0.0667	3.639 ^d ± 0.231
MMS	51.067	27.780 ^a ± 0.153	24.733	19.471 ^a ± 0.676
<i>L</i> -Tyrosine + MMS	1.600	5.690 ^e ± 1.055	1.600	5.690 ^c ± 1.055
<i>L</i> -DOPA	2.533	6.702 ^d ± 0.831	2.067	6.187 ^{bc} ± 0.467
Dopamine + MMS	4.800	8.953 ^c ± 0.409	1.600	5.846 ^c ± 0.696
Epinephrine + MMS	9.467	12.277 ^b ± 1.345	3.067	7.021 ^b ± 1.047

Note: Values are based on the average of readings of 5 slides per mouse per 5 trials
Means having the same letter are not significantly different at $\alpha = 0.05$ DMRT

The possible mechanism of the molecular interaction of the alkylating agent (CH₃⁺) with DNA is shown Fig. 1. When guanine is alkylated at N-7 (Fig. 2), apurinic sites were produced and this will happen if repair is not carried out immediately.

These apurinic sites may lead to the destabilization of base pairs of the DNA. It has also been found that when alkylation takes place at O-6 (Fig. 3) of the purine base, guanine, mispairs could induce destabilization of the DNA, leading to a chromosome

breakage. Alkylation does not only weaken the hydrogen bonds between the guanine and cytosine base pairs but also weakens the N-C bonds glycosidic bond.

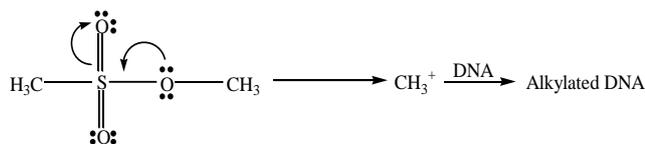


Fig. 1: Metabolic activation of methyl methanesulfonate

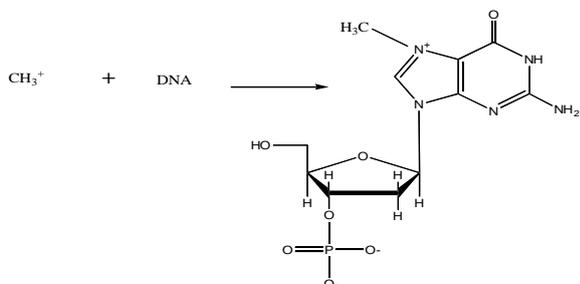


Fig. 2: N-7 alkylation of guanine base

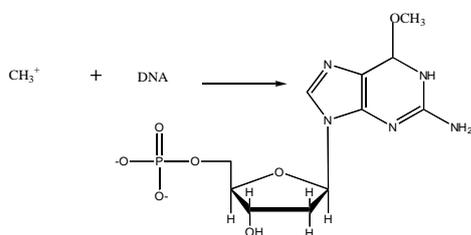


Fig. 3: O-6 alkylation of guanine base

Figs. 4, 5, 6, and 7 shows the possible mechanism of action of the test substances towards the chromosome breaking effects induced by methyl methanesulfonate.

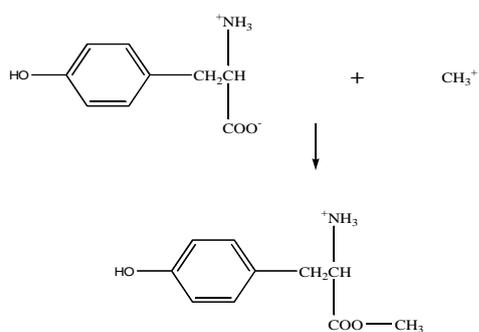


Fig. 4: Possible mechanism of action of *L*-Tyrosine towards MMS

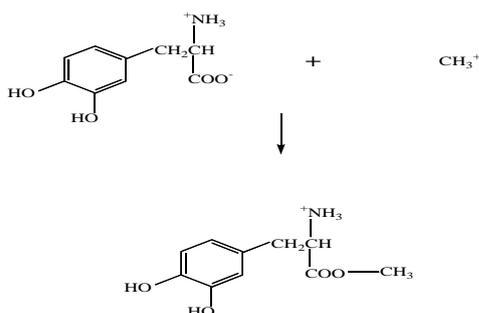


Fig. 5: Possible mechanism of action of *L*-DOPA towards MMS

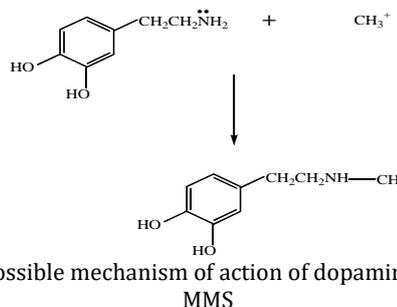


Fig. 6: Possible mechanism of action of dopamine towards MMS

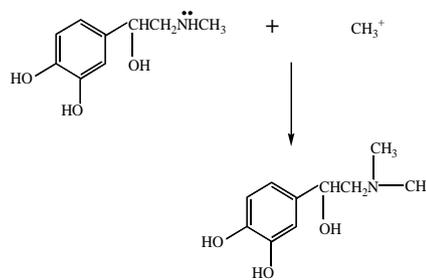


Fig. 7: Possible mechanism of action of epinephrine towards MMS

Another possible mechanism of action on trapping the carbocation of the alkylating agent by the test substances is through electrophilic substitution on the aromatic ring. The electron cloud of the pi system of the benzene ring of the test substances can attract the carbocation; hence substitution of the hydrogen atom can take place, thus preventing the carbocation (CH_3^+) from alkylating the N-7 and O-6 of the guanine base of the DNA (Fig. 8).

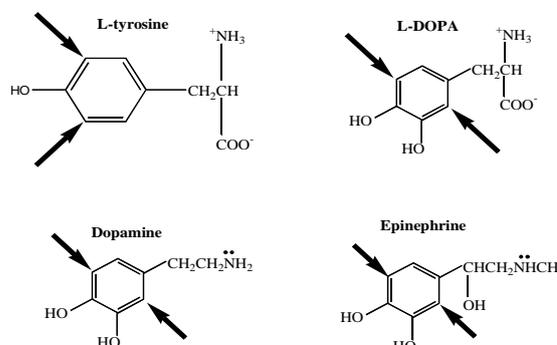


Fig. 8: Possible sites for the interaction of the methyl carbocation and the test compounds

The next following Figs. 9, 10, 11, and 12 will show the electrophilic substitution on the benzene ring of the respective test compounds.

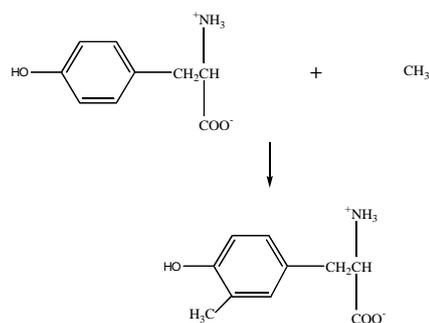


Fig. 9: Electrophilic substitution at the ortho position of *L*-tyrosine

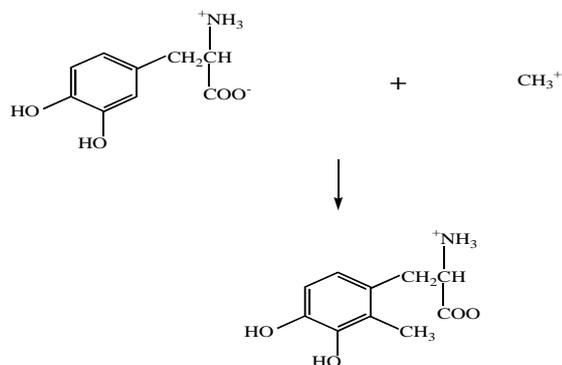


Fig. 10: Electrophilic substitution at the ortho position of *L*-DOPA

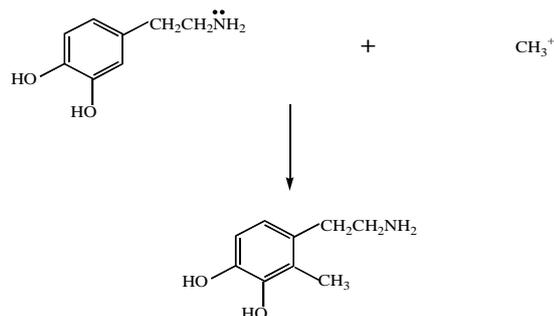


Fig. 11: Electrophilic substitution at the ortho position of dopamine

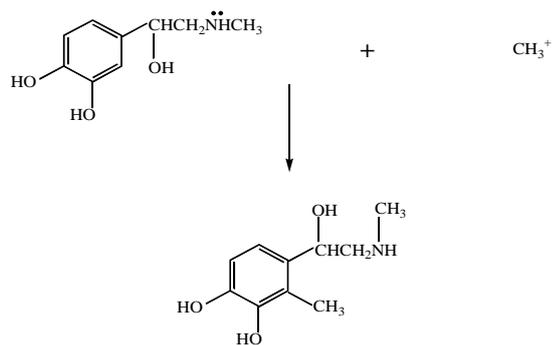


Fig. 12: Electrophilic substitution at the ortho position of epinephrine

Generally the results show that *L*-Tyrosine has the highest inhibitory effects on the chromosome damaging capacity of methyl methanesulfonate both on the peripheral blood reticulocytes and bone marrow cells. It is also found that epinephrine has the lowest inhibitory effects among the test substances both in peripheral blood reticulocytes and bone marrow cells.

The low inhibitory activity of epinephrine may be accounted to the lack of carboxylate group on its structure and less availability of the lone pair in the amino group of the β -carbon.

3.2. In vitro effect

The effects of *L*-Tyrosine and its metabolites on the mutagenic activity induced by methyl methanesulfonate was assessed on the number of growth of revertants using Ames test utilizing TA98 and TA104 as its test microorganisms.

Overall, [Table 3](#) shows that the test substances are significantly different from the positive control in both TA98 and TA104 strains which implies that the test compounds did not induced reversion of the *his*⁻ to *his*⁺ strains. This suggests that the test compounds are not mutagenic.

Table 3: Reversion of the standard tester strain TA98 and TA104 by *L*-Tyrosine and its metabolites

Test compounds	Microorganisms	
	TA98 revertants	TA104 revertants
	Transformed data	Transformed data
Distilled water	21.111 ^b ± 5.810	13.395 ^b ± 3.025
MMS	69.933 ^a ± 1.010	55.753 ^a ± 3.005
<i>L</i> -Tyrosine	2.122 ^d ± 0.000	5.399 ^{bc} ± 4.185
<i>L</i> -DOPA	4.474 ^d ± 4.075	9.054 ^{bc} ± 4.813
Dopamine	4.704 ^d ± 4.474	2.121 ^c ± 0.000
Epinephrine	12.511 ^c ± 5.602	10.009 ^{bc} ± 6.846

Note: Values are based on the average of readings of 3 replicates per 3 trials. Means having the same letter are not significantly different at $\alpha = 0.05$ DMRT.

The test compounds did reduce and inhibit the number of revertants induced by methyl methanesulfonate both in the standard tester strains TA98 and TA104 as shown in [Table 4](#). This suggests that the test compounds have antimutagenic potentials. As such, epinephrine accounted the lowest activity in reducing and inhibiting the number of revertants induced by MMS both in the standard tester strains TA98 and TA104.

Generally, *L*-tyrosine and *L*-DOPA in standard tester strain TA98, and *L*-tyrosine, *L*-DOPA, and dopamine in standard tester strain TA104 has the highest activity in reducing the number of revertants induced by MMS.

Table 4: Reversion of the standard tester strain TA98 and TA104 by *L*-tyrosine and its metabolites plus MMS

Test compounds	Microorganisms	
	TA98 revertants	TA104 revertants
	Transformed data ± S.D.	Transformed data ± S.D.
Distilled water	21.111 ^b ± 5.810	13.395 ^b ± 3.025
MMS	69.933 ^a ± 1.010	55.753 ^a ± 3.005
<i>L</i> -tyrosine+MMS	24.241 ^c ± 5.089	24.216 ^c ± 4.621
<i>L</i> -DOPA+MMS	27.465 ^c ± 0.950	24.347 ^c ± 1.816
Dopamine+MMS	12.104 ^d ± 2.424	25.793 ^c ± 1.777
Epinephrine+MMS	42.250 ^b ± 3.927	37.467 ^b ± 0.594

4. Conclusion

Indeed, the use of the amino acid *L*-Tyrosine and its metabolites, *L*-DOPA, dopamine and epinephrine, were proven to be an attractive, effective and promising approach in reducing the number of micronuclei and number of revertants induced by MMS. In general, *L*-Tyrosine and its metabolites have an antimutagenic potential.

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