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ISSN: 0976-3031

International Journal of Recent Scientific Research
Vol. 6, Issue, 6, pp.4483-4485, June, 2015

International Journal of Recent Scientific Research

RESEARCH ARTICLE

EFFECT OF CHRONIC EXPOSURE OF MONOSODIUM GLUTAMATE (MSG) ON VIABILITY AND RATE OF FEEDING IN TWO DIFFERENT STRAINS OF *DROSOPHILA MELANOGASTER*

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ARTICLE INFO

Article History:

Received 5th, May, 2015
Received in revised form 12th, May, 2015
Accepted 6th, June, 2015
Published online 28th, June, 2015

Key words:

LD50, Monosodium Glutamate (MSG), Excitotoxin, *Drosophila melanogaster*.

ABSTRACT

Monosodium Glutamate (MSG) is most widely used as a flavor enhancer. It has been used as food additive for over 100 years; however, its safety is a much debated issue. In this study, we assess the developmental toxicity of MSG on two strains, Oregon K and Oregon R of *Drosophila melanogaster* to determine the Median Lethal Dose (LD50). LD50 was found to be 10.03% for Oregon K and 12.83% for Oregon R. Significant difference in LD50 was observed between the strains. Further, significant variation in the larval rate of feeding was noticed in different concentrations of MSG.

INTRODUCTION

Monosodium Glutamate (MSG) is sodium salt of Glutamic acid, widely used as a food additive. MSG is chemically similar to Glutamate, an important neurotransmitter which plays a major role in brain development, signal transduction. MSG is an excitotoxin, which is absorbed easily in gastrointestinal track and is metabolized in the tissues by oxidative deamination, or by transamination with pyruvate. MSG was first described to cause the Chinese restaurant syndrome causing a burning sensation, chest tightness, nausea and sweating after a Chinese meal in a restaurant. Since then Investigations have been made to test the effect of MSG on health. If large amounts of glutamate are ingested, portal glutamate levels increase (Daabees *et al*, 1984). This elevation result in the increased hepatic metabolism of glutamate, leading to release glucose, lactate, glutamine, and other amino acids, into systemic circulation (Stegink *et al*, 1983). The present study was undertaken to evaluate the toxicity of chronic MSG exposure on two different strains of *Drosophila melanogaster*, Oregon K and Oregon R.

MATERIALS AND METHODS

Establishment of stock

The experimental stock of Oregon K and Oregon R strains of *Drosophila melanogaster* was obtained from Drosophila Stock Center, Department of Zoology, University of Mysore, Mysuru. These stocks were cultured in standard wheat cream agar medium and maintained at a constant temperature of 22± 1° with relative humidity of 70% in 12D:12L photoperiod cycle.

Feeding assay

Dye tracker method

Food intake was measured by labeling food with Coomassie Brilliant Blue R 250 followed by colorimetric analysis. 2.5% Dye was substituted to 3, 5, 7, 9, 11, 13 and 15 percent concentrations of MSG substituted media. This Dye is non-toxic, non-absorbable and unaffected by gut enzymes, pH and remain in digestive track until excreted. Ten second instar larvae were allowed to feed on this colored media for 30 minutes.

Then homogenized in 200µl distilled water centrifuged to remove debris and supernatant was used to measure absorbance at 595 nm using 96 well plate reader. Dye absorbance of known concentrations was used as reference to plot standard graph and experimental absorbance was plotted on the standard curve to determine experimental concentration. The assay period was

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confined to 30 minutes to retain the whole of ingested dye in the gut (to avoid excretion of dye) (Richard Wong *et al*, 2009).

Proboscis Extension Assay

Proboscis extension on food surface is a direct indicator of food intake (Mair *et al*, 1969). Second instar larvae were placed in a vial containing MSG substituted media of different concentrations and observed under a stereomicroscope. The back and forth movement of the proboscis was recorded for a minute for both Oregon K and Oregon R strains. A total of 10 replicates were observed for each concentration.

Determination of Median Lethal Dose (Ld50)

Eggs were collected from the parental culture using Delcour's procedure (Delcour J, 1969). 100 eggs were sealed in each of seven different concentrations of MSG substituted standard wheat cream agar media. Eggs were allowed to hatch and develop on this MSG substituted media and their viability was scored to determine LD50. Ten replicates were prepared separately for each concentration (3, 5, 7, 9, 11, 13 and 15 percentage) for both Oregon K and Oregon R strains.

Statistical Analysis

Mean, Standard Error, Probit analysis, One-way ANOVA and Tukey's Post-Hoc test were carried out using SPSS version 20.0 (IBM corp. Released 2011. IBM SPSS Statistics for windows, Version 20.0. Armonk, NY: IBM Corp).

RESULTS AND DISCUSSION

Feeding assay

Colorimetric estimation of food intake

Larvae in 5% MSG media showed maximum feeding in both Oregon K and Oregon R strain, while larvae in 13% MSG media showed lowest feeding in Oregon K and 15% showed lowest feeding in Oregon R (Fig.1). Both Oregon K and Oregon R strain showed an initial decrease in food intake at 3% when compared to control, followed by maximum feeding at 5%. The graph showed no specific trend. The feeding rate of *Drosophila melanogaster* varied within various concentrations and also between the strains.

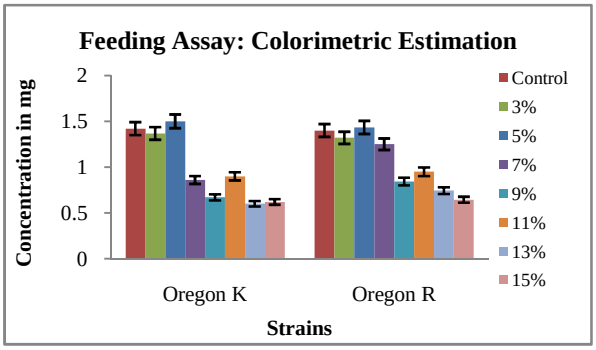

Fig. 1 Feeding of Oregon K and Oregon R strain of MSG substituted media per thirty minute of assay time.

Proboscis Extension Assay

Proboscis Extension (PE) data was subjected to One-way ANOVA followed by Tukey's Post Hoc Test using IBM SPSS Statistics Version 20.0. Significant difference was seen at 0.05 level. (Fig. 2) Feeding was seen to be more in 5% MSG media, for both Oregon K and Oregon R strain when compared to control. Lowest feeding was seen at 13% MSG media for Oregon K and 15% MSG media for Oregon R. There was a linear relation between proboscis extension and food intake as compared to the calorimetric dye estimation method. Difference was observed both between concentrations and between the strains Oregon K and Oregon R.

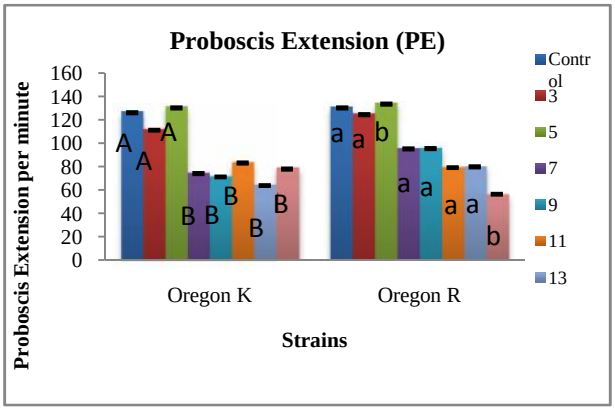

Fig 2 Proboscis Extensions (PE) in different concentrations of MSG substituted media. Different letters on the bar graph indicate significance at 0.05 level by Tukey's Post Hoc Test.

Table 1 Analysis of Variance (ANOVA) of Proboscis Extension.

Strains		Sum of Squares	df	Mean Square	F
Oregon K	Between Strains	34769.743	6	5794.957	9.767*
	Within Strain	37377.400	63	593.292	
	Total	72147.143	69	-	
Oregon R	Between Strains	34809.371	6	5801.562	12.568*
	Within Strain	29081.500	63	461.611	
	Total	63890.871	69	-	

* Significant at 0.001 level

Median Lethal Dosage

Mortality data was corrected using Abbott's formula (Abbott, 1925) and expressed as corrected mortality. The corrected mortality data were subjected to regression analysis of Probit mortality on log dosage (Finney, 1952). LD50 values were considered to be significantly different if the 95% FLs of two LD50 values did not overlap each other (Yang *et al*, 2002).

F1 adult progeny was seen to decrease with increase in concentration of MSG (Fig. 3). Oregon K is found to be more sensitive to MSG than Oregon R. (Table 1) shows the LD50 values of 10.03% and 12.83% for Oregon K and Oregon R respectively calculated from Probit Analysis. Different LD50 values for Oregon K and Oregon R indicate that there is strain difference in toxicity to MSG

Table 1 Median Lethal Dose (LD50) of Oregon K and Oregon R strains of *Drosophila melanogaster* as calculated using Probit Analysis.

Strain	LD50	Standard Error	Significance level	Chi-Square	Degrees of Freedom	P-Value
Oregon K	10.0385	0.2669	0.5	12.4741	5	0.0288
Oregon R	12.8333	0.3733	0.5	2.5894	5	0.7630

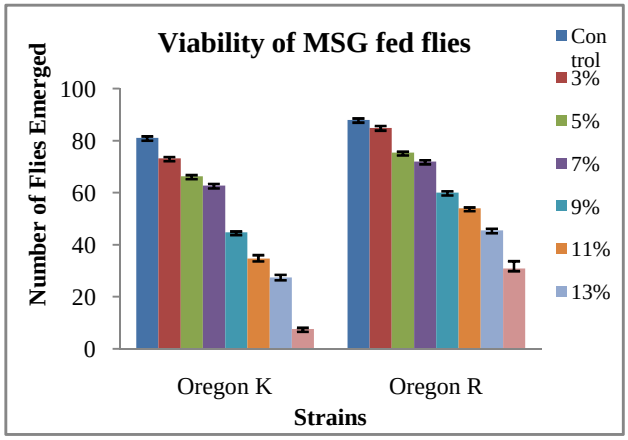

Fig. 3 Viability of Oregon K and Oregon R strain of *Drosophila melanogaster* against various concentrations of MSG.

Acknowledgement

The authors extend their gratitude to the Chairman, Department of Studies in Zoology, University of Mysore, Manasagangothri, Mysore and *Drosophila* Stock Center, University of Mysore for providing facilities to carry out the above work.

We also thank Stress Biology Laboratory, Institute of Excellence (IOE), University of Mysore, Mysore for providing help to complete this work.

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How to cite this article:

Deepak D and M S Krishna., Effect of Chronic Exposure of Monosodium Glutamate (msg) on Viability And rate of Feeding in two Different Strains of Drosophila Melanogaster. *International Journal of Recent Scientific Research Vol. 6, Issue, 6, pp.4483-4485, June, 2015*

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