



RESEARCH ARTICLE

EFFECT OF ENERGY DRINKS' (SYNTHETIC AND NATURAL) ON LIFE SPAN OF *D.MELANOGASTER*

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ABSTRACT

Nowadays popularity of using energy drinks is more common but quality used in these energy drinks has either detrimental or benefits on organisms fitness. Present study was carried out *D.melanogaster* to study effect of synthetic and natural energy drink on longevity. Here the larval feeding rate and adult longevity were studied and it revealed that, flies fed on natural energy drink had consumed greater quantity of food and lived significantly longer compared to flies grown in synthetic energy drink and normal food media. This shows that the nutrients present in the natural energy drink had played a vital role in increasing life span of the fly. Thus these studies suggests that consumption of natural energy drink is more beneficial over synthetic energy drink in *D.melanogaster*.

INTRODUCTION

Diet of an organism is an important extrinsic factor known to affect growth, development and survival of an organism (Sisodia and Singh, 2012). It was shown that dietary restrictions without malnutrition affect lifespan and reproductive output across a wide range of organisms from nematode worms to mammals (Piper *et al.*, 2011) These studies have stressed that it was diet composition had significant influence on life span and reproductive output (Piper *et al.*, 2011; Taylor and Fanson, 2011) Further it was also shown that diet consumed by an organism and its effect on life history traits can be grouped into two types, quantitative which is dependent on food availability, qualitative which is dependent on nutritional compositions of food (Sisodia and Singh, 2012). In recent times there is growing popularity of using synthetic energy drinks by teenagers and also by peoples of all age classes. They presume that it provides instant energy and relief their stress, however the Synthetic Energy drinks contain additives, for example caffeine, Touraine etc, which may cause, obesity, Type II diabetes and it may also leads to cancer. Therefore it is necessary to develop natural energy drinks which do not contain any additives and it should have health benefits. Hence present study has been undertaken, In

D.melanogaster to study the effect of synthetic and natural energy drink on life span.

MATERIALS AND METHODS

Establishment of Stock

The experimental stock of *D.melanogaster* was established from progenies of 105 naturally inseminated females collected at Chamundi hills, Mysore, India. In each generation flies obtained from these culture bottles were mixed together and redistributed to Twenty different culture bottles containing wheat cream agar media (100g of jiggery, 100g of wheat powder, 8g of Agar-Agar was boiled in 1000ml of double distilled water and 7.5ml of propionic acid was added) 20 flies per culture bottle were maintained at 22°C with a relative humidity of 70% in a 12 hrs dark; 12 hrs light cycle. This procedure was carried out for 3 generations to acclimatize flies to lab condition. At fourth generation eggs were collected using Delcour's procedure (1969) and 100 eggs were placed separately for normal media/ natural drink based media (chop the four fruits apple, pomegranate, orange, banana, juices are prepared separately each of 50ml is mixed together and 10ml of vitamin B12 and 60ml of carbonated water are been added a volume of 270ml is finalized for the further analysis and treatments) and synthetic drink based media (Red Bull). Flies obtained from these eggs were used in the present experiment.

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Quantification of Food intake in Larvae using dye method

Ten Second instar larvae obtained from normal media were used to study feeding behavior. Each larva was placed in a vial containing normal /Natural/ synthetic energy drink based media treated with 2.5% (w/v) blue food dye (FD & C Blue Dye no. 1). The larvae were allowed to feed for 15 minutes. Then they were transferred to eppendorf tube and frozen. These frozen larvae were homogenized by adding 200 µl of distilled water further 800 µl of distilled water was added. The absorbance was measured at 629 nm using calorimeter. The larvae which were not treated with blue dye used as the blank. The amount of food taken was measured from the standard graph made from serial dilution of a blue dye.

Effect of synthetic and natural energy drinks on longevity of *D.melanogaster*

To study longevity, flies obtained from synthetic/natural/normal media were individually transferred to new vials once in 7 days containing respective media. This process was continued until death of the individual. The time between eclosion till death was considered as longevity.

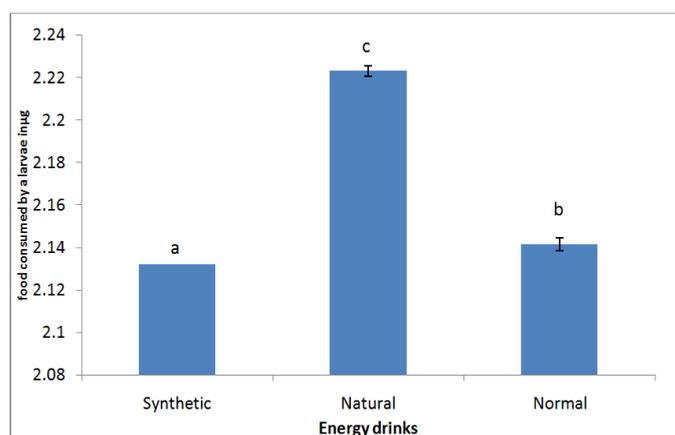


Figure 1. Effect of synthetic and natural energy drink on feeding behavior in larvae of *D. melanogaster*. Different letters on the bar graph indicates significance at 0.05 level by Tukey's Post Hoc test

RESULTS

Figure 1 shows Food intake by a larvae was measured using dye method. It was found that the larvae which were grown in Natural energy drink based media have consumed more

Table 1. One way ANOVA of ' Synthetic and Alternative energy drink' effect on larval feeding, in *D melanogaster*

Dependent Variable	Energy drink	Source	Sum of Squares	Df	Mean Square	F-Value
Larval feeding in (µg)	Synthetic	Media	1.12848	3	0.37616	1684.48**
		Error	0.025904	116	0.000223	
		Total	1.154383	119		
	Alternative	Media	0.150647	3	0.050216	295.6858**
		Error	0.0197	116	0.00017	
		Total	0.170347	119		

** significant at 0.0001 level

Table B. Effect of synthetic and natural energy drinks on Means and Medians for Survival Time in *D.melanogaster*

Media	Mean(a)				Median			
	Estimate	Std. Error	95% Confidence Interval		Estimate	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound			Lower Bound	Upper Bound
Synthetic	45.396	1.239	42.968	47.824	47.000	.000	.	.
Natural	61.964	1.431	59.159	64.769	63.000	2.068	58.946	67.054
Normal	60.063	1.284	57.546	62.579	61.000	.000	.	.
Overall	60.430	1.327	57.828	63.031	63.000	1.853	59.368	66.632

Estimation is limited to the largest survival time if it is censored.

Table C. Overall comparisons

	Chi-Square	df	Sig.
Log Rank (Mantel-Cox)	17.187	2	.000
Breslow (Generalized Wilcoxon)	14.722	2	.001
Tarone-Ware	15.939	2	.000

Test of equality of survival distributions for the different levels of longevity

Statistical analysis

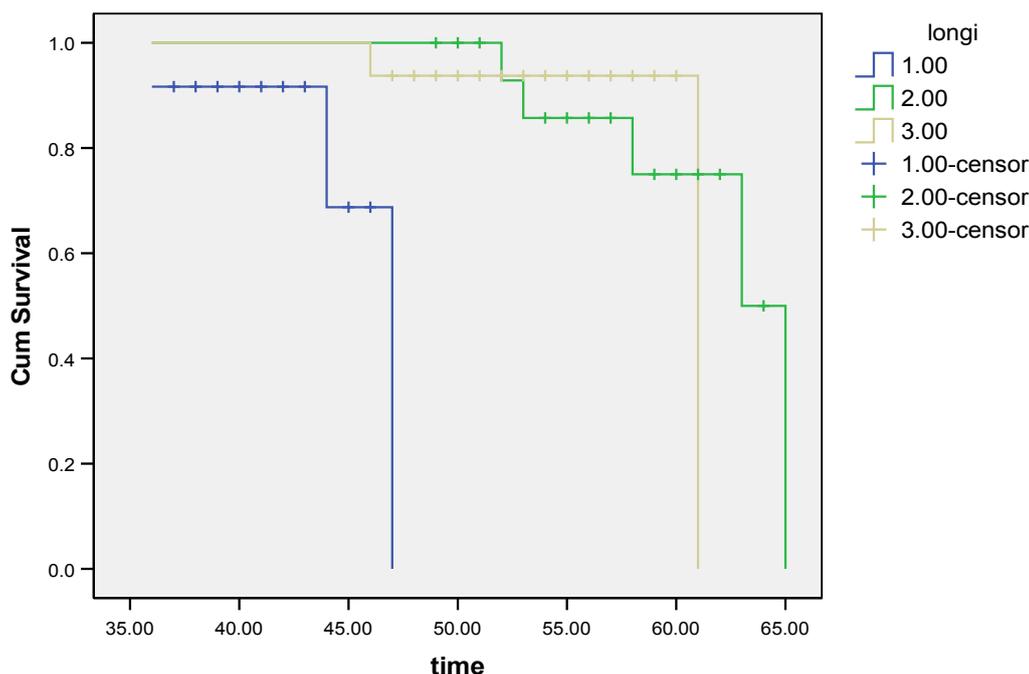
Survival curve was calculated for longevity of males and females. Two functions that are dependent on time are of particular interest: The survival function and Hazard function. The survival function $S(t)$ is defined as the probability of dying at time t having survived until that time. The graph of $S(t)$ against t is called the survival curve. The Kaplan- Meir method was used to estimate this curve from observed survival times without assuming an underlying probability distribution. Two survival curves were compared using a statistical hypothesis test called the log-rank test, which is used to test null hypothesis that there is no difference between survival curves, i.e., the probability of an event occurring at any point of time is for each media 50 trials were made for each of the synthetic/natural and normal media.

amount of food compared to larvae which were grown in Synthetic energy drink based media and wheat cream agar media. One-way ANOVA followed by Tukey's Post Hoc test carried out using SPSS version 14.0 on the above data showed significant variation in feeding rate (Table 1). Figure 2 and table A and B shows the analysis of survival curve of flies grown in synthetic energy drinks, natural energy drink based media and normal media, It was found that flies grown on natural energy drinks lived significantly longer than those flies grown in synthetic energy drink based media and normal media.

DISCUSSION

The Figure 1 and Table 1 reveal that the feeding rate of *D.melanogaster* was varied between Natural energy drink and

Survival Functions



Synthetic energy drinks. Feeding rate was found to be highest in the flies grown in Natural energy drink followed by Normal and synthetic energy drink. This suggests that there is a significant influence of energy drinks on larval feeding rate. In *Drosophila* it was shown that it is a larval stages shows an inhibition threshold when consuming a new or foul tasting foods (Meleher *et al.*, 2007) however such inhibition threshold is not observed in larvae fed on Natural energy drink when compared to Synthetic energy drinks as well as Normal media since the rate of larval feeding was highest among larvae fed on Natural energy drinks. Difference in feeding rate itself an important life history traits because it reveals the amount of food consumed by an organism and the level of nutrient intake by an organism (Weindruch and Walford, 2015; Partridge *et al.*, 2005; Wafa and Krishna, 2015; Alwyn and Krishna, 2015). For example, nutrient intake has a profound influence on lifespan across a wide range of species, with both very low and very high levels of dietary protein (Partridge *et al.*, 2005; Piper and Partridge, 2007). In the present studies flies grown on natural energy drink had consumed greater quantity of food and lived significantly longer than those flies grown on synthetic energy drink and normal media (Figure 2 and 3, table A and B). Further least longevity was observed in flies grown in synthetic energy drinks. This is because the additives such as caffeine etc found in synthetic energy drink might had negative effect on longevity. On the other hand nutrients found in natural energy drinks could support the longevity thereby flies grown on natural energy drink had significantly lived longer. Thus these studies suggests that natural energy drink has health benefit in *D.melanogaster*.

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