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Full Length Research Article

Effect of Energy Drinks' (Synthetic and Natural) on locomotor activity of *D.melanogaster*

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ABSTRACT

Diet used by an organism provides energy which is required for various activities like, locating food, mate, escape from predators, defense of territories and response to stress. In the present study flies reared in natural and synthetic energy drinks were used to study the effect on locomotor ability so it was found out that feeding rate was found to be greater in flies reared in natural energy drinks compared to flies reared in synthetic energy drinks as well as normal media, Further it was also noticed that larval crawling and adult climbing ability was found to be significantly greater in flies reared in natural energy drinks, compared to synthetic and normal, Thus these studies suggests that natural energy drinks had beneficial effect compared to synthetic energy drinks and it may provide greater energy.

Key words: Natural energy drink, Synthetic energy drink, Locomotory Activity, Climbing assay, Crawling assay.

INTRODUCTION

For many of the daily activities of an organism such as courtship, searching of food, searching for mate all these activities requires energy, in addition to this the development, growth, reproduction, and survival also requires energy, locomotion is one of the important activity of an organism because, it is through these activities, organism can locate, food, mate, escape from predators, defense of territories and response to stress. + Organisms obtain energy for activities from their food. Diet effect are of two types quantitative effect, which involves quantity of food consumed by an organism whereas qualitative effect involves nutrient composition found in the food (Sisodia and Singh, 2012). The type of nutrients found in different foods is very much important because they contribute differentially in energy production. Further the balance between energy intake and expenditure of that energy to different activities is very important (Pough, 1989; Sibly, 1991; Karasov, 1989). In recent times the use of synthetic energy drinks is of more frequent among all the age groups, they presume that these energy drinks provides immediate energy and the relief from stress, therefore their assumption is that these are healthy drinks because they contain additives such as caffeine, taurine etc. these compounds actually cause Type II Diabetes, obesity and sometimes it also leads to cancer. Therefore there is a need to develop a natural energy drink which does not contain any additives. However the effect of synthetic and natural energy drinks on locomotory activity and health benefits has not been accessed since *Drosophila* is one of the very good model organism to study nutritional requirements of an organism and it also being used as model system for many of the human disorders because of the mechanisms involved in metabolism are very much conserved between *Drosophila* and humans therefore present study has been undertaken to study the effect of synthetic and natural energy drinks on locomotory activity of *D.melanogaster.*

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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 prop ionic 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.

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.

Climbing Assay

To study climbing ability, Ten mated and unmated flies were placed in the bottom of the measuring cylinder and they were given 20sec to climb up. At the completion of 20sec, the number of flies that climbed up to a vertical distance of >8cms was recorded, climbing ability was carried at 10th and 40th day for the same set of flies, a total of three trials were made for flies obtained from each of three different media .

Larval crawling assay

To study larval crawling ability, Second instar larvae were collected separately from different media, with the help of brush individual larva was transported to a 15cm Petri dish containing 2% agarose (previously poured and allowed to harden) over graph paper with a 0.2cm grid, the number of grid lines crossed by a larvae in 1 minute was recorded under a dissection microscope, a total of thirty trials were made for larvae obtained from synthetic/natural/normal media.

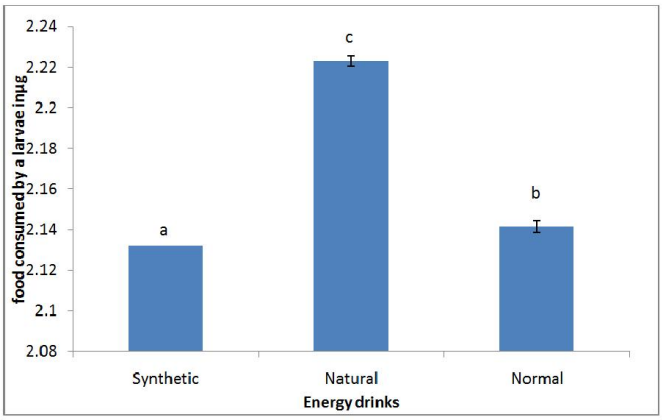
Statistical Analysis

Mean, standard error, One-way ANOVA, Two-way ANOVA and Tukey's Post-Hoc test were carried out on the obtained data using SPSS version 14

RESULTS

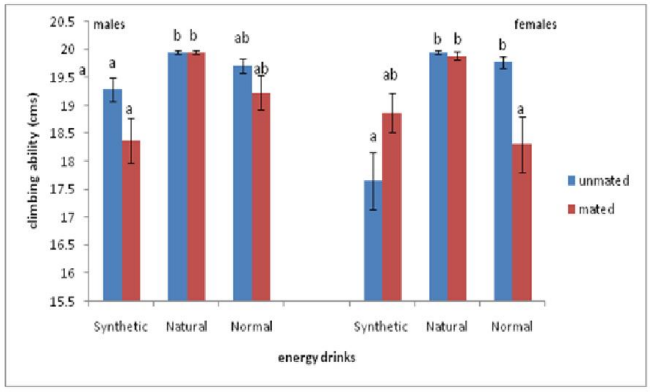
Figure 1 shows Food intake by larvae was measured using dye method. It was found that the larvae which were grown in Natural energy drink based media have consumed more amount of food compared to larvae which were grown in Synthetic energy drink based media and wheat cream agar media. One-way ANOVA followed 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 3 shows that mean climbing ability of 10th and 40th day old flies grown in synthetic/natural/normal media, it was noticed that both unmated and mated flies grown on natural energy drinks had greater climbing ability over flies grown on synthetic energy drinks and normal media further unmated flies had greater climbing ability than those of mated flies in all the media studied, Data of climbing assay was subjected to two way ANOVA followed by Tukey's Post-Hoc test showed that except females of 10th day old remaining flies showed significant variation in climbing ability between flies obtained from different media, Tukey's Post-Hoc test also showed that flies grown on natural energy drinks had significantly greater climbing ability than those flies obtained from synthetic energy drinks and normal media.

Data of synthetic and natural energy drinks effect on larval crawling ability is provided in Figure.4.



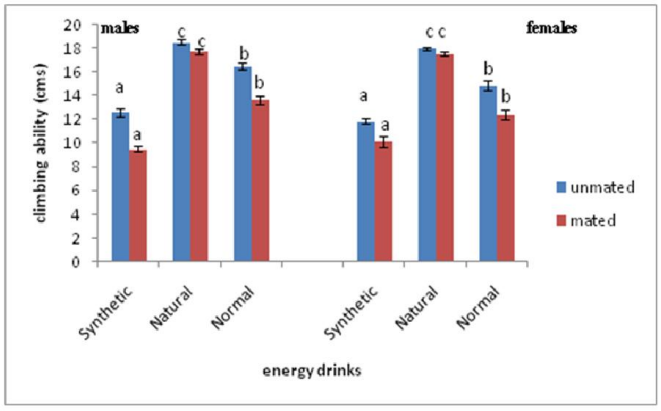
Different letters on the bar graph indicates significance at 0.05 levels by Tukey's Post Hoc test

Figure1. 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 levels by Tukey's Post Hoc test

Figure 2. Effect of synthetic and natural energy drinks on climbing ability of 10th day old mated and unmated flies of *D.melanogaster*



Different letters on the bar graph indicates significance at 0.05 levels by Tukey's Post Hoc test

Figure 3. Effect of synthetic and natural energy drinks on climbing ability of 40th day old mated and unmated flies of *D.melanogaster*

It was found that larval crawling ability was found to be greater in flies grown in natural energy drink based media and least crawling ability was noticed in flies obtained from synthetic energy drink based media.

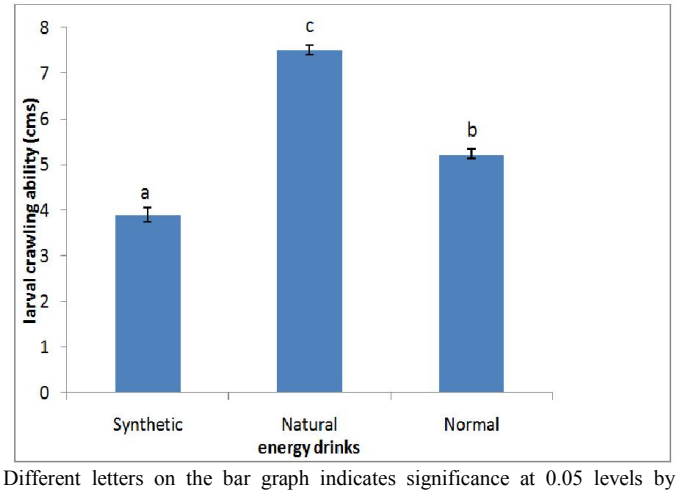
Table 1. One way ANOVA of' Synthetic and Natural energy drink' effect on larval feeding and larval crawling in *D melanogaster*

Dependent Variable	Energy drink	Source	Sum of Squares	Df	Mean Square	F-Value
Larval feeding in (µg)	Synthetic energy drinks	Media	1.12848	3	0.37616	1684.48**
		Error	0.025904	116	0.000223	
		Total	1.154383	119		
	Natural energy drinks	Media	0.150647	3	0.050216	295.6858**
		Error	0.0197	116	0.00017	
		Total	0.170347	119		
arval crawling (cms)		Media	198.756	2	99.378	227.244
		Error	38.047	87	0.437	
		Total	236.802	89		

Table 2. Two way ANOVA of mated and unmated 10 days and 40 days old flies of *D.melanogaste*

Dependent Variable	Energy drink	Source	Sum of Squares	DF	Mean Square	F-Value
Climbing assay for mated and unmated 10th day old flies	males	status	9.8	1	9.8	6.158921*
		source	38.275	2	19.1375	12.02718**
		status*source	6.308333	2	3.154167	1.982272NS
		Error	276.8667	174	1.591188	
		Total	68192.5	180		
	females	Status	0.501389	1	0.501389	0.151797NS
		source	82.61944	2	41.30972	12.50666**
		status*source	54.03611	2	27.01806	8.179811*
		Error	574.725	174	3.303017	
		Total	66167.75	180		
Climbing assay for mated and unmated 40th day old flies	males	Status	488.4014	1	488.4014	8.347969*
		Source	888.6583	2	444.3292	7.594668*
		Status*source	249.2361	2	124.6181	2.130026NS
		Error	10179.94	174	58.50541	
		Total	54172.25	180		
	females	Status	104.2722	1	104.2722	31.53449**
		Source	1406.053	2	703.0264	212.6125***
		Status*source	30.35278	2	15.17639	4.589714*
		Error	575.35	174	3.306609	
		Total	38099.5	180		

One way ANOVA followed by Tukey's Post-Hoc test carried out on above data showed significant variation in larval climbing ability between flies obtained from different media.



Different letters on the bar graph indicates significance at 0.05 levels by Tukey's Post Hoc test

Figure 4. Effect of synthetic and natural energy drinks on larval crawling ability of *D.melanogaster*

Tukey's Post-Hoc test also showed that crawling ability of flies grown on natural energy drinks had significantly greater ability than those flies grown in synthetic energy drink based media and normal media.

DISCUSSION

D. melanogaster is one of the most popular animal models to study beneficial and detrimental effects of energy drinks under different physiological and pathophysiological conditions, while the beneficial effects of natural energy drinks over synthetic energy drinks on locomotor ability in *D. melanogaster* was not tested, hence flies grown on different energy drinks based media(normal, natural and synthetic) were subjected to experiment to determine their locomotor ability (larval crawling and adult climbing). It was noticed that flies reared on different energy drink based media had a significant influence on larval feeding (Fig. 1 and Table 1). In *Drosophila*, it was shown that the larvae can show inhibition threshold when consuming a new or foul tasty food (Melcher *et al*., 2007). In this study also such an inhibition threshold was found when the larvae fed on synthetic energy drinks. Hence larvae fed on synthetic energy drinks had taken a significantly lesser quantity of food.

Nowadays the consumption of energy drinks has become more popular among all the age groups and It is presumed by them that the consumption of synthetic energy drinks gives energy and it reliefs from stress but there are no experimental evidences that these caffeinated energy drinks either gives energy or reliefs from stress. Hence, in the present study, we subjected larvae and flies fed on different energy drinks media to locomotor activity (larval crawling and adult climbing) to study its implication on locomotor behavior.

The Fig 4 shows that the larval crawling ability was found to be highest in natural energy drink and it was found to be least in larvae fed on synthetic energy drink based media (Table 1). This is because the amount of food consumed by the natural energy drinks fed larvae was greater than other normal and synthetic energy drinks based media fed larvae hence the food consumed found to have a direct effect on the crawling ability of larvae. In the present study adult flies locomotor ability was tested by performing climbing assay. Natural energy drinks based media fed flies had significantly greater climbing ability compared to synthetic energy based media fed flies till the age of the 10th (Fig. 3 and Table 2). In (Fig. 3) it is shown that the 10^{th} day old flies has greater climbing ability in unmated flies compared to mated flies but in natural energy drink based media fed flies both mated and unmated males and females shows significantly similar climbing ability whereas the female flies grown in synthetic energy drink based media mated flies has significantly greater climbing ability over unmated flies. In (Fig 4) it was shown that the 40^{th} day old flies which are reared in natural energy drink based media has greater climbing ability irrespective of mated and unmated males and females compared to flies reared in synthetic and normal media, this shows that the flies which consumed natural energy drink lasts energy even when they get older because they do not contain any additives which are present in synthetic energy drinks.

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REFERENCES

Alwyn D'souza and M. S. Krishna*. Effect of energy drinks' (Synthetic and Alternative natural) on Pre-adult development of *D.melanogaster*

Anderson, M., 1994. Sexual selection, Princeton University

Chapman, C. A,, Chapman, L. J., Rode, K. D., Hauck, E.M., McDowell, L. R. 2003. Variation in the Nutritional Value of Primate Foods: Among Trees, Time Periods, and Areas. International. *Journal of Primatology* 24(2):317-333.

Fanis Missirlis, Sterner, R.W., Schulz, K.L., 1998. Zooplankton nutrition: recent progress and a reality check. Aquatic Ecol 32: 261–279.

James, H., sang and Robert, C. King, 1961. Nutritional requirements of axenically cultured drosophila melanogaster adults. Agricultural Research Council Poultry Research Centre, Edinburgh 9, Scotland.

Krishna, M. S. and Hegde, S.N. 2003. Influence of body size in mating success in three sympatric species of *Drosophila, Italian Journal of Zoology*, 70(1). pp. 47-52.

Luciano M. Matzkin, Sarah Johnson, Christopher Paight and Therese A. Markow Mathew, D. W. Piper and Linda Partridge. Dietary restriction in *Drosophila*: Dela

Odegaard, Andrew, O. Koh, Woon-Puay, Arakawa, Kazuko, Yu, Mimi C. Pereira, 2010. Soft drink and juice consumption and risk of physician-diagnosed incident Type 2 diabetes.

Pough, F. H., 1989. Organismal performance and Darwinian fitness: approaches and interpretations. Physiol Zool 62: 199–236.

Preadult Parental Diet Affects Offspring Development and Metabolism in *Drosophila melanogaster*

Sibly, R.M., 1991. The life-history approach to physiological ecology. Func Ecol 5: 184–191. doi: 10.2307/2389256

Sisodia, S. and Singh, B. N. 2012. Experimental Evidence for Nutrition Regulated Stress Resistance in Drosophila ananassae. PLoS ONE 7(10):1-9.

Subramani Paranthamam Balasubramani, Jayaram Mohan, Arunita Chatterjee, Esha Patnaik, Subrahmanya Kumar kukkupuni, Upendra nongthomba, Padmavathy Venkatasubramanian. Pomegranate Juice enhances healthy lifespan in *D.melanogaster:* An exploratory study

Taylor, E.N., Malawy, M.A., Browning, D.M., Lemar, S.V., DeNardo, D.F., 2005. Effects of food supplementation on the physiological ecology of female Western diamond-backed rattlesnakes (*Crotalus atrox*). Oecologia 144: 206–213. doi: 10.1007/s00442-005-0056-x

Wafa Faroki, M.S. Krishna, 2014. Organically grown fruits' effect on reproductive fitness of *Drosophila melanogaster. Cancer Biology,* 4(4):48-55]. (ISSN: 2150-1041). http:// www.cancerbio.net. 7

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