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Research Article

Effect of Minerals on Free Radicals Induced Damages in *Drosophila melanogaster*

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ABSTRACT

A free radical is a molecule or molecular fragments containing one or more unpaired electrons in its outermost atomic or molecular orbit, and they are very reactive, capable of independent existence. Free radicals are well documented for playing a dual role in our body as both deleterious and beneficial species. Minerals are inorganic substance required by the body in small amount for a variety of function. The present study was designed to investigate the toxicity of free radicals on Drosophila melanogaster and free radicals scavenging activity of minerals which is most essential for routine metabolism. Minerals and free radicals effect on drosophila growth, body weight and longevity changes, free radicals scavenging activity assay were evaluated. The present study results reveal that addition of minerals enhances the growth and survival. Test organisms exposed to an array of concentrations of DPPH showed statistically significant eclosion inhibition from 10µM to 250 µM and 100% inhibition was observed in 500 µM. This study results enlightened the biological significance of free radicals in living organism's physiology and maintenance.

Keywords: *Drosophila melanogaster*, Scavenging activity, DPPH, Free radicals, Minerals

INTRODUCTION

A free radical is a molecule or molecular fragments containing one or more unpaired electrons in its outermost atomic or molecular orbit, and they are very reactive, capable of independent existence. The first organic free radical identified was triphenylmethyl radicals discovered by Moses Gomberg in 1900. The simplest free radical is an atom of the element hydrogen, with one proton and a single electron (H.)[1]. Examples of oxygen centered free radicals are superoxide's, hydroxyl, peroxyl, alkoxyl, and hydroperoxyl radicals. Nitric oxide and nitrogen dioxide are two nitrogen centered free radicals. Thiyl radicals are sulfur centered free radicals and trichloromethyl is example of carbon centered free radical[2]. Free radicals can be generated in the body from various sources that can either be endogenous or exogenous. Exogenous sources of free radicals include UV radiations, X-rays, gamma rays, microwave radiations, metal-catalyzed reactions, pesticides, food preservatives, smoking, alcohol, etc. There are several endogenous source free radicals include, inflammation initiates neutrophils and macrophages to produce ROS and RNS, in mitochondria-catalyzed electron transport reaction, oxygen free radicals produced as by product[3]. Free radicals are well documented for playing a dual role in our body as both deleterious and beneficial species. In low or moderate concentrations free radicals are involved in normal physiological functions such as involve in immune response, controlling the blood flow through our arteries, act as cell signaling molecule, some free radicals kill cancer cell etc.[5]. Excess production of free radicals or decrease in antioxidant level leads to oxidative stress. Free radicals are very unstable and react quickly with other compounds, and try to capture the needed electron to gain stability, a chain reaction thus get started. Once the process is started, it can cascade, and finally results in the disruption of living cells[4]. Generally harmful effects of reactive oxygen species on the cells are most often like damage of DNA, oxidation of polyunsaturated fatty acids in lipids, oxidation of amino acids in protein, oxidatively inactivate specific enzymes by oxidation of cofactors. Lipid peroxidation is one of the deleterious effects of free radicals[6]. Antioxidants are any substance that delay or inhibits oxidative damage to a target molecule. At a time one antioxidant molecule can react with single free radicals and are capable to neutralize free radicals by donating one of their own electrons, ending the carbon-stealing reaction. Antioxidant prevents cell and tissue damage as they act as scavenger. A variety of components act against free radicals to neutralize them from both exogenous and endogenous in origin. These include endogenous enzymatic antioxidants, non-enzymatic, metabolic and nutrient antioxidants, metal binding proteins like ferritin, lactoferrin, albumin and cerulopasmin and phytoconstituents and phytoproteins[7]. There are natural and synthetic antioxidants. The natural antioxidants include vitamin E, vitamin C, vitamin A, ascorbic acid, green tea, pomegranate etc. The synthetic antioxidants include butylated hydroxyl toluene (BHT), butylated hydroxyl anisole (BHA), Gallic acid etc. Regular physical exercise enhances the antioxidant defense system and protects against exercise induced free radical damages. These changes occur slowly over time and appear to be

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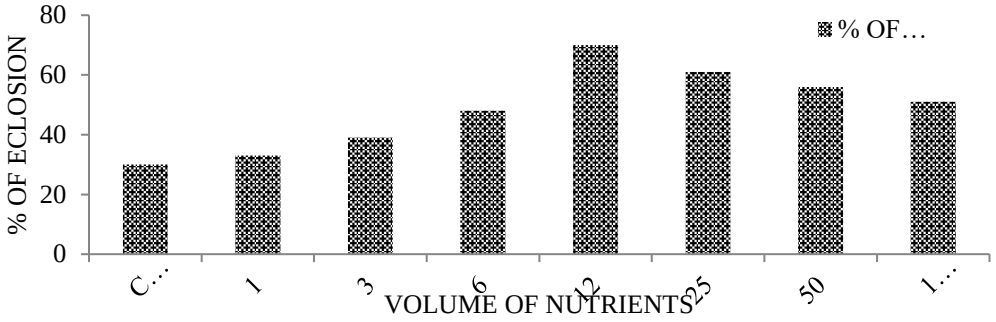

Figure 1: showing the effect of minerals on *Drosophila*eclosion.

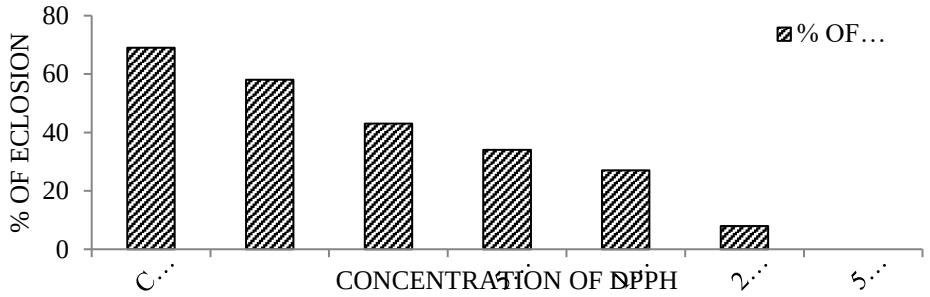

Figure 2: Showing the effect of free radicals on *Drosophila* eclosion.

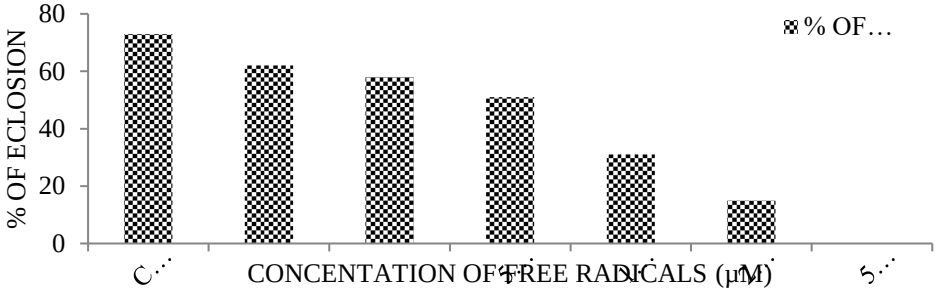

Figure 3: Showing the combined effect of free radicals and minerals on *Drosophila* eclosion.

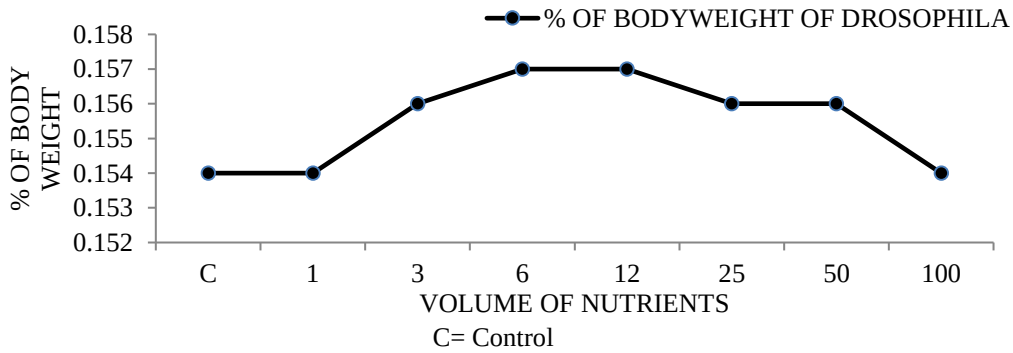

Figure 4: Showing the Weight of *Drosophila* treated with minerals.

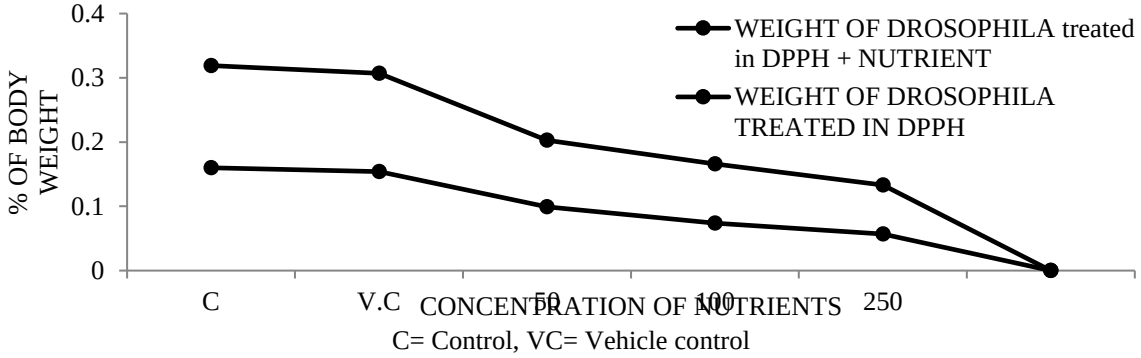

Figure 5: Showing the weight of *Drosophila* treated with DPPH and both DPPH and minerals.

parallel other adaptations to exercise. Endurance exercise can increase oxygen utilization from 10 to 20 times over the resting stage. This greatly increases the generation of free radicals, prompting concern about enhanced damage to muscle, and other tissues[8]. Minerals are inorganic substance required by the body in small amount for a variety of function. Minerals play a different role in the fight against free radicals pathology. Minerals works synergistically with the superoxide dismutase (SOD) enzyme to protect the body cells from free radicals damage. Oxidative changes in low density lipoprotein cholesterol (LDL-C) have been linked with atherosclerosis. But trace elements, such as copper and zinc are reported to prevent this phenomenon via their direct effect on LDL-C, or more likely as part of the SOD

	Micro minerals		Macro minerals	
S. No.	Compound	mg/l (ppm)	Compound	mg/l (ppm)
1.	$Fecl_3$	200	NH_4Cl	1400
2.	$Mncl_2.2H_2O$	500	KH_2PO_4	1250
3.	Mg_2EDTA	500	$MgSO_47HO$	500
4.	$NaSeO_3$	500	$CaCl_2.2H_2O$	50
5.	H_3BO_4	100	$NaHCO_3$	2000
6.	$Zncl_2$	50		
7.	$NH_4MoO_{24}HO$	50		
8.	$Cocl_2.6H_2O$	50		
9.	$Cncl_2.2H_2O$	50		

Table 1: Eclosion rate of *Drosophila* treated with minerals.

Volume of minerals in µl	No. of eggs exposed	% of eclosion	% differences than control
Control	100	30	100.0
1	100	33	110.0
3	100	39	130.0
6	100	48	160.0
12	100	70	233.3
25	100	61	203.3
50	100	56	186.7
100	100	51	170.0

enzyme shown to block free radical formation. In the present study an attempt has been taken to investigate the toxicity of free radicals on *Drosophila melanogaster* and free radicals scavenging activity of minerals which is most essential for routine metabolism.

METHODOLOGY

Test organism

The test organism selected for the present work was *Drosophila melanogaster* commonly known as fruit fly or vinegar fly. The healthy culture of the *Drosophila* were procured from Mangalore University, Department of Zoology and transported by ensuring minimum stress

Table 2: The eclosion rate of *Drosophila* treated with minerals.

Concentration of free radicals DPPH (µM)	no. of eggs exposed	% of eclosion
Control	100	69
Vehicle Control	100	58
10	100	43
50	100	34
100	100	27
250	100	8
500	100	0

Table 3: Eclosion rate of *Drosophila* treated with both minerals and free radicals.

Concentration of free radicals (µM)	No. of eggs exposed	% of eclosion	% of inhibition
Vehicle Control	100	62	15.1
10	100	58	20.5
50	100	51	30.1
100	100	31	57.5
250	100	15	79.5
500	100	0	100.0

Table 4: Weight of *Drosophila* treated with minerals.

Volume of Nutrients	Weight of *Drosophila* (weight in gm)
CONTROL	0.154
1	0.154
3	0.156
6	0.157
12	0.157
25	0.156
50	0.156
100	0.154
Mean±Standard Deviation	0.1571±.00125

during transportation and maintained in the laboratory for the entire work.

Chemicals

DPPH solution

DPPH is common abbreviation for an organic chemical compound 2, 2-diphenyl-1-picrylhydrazyl (also known as 1,1-diphenyl-2-picrylhydrazyl radicals, 2,2-diphenyl-1-(2,4,6-trinitrophenyl) hydrazyl, diphenylpicryl-hydrazyl). It is a dark colored crystalline powder composed of stable free radicals molecule. 15mg DPPH powder weighed in semi analytical electronic balance (SHIMADZU -Ax200, Japan) and make up to 100ml methanol and kept in dark so as to avoid photo bleaching.

Minerals

In the present study micro and macro mineral solutions were prepared by weighing each components using semi analytical electronic balance (SHIMADZU Japan, Ax200) and dissolve in one liter distilled water and stored in amber

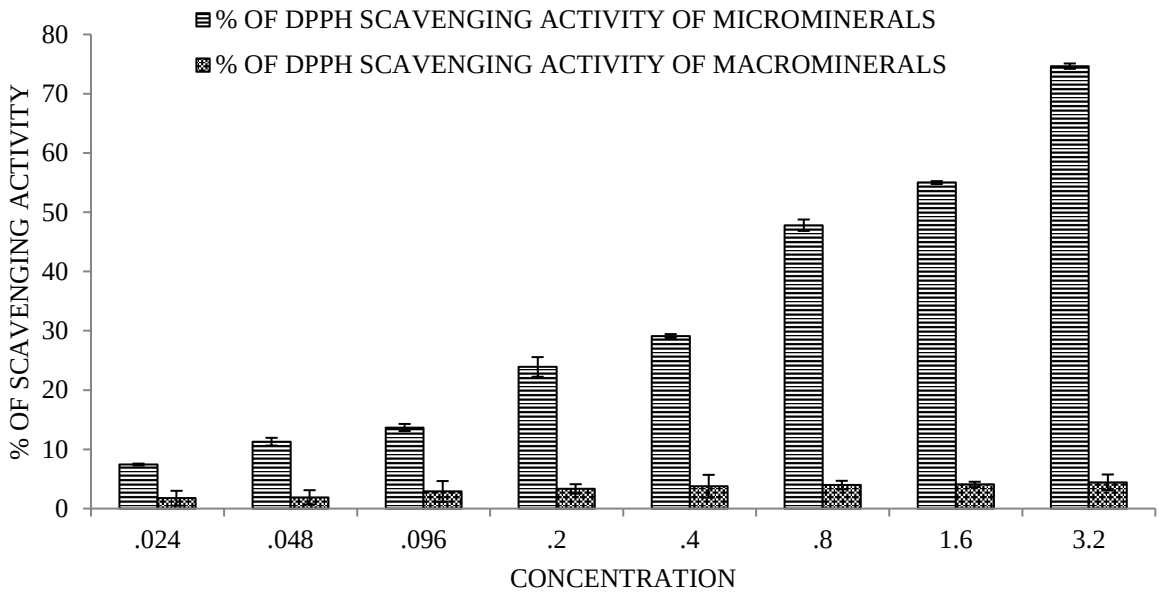

Figure 6: showing the free radicals scavenging activity of minerals.

Table 5: Weight of Drosophila treated with DPPH and both DPPH and minerals.

Concentration of DPPH	No. of Drosophila examined	Weight of Drosophila treated in DPPH + nutrient	Weight of Drosophila treated in DPPH
Control	100	0.159	0.16
Vehicle control	100	0.153	0.154
10	100	0.127	0.115
50	100	0.104	0.099
100	100	0.092	0.074
250	100	0.076	0.057
500	100	0	0
MEAN±STANDARD DEVIATION		.0798±.048	0.0690±.044

Table 6: Percentage of death rate of drosophila treated in DPPH.

Concentration	% of death in days								
	1st day	3rdday	6thday	9th day	12th day	15th day	18th day	21st day	24th day
Control									
Vehicle control	0	0	0	0	0	0	13.04	36.23	50.72
10	0	2.33	27.91	53.49	13.953	2.33			
50	0	18.52	52.94	26.47	5.88				
100	0	29.63	59.26	11.11					
250	0	75.00	25.00						

bottles. The ratios of components per liter was tabulated below,

Effect of minerals and free radicals on drosophila growth

The experiment detecting the optimum concentration of minerals for maximum *Drosophila* growth conducted by setting concentrations include control (without nutrients), 1µl, 3µl, 6µl, 12µl, 25µl, 50µl, and 100µl volume of both macro minerals and micro minerals per 25ml of medium and transferred to the pre-sterilized bottles. For detecting free radicals effect on *Drosophila* growth, setting an array of concentrations includes control (with minerals only), vehicle control (with methanol and minerals), 10µM, 50µM, 100µM, 250µM, 500µM of DPPH per 25ml of medium and transferred into pre-sterilized bottles. Along with these experiments also detect minerals effect on free radicals treatment. For this work setting concentrations include control (with minerals only), vehicle control (with methanol and minerals), 10µM, 50µM, 100µM, 250µM, 500µM of DPPH per 25ml of medium and transferred into pre-sterilized bottles. Along with the DPPH 12µl both micro and macro minerals are added. Four replica of each experiment was conducted were maintained so as get 100 number of test organisms per each concentrations. 25 numbers of healthy eggs were transferred to each bottle and ensure minimum disturbance to the eggs. Developments of test organisms were observed day by day and record the major events like pupations and eclosion.

Body weight of Drosophila

Table 7: Free radicals scavenging activity of minerals.

	Conc. (µL)	% OF DPPH scavenging activity of microminerals	% OF DPPH scavenging activity of macrominerals
1.	0.024	7.4307±.16130	1.7482±1.24938
2.	0.048	11.2862±.65671	1.9062±1.20121
3.	0.096	13.6843±.60071	2.8858±1.75948
4.	0.2	23.9032±1.65845	3.3193±.79729
5.	0.4	29.1195±.32378	3.7803±1.91626
6.	0.8	47.8163±.96079	3.9705±.71492
7.	1.6	55.0138±.24333	4.0772±.45155
8.	3.2	74.6557±.45162	4.4352±1.31999

Values are expressed as mean ± standard deviation

The three day old female flies emerges from above experiments were taken for body weight measurement. 100 female body weights from each experiment is measured using weighing balance.

Longevity assay

Newly emerged flies from above described experiment are transferred to new vials containing fresh medium. The death of flies was recorded every day. 100 young flies were used for longevity assay.

Free radicals scavenging activity assay

The free radicals scavenging activities of minerals were assayed using a stable DPPH, following standard method[9] with slight modification. The reaction mixture contain 200µL of 100µM DPPH solution and 50µL different concentration of (0.2µL, 0.4µL, 0.8µL, 1.6µL, 3.2µL) micro and macro minerals. The combined effect of minerals was also tested. The mixture was then incubated in the dark for 30 minutes at 37°C and the absorbance at 517nm was recorded as sample (A_{Sample}), using 96 well micro plate reader (SYNERGY HT, USA). A blank experiment was also carried out applying the same procedure to a solution without the minerals and the absorbance was recorded as blank (A_{Blank}). The free radicals scavenging activity of each solution was then calculated as percent inhibition according to the following equation.

$$\% \text{ of inhibition} = 100 \times (A_{Blank} - A_{Sample})/A_{Blank}$$

RESULT

Minerals effect on drosophila growth

From Table 1 and Fig. 1, it was evident that additions of nutrients enhance overall performance of growth and survival of the test organisms. The maximum value of eclosion rate was observed in 12µl nutrients which was 233.3% higher than the normal. The eclosion rate increase with increasing quantity of nutrients up to 12µl and start declaim drastically (Table 1). The highest quantity of nutrients was administrated to the present experiment was 100µl, which was 8.3 times higher than the optimum quantity. The eclosion inhibition recorded between these quantities was 27.2% less than that of the control group and 127.2% lower values than the 12µl administrated group. One way ANOVA showed a significant effect of the nutrients on *Drosophila* eclosion ($F_{7,24}$) =42.984; p=0.000).

Effect of free radicals on drosophila eclosion

From Table 2 and Fig. 2, it was revealed that concentration of free radicals increase with decreasing the eclosion rate. In the lowest concentration (10µM), the percentage of eclosion was 43%. When the concentration reaches to 500 µM the percentage ofeclosion become zero. No flies are emerges in that treatment. The present study result showed that the eclosion rate of *Drosophila* was significantly different from vehicle control and control ($p<0.05$). Negative correlation was observed between the concentration of free radicals and percentage of eclosion (Pearson correlation coefficient, r=-.945, p=.015).

Combined effect of free radicals and minerals on drosophilaeclosion

From Table 3 and Fig.3, it was revealed that concentration of free radicals increase with decreasing the eclosion rate. In the lowest concentration (10µM), the percentage of inhibition on eclosion was 20.5%. When the concentration reaches to 500 µM the percentage of eclosion become zero *ie.* inhibition was 100%. In the present study, the % of eclosion of *Drosophila* was significantly different from vehicle control and control ($p<0.05$). Negative correlation was observed between the concentration of free radicals and percentage of eclosion (Pearson correlation coefficient, r=-.942, p=0.016).

Estimation of body weight of drosophila

By adding nutrients alone, the mean weight of *Drosophila* was 0.1557±.00125 g, which was significantly increasing than the control (t=3.618, p=0.011). No significant correlation was observed between the quantity of nutrients given and the weight of *Drosophila* (Pearson correlation coefficient, r=-0.472, p=0.285). Administration of DPPH and nutrients, the mean weight of *Drosophila* was 0.69±0.45g, which was significantly different from vehicle control (t=-4.264, p=0.013) and control (t=-4.565, p=0.010). Negative correlation was observed between the concentration of DPPH and weight of *Drosophila* (Pearson correlation coefficient, r=-0.984, p=0.002). Addition of DPPH alone, the mean weight of *Drosophila* was 0.69±0.45g, which was significantly different from vehicle control (t=-3.386, p=0.028) and control (t=-3.664, p=0.022). Negative correlation was observed between the concentration of DPPH and weight of *Drosophila* (Pearson correlation coefficient, r=-0.983, p=0.003).

Longevity assay

The table 6, Shows that the free radicals are significantly affecting the *Drosophila* life span. When compare with the control the treated flies had very short life span. The maximum life span was observed in control and vehicle control (24 days). In high concentration showed maximum life span was up to 6 days. The results reveal that free radicals induced stress may decrease the life span of *Drosophila.*

Free radicals scavenging activity

From the Table 7 and fig. 6, it was showed that the free radical scavenging activity of micro minerals increases with increasing the volume of minerals. The IC_{50} value was found at 1.45µl micro minerals. A positive correlation was observed between volume of micro minerals and % of scavenging activity (Pearson correlation coefficient,

r=0.937, p=0.002). Oneway ANOVA showed a significant effect of minerals on free radicals scavenging activity (F (7,40) =5.694, p=0.000). The study results of free radicals scavenging activity of macro minerals showed poor activity no more significance. IC_{50} value was found to be 36.077µl. a positive correlation was observed between volume of macro minerals and percentage of scavenging activity (Pearson correlation coefficient, r=0.0748, p=0.053). Oneway ANOVA showed a significant effect of minerals on free radicals scavenging activity (F (8,39) =3.610, p=0.003).

DISCUSSION

The main objectives of the study were to demonstrate the toxic effect of free radicals on *Drosophila melanogaster* and prove the antioxidant activity of dietary minerals. The present study of minerals effect of drosophila growth showed that, when compare with control (without minerals) the eclosion rate is significantly increase in test experiments. This proved that dietary minerals are important factor in growth of insects. Between test experiments, the eclosion rate is significantly decline when the mineral composition exceeds from optimum level. This reveals that the excess minerals content are unfavorable for the growth of insects. The results showed that, in insect's development the minerals play a vital role. The minerals composition in their diet increase with increasing the insect's growth but accumulation of minerals declines the growth rate. The earlier studies on consequences of specific nutrient deficiencies for growth performance, food processing efficiencies and detoxciation enzyme activities in larvae of the gypsy moth, *Lymantriadispar*. According to them the low protein and low mineral diet leads to prolonged developmental time of females and reduced pupal weight of male and female[10]. Free radicals are the highly reactive molecules causing physiological and pathological effect on organisms. Free radicals attacks most of the macro and micro molecules of the cell and finally leads to the cell death. The present study results showed that free radicals exposure increases with decreasing the eclosion rate of *Drosophila*. Among experiments, in high concentration (500 µM) the complete eggs are failed to hatch. The results showed that the free radicals concentration determines the egg development. The free radicals causes' larval mortality may be due to toxic effect on the growth of larval tissues. In free radicals treated experiments the larval development becomes slowed down. The developmental time from egg to adult become exceeded. During the development of organism oxygen may react directly with some cellular components and be metabolized to form highly reactive free radicals. Reactive intermediates of oxygen metabolism and the cellular responses to their accumulation may be and the cellular responses to their accumulation may affect development of organism[11]. Studies on developmental delay induced by low-KeV energy ion in early larva of the nematode *Caenorhsbditis elegans* shows that the ionizing radiation induces the excess generation of free radicals. This leads to the developmental delay, decrease in mean brood size when compare with control of test organism. In the present study, when compare the DPPH alone treatment with the combined mineral-DPPH treatment showed that DPPH-mineral combination reduces the effect of free radicals. The eclosion rate, body weight and the life span of *Drosophila*was increase in combined DPPH-mineral treatment than DPPH alone treatment. An in vitro study of scavenging activity of minerals proved that the minerals are the good antioxidants, they can scavenge the free radicals significantly. Minerals have vital role in metabolism. They are cofactors of many enzymes including free radicals scavenging enzymes such as superoxide dismutase, glutathione (GSH) etc. In DPPH mineral combined experiments, the minerals can scavenge the DPPH independently and also these minerals enter to the test organism through food. The extent of the tissue damage is the result of the imbalance between the free radicals generated and antioxidant protective defense system. The mineral helps defense against free radicals damages. They are the cofactors of free radicals scavenging enzymes. Selenium is help for the activity of glutathione peroxidase, iron for the catalase and superoxide dismutase needs copper, zinc and manganese[12]. Takashi *et al*, (2009) studied the useful properties of macro algal beach- casts for food. They determined the mineral composition and antioxidant properties of aqueous solution obtained from frond of eight brown and four green algae. The solutions mainly contain high content of potassium, magnesium, and calcium ions. These solutions showed strong DPPH scavenging activities and reducing power[13]. Manzoor*et al*, (2012) studied the variations of antioxidants characteristics and mineral contents in pulp and peel of different apples. According to their studies apple peel is more rich in minerals than pulp. Amount of potassium to be highest, followed by magnesium, calcium, iron, sodium and zinc. Their results reveal that apple peel shows superior antioxidant activity than pulp[14]. The present study results prove free radicals effects causes premature aging and wait losses in adult oraganisms. The free radicals exposure causing the delayed larval development. Minerals are the essential nutrients which helps to reduce the toxic effect of free radicals they able to scavenge the fee radicals and protect the body from damages.

CONCLUSION

On the basis of result it may conclude that 12 µl cocktail minerals administrated in the experiment showed highest eclosion rate and which 233.3% higher than the control group. Being a laboratory experiment model this concentration of cocktail can be incorporated to the medium for the better survival and longevity. The present study results reveal that addition of minerals enhances the growth and survival. Test organisms exposed to an array of concentrations of DPPH showed statistically significant eclosion inhibition from 10µM to 250 µM and 100% inhibition was observed in 500 µM. This study results enlightened the biological significance of free radicals in living organism's physiology and maintenance. In addition, further study is required for the increment of

depth knowledge regarding the in vivo scavenging activity of minerals on *Drosophila melanogaster*.

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