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Effects of dietary iron on phenotypic characteristics of fruit fly (*Drosophila melanogaster*)

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Abstract
The effects of different concentrations of iron sulfate on progeny production, life cycle, and lifespan of *Drosophila melanogaster* were analyzed in this study. Effects of iron accumulation on progeny production and life cycle were analyzed by culturing fruit flies with different doses of iron. Compared to the control group, the result showed a significantly increasing number of the progeny in both F1 and F2 generations when using 1 mg/l iron dose. A significant lengthening of the life cycle was observed from F1 to F3 in case of 5 mg/l and from F1 to F4 for 10 mg/l iron fortified media. Lifespan experiments indicated that the female flies have a slightly longer lifespan in 0.3 mg/l and 1 mg/l group while male flies showed slightly longer lifespan than female in case of 10 mg/l group

Keywords: Iron, Hemochromatosis, Toxicity, Reproduction, Lifespan, *Drosophila melanogaster*

1. Introduction
Iron is one of the integral components of many biochemical properties which is maintained normal physiological activities for the healthy life [1-3]. Insufficient iron causes different effects at the cellular level like limited oxygen supply, meager work performance and reduces immunity [4, 5]. The most common sign of iron deficiency is anemia; resultantly reduces the size and number of red blood cells [6, 7]. Conversely, iron can toxicant even life threatened when it is present at an excessive level [8]. Iron presents as a natural nutrient in all foods of plant and animal origin and even in drinking water. Body iron amounts range from around 3 to 5 g under normal physiological conditions [9]. Iron and its ingredients can cause harmful effects to humans, animals, and plants when it is observed in the environment as pollutants. Deviation from the normal iron range can lead to tissue damage, resulting in the formation of free radicals if iron homeostasis is not tightly regulated. In human, the concentration of tissue iron is regulated primarily by absorption due to the lack of a proper physiological mechanism to excrete excess iron [10, 11]. Iron absorption in *D. melanogaster* is of vital significance for their physiological activities [12]. Iron is one of the most indispensable micronutrients for the growth and development of fruit flies [13-15]. Iron homeostasis is regulated by a complex mechanism in *Drosophila* which is similar in the manner for maintenance of iron in the human body [16].
Hemochromatosis is a hereditary disorder and controlled by a distinct hemochromatosis gene (HFE gene) [17]. This disorder is most often considered as the major cause of toxicity and often characterized by excessive absorption and toxic accumulation of dietary iron in different organs [18]. High levels of iron saturation in the tissues can often lead to tissue damage, particularly in the liver where excess iron primarily stored to protect other tissues [19-21]. The iron toxicity could affect different endpoints of nematodes in a dose-dependent manner to cause multiple biological defects [22]. In case of iron-exposed *Caenorhabditis elegans*, it is revealed that most of the multiple biological defects could be transferred from parents to their progeny [23]. Previously, harmful effects of excessive iron absorption in reproduction were observed but the possibility of transferring multiple biological toxicities of excessive iron exposure to progeny remains little known [23]. In the last decade, Bangladesh has been affected by the serious problem of heavy metal contaminations including iron in the drinking water. The normal concentration of iron in the drinking water for humans is reported to be 0.3 mg/l [24]. Nevertheless, in the drinking water of Bangladesh, these values are over the international parameters, oscillate between 0.3 to above 6 mg/l of iron [25]. In a study which covers about

86% of total area of Bangladesh, 41% and 22.5% of studied area exceed the iron concentration of 1 mg/l and 5 mg/l respectively [26].

The great advantages of investigating environmental toxicity are to develop a standard protocol with low cost, rational and fast execution capability [27, 28]. *Drosophila* is a model animal that plays a vital role in the versatile research fields of life sciences [29-31]. *Drosophila* is easy to handle, inexpensive to maintain their life, reproduction and growth give a large quantity of progeny, and its genome sequence and genetic information are well documented [27, 28]. The fruit fly, *D. melanogaster* is most important due to its genetic homology with human [32]. Currently, different mammal diseases have been modeled in this fruit fly which covers a broad range of physiological alterations [33]. *Drosophila* is also considered beneficial to study potential toxic effects of wide range of compounds including metal toxicants [34]. Due to their shorter life cycle, it is easy to analyze the effect of metal toxicity during development and adulthood. In addition, neuronal activities, survival, and behavior assays are easy to perform in this organism [35-37]. Toxicity-mediated mechanisms at the molecular level can be easily searched in *Drosophila*. Although a lot of various studies have been done on *Drosophila*, only very few researches about iron toxicity in *D. melanogaster* have been reported [12, 15, 38-40]. Therefore, the present study was undertaken to determine the short and long-term effects of different concentrations of iron on the reproduction, life cycle and life span of *D. melanogaster*in Bangladesh. This study will reveal the health effects of consuming excessive iron from the drinking water in the perspective of Bangladesh. Ferrous sulfate (Green vitriol, $FeSO_4.7H_2O$) is very often used as laboratory reagents and it was used for the present study.

2. Materials and methods

2.1. Preparation of culture vials

The experiments were conducted in the Animal Biotechnology Laboratory of the Department of Genetic Engineering and Biotechnology at the Shahjalal University of Science and Technology (SUST), Sylhet, Bangladesh. Some vials and glass bottles (205 mm×180 mm, the mouth of the bottle is 100 mm diameter) were used for the experiments which are the best approach for Drosophila culture in the laboratory [41, 42]. These bottles are stronger than other glass bottles and also safe for cooking old media and reusing. These glass bottles are also transparent for observing the growth and development of the fruit fly. Some test tubes were also used for lifespan experiment. All vials and bottles were decontaminated very well by washing with tap water, distilled water, detergent, 70% ethanol and using the sterilizer.

2.2. Collection and maintenance of flies

Tomato, lemon, banana, etc. were used as food for fly trapping. Fruits and vegetables were cut into the slice and kept on the dining table near the kitchen. When fruits and vegetables were about to rotten, fruit flies were flying around the rotting fruits and gobbled up the rotten fruits. The flies were collected with a water glass by inverting it carefully upon the flies so that they could not escape. Fruit flies always moves so the glass mouth was closed by hand to prevent the flies from escaping. Then, the flies were transferred to the bottle by keeping the mouth of the bottle to the mouth of glass inversely. This time the bottle was positioned at the top of the opening of glass and the glass remained on the ground. Before starting the present study, flies were maintained at room temperature on 12 hr light/12 hr dark cycle at 70%-90% relative humidity, with standardized nourishment prepared with agar (Wako Pure Chemical Industries Ltd., Japan), yeast extract (Wako Pure Chemical Industries Ltd., Japan), yellow cornmeal (Quaker Oats Company, USA), jackfruit, water and Natamycin fungicide (Sigma-Aldrich Corporation, USA) for several generations.

2.3. Identification of the experimental species

The experimental fruit fly, *D. melanogaster* is one of the most common flies available in Bangladesh and easily identifiable than other fruit flies such as *D. funebris* and *D. hydei* considering different morphometric characteristics [43, 44]. Male and female *D. melanogaster* was confirmed by studying its life cycle considering different previous observations [45-49].

2.4. Media preparation for *Drosophila* culture

All experiments with *Drosophila* requires media preparation before culturing. Based on Begum *et al*., jackfruit fortified media was used in this study for *Drosophila* culture [50]. The composition of the jackfruit fortified media is as follows: yellow cornmeal (38 g), jackfruit pulp (376 ml), agar (8 g), water (490 ml), propionic acid (Wako Pure Chemical Industries Ltd., Japan) (8 ml), commercial vinegar (Kraft Foods Inc., United States) (75 ml), yeast extract (10 g) and bread (16 g)[50]. Modified Jackfruit fortified media was prepared by mixing iron sulfate fortified water containing different concentrations of iron (Ferrous sulfate, $FeSO_4 \cdot 7H_2O$) (Sigma-Aldrich Corporation, USA) viz. 0.3 mg/l, 1 mg/l, 5 mg/l, 10 mg/l, and 20mg/l of iron with control group. In the control media, the iron concentration was 0 mg/l.

2.5. Experimental set up for the analysis of phenotypic characteristics

Five vials were set up for reproduction and life cycle experiment and six vials for lifespan experiment and labeled all of them with a marker pen for each with respective concentration of iron. Genders, generations, and dates of the beginning of the experiments were added to the label upon requirement. Prepared modified jackfruit fortified media were poured about 100 ml into the clean and dry labeled bottles. A funnel was used for transferring the media into bottles so that no media was clung to the side or mouth of the bottles. A cotton plug was put on the top of the culture bottles and kept it untouched until the media was solidified. After the media was solidified, a few grains of baker's yeast was added to the surface. From the stock of *D. melanogaster*, some newly emerged (2 days of inoculation) were then transferred to an ice pad and put under anesthesia. Flies were then sorted by gender and separated into males and females group. Five unmated males and five virgin females were added per bottle for life cycle and reproduction experiment. Lifespan experiment was carried out with only one sex considering eight individuals. The bottles were kept in a definite place and each experiment was repeated 3 times.

2.6. Phenotypic characteristics analysis

2.6.1. Observation of reproduction and life cycle

Media with different concentrations of iron (0.3 mg/l, 1 mg/l, 5 mg/l and 10 mg/l with control) were used for each experiment. To test life cycle and reproductive performance, five males and five females were taken in each vial in order to produce a new generation of flies. The culture medium was used as a substrate for feeding and females were allowed to oviposit for 4 days. After oviposition, the adults were

removed from the bottle. Ranges of the life cycle were 7-12 for the emergence of a new generation (F1) for each concentration. In the day 2 of emergence, again five males and five females were isolated from each replicate and then transferred to a new glass bottle, maintaining the same conditions, in order to originate F2 generation. The same procedure was repeated until the appearance of the fifth generation to study the effects of the repeated accumulation of iron over time. The number of progeny for each concentration was quantified, collected and analyzed for both male and female flies until approximately two weeks from the initial start of the experiment. The data collected from the affected group was then compared to that of the control group and analyzed. Life cycles for each concentration of iron were recorded very carefully in all successive generations. Subsequently, the adult fly transferring date, adult fly removing date, egg-laying date, egg hatching date, larvae (first, second and third instar) forming date, pupae forming date and adult fly enclosing date were recorded very carefully. Observations and records were made on day basis for the study of the life cycle. All unusual events which did not agree with the expectations such as fungus occurring, the death of larvae, death of pupae, losing of culture etc. were also noted.

2.6.2. Lifespan study of D. melanogaster

Twelve vials (six for each gender) were used for each lifespan experimental trial. All vials were labeled accordingly with their respective concentrations of iron, the gender of the flies, and the starting date of the experiment. From the stock, newly emerged flies were studied for lifespan experiment because mating markedly changes the physiology of female *D. melanogaster*, resulting in a shortened lifespan compared to virgins [51, 52]. Then the flies were sorted by gender and separated into males and females, with eight flies of each gender placed into each vial. Flies chosen for the experiments were healthy, judging by the brightness of the eye color. Different same concentrations of iron were used for this experiment with control and new media were supplied after 8-9 days repeatedly throughout the whole lifespan of this experiment. This process was then repeated twice more to produce more data. The flies of each trial were observed and dated over a period of 32 days and the number of live flies was recorded at intervals approximately twice a week. All flies for the lifespan experiments were cultivated at room temperature (20-28 °C) with a moderate amount of sunlight. The data collected from the affected group was then compared to that of the control group and analyzed.

2.7. Microscopic analysis of fruit flies

Flies should be anesthetized using least harmful and simple cooling method before observing under the biological microscope (Model: CX31, Olympus Corporation, Japan). Culture bottle was placed in the freezer for 8-10 minutes until the flies are not moving. Then the flies were placed on the top of a Petri dish and the bottom of the Petri dish was placed on the top of a zip-lock type icebox. According to a previous report, keeping flies in this cooling system will help them to remain chilled long enough for further experiments [53]. After each experiment, the flies were simply placed back into the culture vial. Observation of growth, development and all the stages of *D. melanogaster* was made out regularly. All development stages such as egg, larvae, pupae and adult fly as well as male and female of *D. melanogaster* was examined under the microscope with 4X/0.10 power magnification lens. Photographs of all stages of *D. melanogaster* with adult male and female were taken cautiously.

2.8. Statistical analysis

Quantitative data was analyzed statistically considering mean, standard error, and significance t-test, etc.

3. Results

Most of the researchers used constant temperature and humidity for *Drosophila* culture but the present study was conducted in the ambient environment during the summer season. During the study period, all experiments were conducted by*D. melanogaster* from the stock under room temperatures (within ranges of 20 °C-28 °C) with relative humidity (within ranges of 70%-90%).

3.1. Iron effect on the reproduction of *D. melanogaster*

The effect of iron on reproduction was assessed by culturing the *D. melanogaster* in the media prepared with different doses of iron-fortified water and finally counting their offspring through five generations in an average of three replications. The average number of progeny produced by culturing fruit flies in different iron concentrations is summarized in table 1.

Table 1: Number of average progeny of three trials in vial by different iron concentrations.

Generation	Control	0.3 mg/l	1 mg/l	5 mg/l	10 mg/l
P1	10	10	10	10	10
F1	124.34	128	152.67	94	90.34
F2	125.67	133	142.34	98.67	83.67
F3	121	126.34	135	105	97.34
F4	119	127.67	124.67	112.34	104.67
F5	129.34	127.34	128	121.67	119.67

Differences in percent between the ranges of survival among the treated groups in relation to the respective control groups on each generation were recorded (Fig. 1). A significant increase in the percentage of survival was observed in case of 1 mg/l concentrations with values of 122.78% (**$p \leq 0.01$) in F1 and 113.27% (*$p \leq 0.05$) in F2 respectively. For equal doses in F3 and F4 generations, there were also increasing of the survival but the results were not significant while in F5, number of survivals were less than the control and it was recorded insignificantly. In case of 5 mg/l concentration, a significant diminishing of the percentage of survival from F1 to F3 with values of 75.59% (**$p \leq 0.01$), 78.51% (**$p \leq 0.01$), and 86.77% (*$p \leq 0.05$) were found respectively. In F4 to F5 higher levels of recuperation of survival with the values of 94.39% and 94.06% respectively were observed which were found insignificant when compared them with the individuals from the control group.

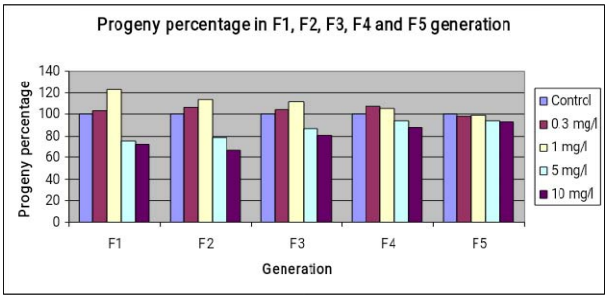

Fig 1: Progeny percentage in F1, F2, F3, F4 and F5 generations by different iron concentrations by comparing with control group.

On the other hand, in case of 10 mg/l dose, a significant diminishing of the percentage of the survivors was found from F1 to F4 with the values of 72.65% (**$p \leq 0.01$) in F1 until 87.95% (*$p \leq 0.05$) in F4. In F2, the diminishing of survival was very highly significant 66.05% (***$p \leq 0.001$). Higher levels of recuperation of survival with the value of 92.52% were found in the F5 generation, which was not significant when compared with the individuals from the control group. There was no significant difference in the survival between the control group and 0.3 mg/l concentration group through successive generations. Morphologically, *D. melanogaster* was slightly smaller (observed by eye) in F4 to F5 of 5 and 10 mg/l concentration groups than other groups.

3.2. Effect of iron on the life cycle of D. melanogaster

Effects on the life cycle were examined for each successive generation throughout the whole study for each dosage of iron and compared with the respective control group. The extreme increase in development time was found as the main effect of the treatment for 5 mg/l (until F3) and 10 mg/l (until F4) groups by comparing with the respective control group in each generation. The development time was determined from egg-to-adult (in days) (Fig. 2).

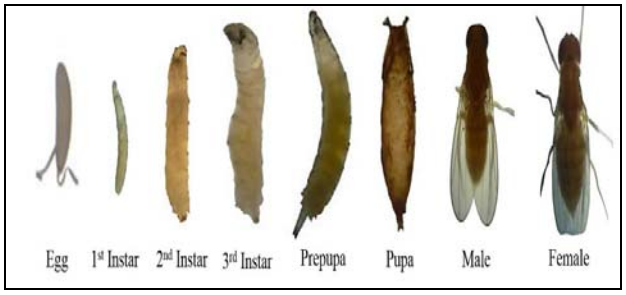

Fig 2: Different stages of *D. melanogaster*.

Average life cycle among the treated groups in relation to the respective control groups on each generation was differentiated and represented in table 2. A significant lengthening of the average life cycle was obtained in the case of 10 mg/l concentrations with the values of 10.67 (***$p \leq 0.001$) in F1, 10.67 (***$p \leq 0.001$) in F2, 10.34 (**$p \leq 0.01$) in F3 and 10.34 (**$p \leq 0.01$) in F4 respectively when compared with the control groups with values of 8.67, 8.34, 8.67 and 9.17 respectively. In F5 of the same group, shortening of the average life cycle with a high level of recuperation was observed than the previous generation which was almost similar to control. A significant lengthening of the average life cycle from F1 to F3 was observed in case of 5 mg/l concentration group with values of 10.17 (**$p \leq 0.01$) in F1, 9.83 (**$p \leq 0.01$) in F2 and 9.67 (*$p \leq 0.05$) in F3 respectively when compared with the control groups. In F4 and F5 of the same group, it was observed that the average life cycle became shortened with higher levels of recuperation than the previous generation which was almost similar to control. There was no significant difference between the average life cycles of control, 0.3 and 1 mg/l concentration groups through successive generations.

Table 2: Average life cycles of *D. melanogaster* at room temperature through five generations.

Generation		Control	0.3 mg/l	1 mg/l	5 mg/l	10 mg/l
F1	R1	8.5	8.5	8.5	10	10.5
	R2	8.5	8.5	8	10	10.5
	R3	9	9	8.5	10.5	11
	Average	8.67	8.67	8.34	10.17 (**)	10.67 (***)
F2	R1	8.5	8.5	8.5	10	10.5
	R2	8.5	8.5	8	10	10.5
	R3	8	8	8	9.5	11
	Average	8.34	8.34	8.17	9.83 (**)	10.67 (***)
F3	R1	8.5	8.5	8.5	9.5	10
	R2	8.5	8.5	8.5	9.5	10.5
	R3	9	9	9	10	10.5
	Average	8.67	8.67	8.67	9.67 (*)	10.34 (**)
F4	R1	9.5	9.5	9.5	10	10.5
	R2	9	9	9	9.5	10.5
	R3	9	9	9	9.5	10
	Average	9.17	9.17	9.17	9.67	10.34 (**)
F5	R1	7	7	7	8	7.5
	R2	7.5	7.5	7.5	8	8
	R3	7	7	7.5	7.5	8
	Average	7.17	7.17	7.34	7.83	7.83

3.3. Effect of iron accumulation on the lifespan of *D. melanogaster*

To test the effects of iron supplementation on lifespan newly emerged adult flies were raised on culture medium supplied with different concentrations of iron. A correlation between the dosage level of iron and the percentage number of flies alive for total three replications over time were studied for male and female respectively. The results of lifespan experiment under different iron concentration are illustrated in table 3.

Table 3: Total number of live male and female for the three replications with their percentage in the lifespan experiment of *D. melanogaster*.

F	20 mg/l	24	100	22	91.7	18	75	9	37.5	2	8.3	0	0	0	0
M		24	100	21	85.5	18	75	10	41.7	2	8.3	0	0	0	0
F	10 mg/l	24	100	22	91.7	20	83.3	18	75	10	41.7	3	12.5	0	0
M		24	100	22	91.7	19	79.2	17	70.8	10	41.7	4	16.6	0	0
F	5 mg/l	24	100	23	95.8	22	91.7	18	75	16	66.7	12	50	6	25
M		24	100	22	91.7	20	83.3	18	75	16	66.7	10	41.7	6	25
F	1 mg/l	24	100	24	100	22	91.7	19	79.2	16	66.7	15	62.5	12	50
M		24	100	24	100	23	95.8	21	85.5	19	79.2	16	66.6	10	41.6
F	0.3 mg/l	24	100	23	95.8	23	95.8	21	87.5	20	83.3	17	70.8	14	54.1
M		24	100	24	100	23	95.8	20	83.3	17	70.8	15	62.5	12	50
F	Control	24	100	23	95.8	23	95.8	20	83.3	17	70.8	15	62.5	12	50
M		24	100	23	95.8	22	91.7	19	79.2	16	66.6	14	58.4	12	50
Day		1	%	10	%	15	%	20	%	25	%	28	%	32	%

Lifespan experiments showed that as the percentage of iron in water of the media increased beyond 5 mg/l, the number of flies alive drastically declined after a set amount of time which was seen to be approximately two weeks. In 10 mg/l group, drastic reduction of flies started after 20 days but in 20 mg/l group, drastic reduction of flies started from 15 days from the start of the experiment. In 5 mg/l group, gradual reduction of flies continued for 25 days and after that drastic reduction was started but not as significant as 10 and 20 mg/l groups. Most importantly, the lifespan experiments clearly showed that flies in the control groups and the lower end of the dosage spectrum (0.3 mg/l- 1 mg/l) was not reduced drastically compared to their higher dosage. Female showed slightly longer lifespan in 0.3 mg/l and 1 mg/l group. On the other side, the male showed slightly longer lifespan than female in case of 10 mg/l group. Male and female flies showed equal longevity in case of control, 5 mg/l and 20 mg/l groups.

4. Discussion

In this study, it was observed that the iron has a deep effect on the descendants among the treated groups in relation to the respective control groups on each generation. Iron is an integral part of many proteins and enzymes that maintain good health [54] and it is essential for the regulation of cell growth and differentiation [1, 3]. Some previous studies recommended that higher dosage (750 mg/day) of vitamin C, iron, antioxidants, and arginine supplements play a crucial role in females to achieve pregnancy [55-57]. In *Drosophila* with the hypomorph alleles dmfrnEY01302 and dmfrnBG00456over the deletion Df (3R) ED6277, increased male sterilityoccurred by iron chelation with while iron supplementation through foodscaused the suppression [58]. These results suggested that iron plays a vital role in reproduction, growth, and differentiation of tissues in *D. melanogaster*. Results obtained in this study also showed a similar trend with these previously reported results. Previously it was reported that excess iron accumulation in a dose-dependent manner has a negative impact on reproduction and even promotes death in severe case [8, 23]. Results from the present study also strongly support the similar negative effect of accumulating excess iron in the reproduction of fruit flies. High concentration of ferrous ion (0.05% wt/wt) released from the diet is enough to disturb embryotoxicity [59]. Consumption of ferrous ion caused to reduce the production by damaging ovarian cells and hepatic tissue through the release of reactive oxygen species. The reduction of offspring number in this study also harmonized with many previous findings although the conditions applied in this study didn't agree with their work. Previously, Islam *et al*. reported significantly reduced hatchability of the *D. melanogaster* due to the effect of different concentrations of iron sulfate and copper sulfate at 25–0.5 °C ($P<0.001$) [60]. Turner and Gardner examined the effects of iron salts (Fe (NH_4) $(SO_4)_2$) on the expression of head abnormalities, melanotic tumors and general mortality of flies [61]. They reported that the total mortality of all the groups increased with the increasing concentration of the iron compound and found a correlation of 0.67 (r (0.05)=0.576) between mortality and melanotic tumor incidence.

The lifespan experiments in the present study suggest that iron sulfate has a significant impact on the longevity of the *D. melanogaster*. Previously, Vecchio *et al.* (2012) evaluated the toxicity of monodisperse citrate-capped gold nanoparticles (AuNPs) in *D. melanogaster,* upon ingestion and they found a strong reduction of *Drosophila* lifespan and fertility performance [62]. Huang (2013) also found that the flies exposed to dosages exceeding that of 1% Ace K produce significantly fewer progeny and significantly shorter lifespan [63]. All these findings are in good agreement with the results of the present study. It was also observed that there was no significant difference in the survival between the control group and 0.3 mg/l concentration group through successive generations which indicate that iron is more tolerable in 0.3 mg/l concentrations. The typical lifespan of *D. melanogaster* is approximately 30 days at 29 °C [64, 65], 37 days at 25 °C [66, 67] and may be several weeks [68]. In the present study, the majority of the flies in the control, 0.3 mg/l, and 1 mg/l groups remained alive at 32 days, longer than that for the 10 and 20 mg/l concentrations, suggesting that the flies were healthy in the control to 1 mg/l concentration. This suggests that the iron sulfate must be consumed over time to have any adverse health effects and any dose past 1 mg/l in the food is potentially toxic, at least in populations of *D. melanogaster*. One possible mechanism to explain this result is based on the iron homeostasis concept during aging [69]. Due to the imbalance of iron concentration in the body, catalysis occurred by iron through Fenton reaction to generate reactive oxygen species including superoxide, hydroxyl radicals and hydrogen peroxide ($Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + \bullet OH + OH^-$) [70]. The abstraction of hydrogen atoms occurred on the deoxyribose sugar backbone of DNA to leave a DNA radical adduct that results in strand scission [71]. In an alternative manner, these hydroxyl radicals caused to damage nucleotide bases [72, 73]. Both cases of DNA damage resulting in genetic mutations, cell and tissue damage, or even cell death [74]. In the present study, the female showed slightly longer lifespan in 0.3 mg/l and 1 mg/l group while male showed slightly longer lifespan than female in case of 10 mg/l group. Similarly, the results of the present study also postulated that the toxicity of iron overdoses can cause a reduction in lifespan of *D. melanogaster* population.

In the present study, a difference between the average life cycle ranges was obtained among the treated groups in relation to the respective control groups in each generation. Lengthening of life cycle occurred due to the longer larval stage than mature fly. Due to the presence of iron, eclosion of pupa was also lengthening than normal. This result supported the other findings where the iron compound prolongs the developmental period and eclosion which started in 15 days after the eggs were laid in comparison with 10 days for the controls [60, 61]. The present result also partly supported by the result of Akins *et al.* (1992), where some heavy metals, such as lead and cadmium caused a developmental delay in *D.melanogaster* at the phase from larva to pupa[77]. It appeared from the observations that the effect of the addition of iron to the medium of *D. melanogaster* caused the lengthening of the developmental periods. In this study, effects were not examined for five stage of life cycle but it was seen through the developmental time from egg to adult that larval stage are more sensitive to iron and it requires more days to the pupal stage than the respective control group.

5. Conclusion

In this study, short and long-term effects of different concentrations of iron on progeny production, life cycle, and lifespan of *D. melanogaster* were analyzed and it may be the beginning of further investigations to establish the real effects of iron in living organisms. Iron isa microelement essential

for growth and development, but in high doses can significantly alter the patterns of development, reproduction, aging and even promotes death. The fruit fly, *D. melanogaster* is an important model animal which is genetically similar to human. Results of this study suggest that moderate consumption of iron and its salts may have the potential to improve the mammalian reproduction, growth, and development and consequently, have no toxic effects. But an excess amount of iron intake is adverse problematic for life. This present study demonstrated that excess accumulation of iron caused to reduce reproduction ability, decrease lifespan andshorten the life cycle of fruit flies. These findings on *D. melanogaster* could be taken as a reference about the effects of iron in human population and establish astrong scientific basis for creating better management steps to prevent the effects of drinking water withhigh levels of iron.

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Availability of data and materials
Data and materials related to this work are available upon request.

Authors' contribution
All authors contributed equally to this research study and during the preparation of the manuscript. All authors read and approved the final manuscript.

Competing interests
The authors declare that they have no competing interests.

Consent for publication
All authors approve the manuscript for publication.

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