



Dietary Supplementation of Citric acid (monohydrate) Improves Health Span in *Drosophila melanogaster*

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ABSTRACT

Citric acid is a biochemical compound present in variety of citrus fruits. Citrate plays a vital role in production of energy (ATP) via Tricarboxylic acid (TCA) cycle and also involve in Fatty acid (FA) synthesis in cytoplasm. The objective of this study is to evaluate the effect of citric acid supplementation on health span in *Drosophila melanogaster*. *OregonR*⁺ fly used in most of the experiments. Body weight of male and female flies fed on normal and citric acid mixed diet was determined, locomotor assay and anti-oxidant activity associated with citric acid was measured by performing superoxide dismutase (SOD) and catalase (CAT) enzymatic assay. Fat content in citric acid fed flies was evaluated by Oil Red O staining in fat body tissues of adult *Drosophila*. Reverse transcriptase PCR (RT-PCR) analysis was performed to check the expression level of *Brunner (bmm)* and *Fatty acid Synthase (FAS)* genes. In the present study we have shown that supplementation of citric acid reduces body weight of male and female flies, improves locomotor activity of Alzheimeric flies, reduces oxidative stress and increases FA synthesis in *Drosophila*. Here, we concluded that supplementation of citric acid (monohydrate) improves health span in *Drosophila*.

1. INTRODUCTION

Citric acid is a biochemical compound and one of the major products of *Citrus* family. Citric acid is present in variety of fruits such as lemon, lime, oranges, grapes, kiwis, strawberries, apple, pears, raspberries and also available in vegetables and plants (roots & leaves) [1, 2]. It is commonly used as a food additive to provide acidity and sour taste to foods and beverages, also used as an acidity regulator [3]. Citrate is a weak acid and plays an essential role in production of energy (in the form of ATP) via mitochondrial Krebs' or tricarboxylic acid (TCA) cycle. It is synthesized in mitochondria by Krebs' cycle (TCA cycle) and comes out in the cytoplasm with the help of citrate transporter to provide cellular energy in the form of ATP

[4, 5]. In addition, citrate also provides NAD⁺, required for the glycolysis [6]. The other role of citrate is the synthesis of Acetyl-CoA, the lipid building block and converts it into malonyl acid for formation of fatty acid (FA) and sterols [7]. Citrate is also known to play a vital role in maintenance of normal brain function as a neurotransmitter precursor and in energy production [6, 8]. It is well studied that supplementation of antioxidant compound protects cells against the damaging effect of Reactive Oxygen Species (ROS) [9]. Antioxidant compounds such as vitamin C, E, carotenoids and several polyphenolic compounds used as dietary supplements have ability to scavenge the oxygen free radicals and save the cells from any damage i.e. lipid peroxidation, protein oxidation and DNA damage [10]. Herbs, plants and plants based components are rich source of antioxidant and used from ancient time to treat several human diseases. Citric acid synthesized naturally (from lemon, orange) and intracellular during metabolism, act as an antioxidant and present in many foods i.e. jelly sweet, jam, marmalade, baked nutrients, candy, soft drinks, tinned vegetable and fruit food [11]. In the present study, we used *Drosophila melanogaster* as an animal model to study the effect of citric acid on physiology of *Drosophila melanogaster*.

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Drosophila melanogaster commonly known as “fruit fly” is one of the oldest animal models (more than 110 years) with fully sequenced genome [12]. It has shorter life cycle (10-12 days at 25°C), lesser no. of chromosomes (4 pair of chromosomes), high fecundity (females lay ~800 eggs in a life time), ease to handle and possess ~75% homology with human disease genes [13, 14]. After genome sequencing in 2000, It was inferred that *Drosophila* is one of the closest invertebrate models to the human [15] *Drosophila* has a complex nervous system [16], complex behavior and exhibit daily cycles of activity and inactivity, a behavioral rhythm governed by animal's endogenous circadian system. In recent years, *Drosophila* has been used as a model of choice to study the basic mechanism of neurodegenerative disease, circadian rhythms and in search of medicinal property of different medicinal plants [17-21].

In this study, using *Drosophila melanogaster* as an animal model, we have shown that, supplementation of citric acid mixed diet reduces body weight, improves locomotor activity, reduces oxidative stress and increases fatty acid synthesis in *Drosophila*. Thus, on the basis of the results it was concluded that dietary supplementation of citric acid improves health span in *Drosophila melanogaster*.

2. MATERIALS AND METHODS

2.1 Compound name

Chemical compound studied in our work is Citric acid monohydrate (PubChem CID: 22230)

2.2 Fly strains and culture conditions

OregonR⁺ (wild type) strain of *Drosophila melanogaster* was used for most of the experimental studies conducted in this paper. We also used *GMRAB₄₂^{K52}*; *GMRAB₄₂^{K53}* a transgenic *Drosophila* line that over-express *Amyloid β ₄₂* protein in *Drosophila melanogaster* and used *Drosophila* model of Alzheimer's Disease (AD). This fly stock was used to examine the effect of citric acid on locomotor activity in AD flies.

The flies were cultured at 22±1°C in BOD incubator.

2.3 *Drosophila* culture medium

The flies were cultured on *Drosophila* food medium containing agar-agar, corn meal, sugar, yeast, propionic acid (anti-bacterial), and nepagin (anti-fungal agent). One unit (360mL) of *Drosophila* food was prepared by dissolving the 3g agar-agar (SRL, Cat# 0140186), 17g corn meal, 15g sugar, 6g yeast, 1mL propionic acid (Merck) and 1g nepagin (Methyl-p-hydroxybenzoate sodium salt, Himedia, Cat# GRM129-500G) in water and total volume was make up to 360mL. The additional yeast suspensions were provided for healthy growth.

2.4 Preparation of citric acid (monohydrate) containing food

Citric acid (monohydrate) (SRL Cat# 0348216) mixed diet were prepared by dissolving varying concentration of citric acid solution (0.15M, 0.3M, 0.45M and 0.6M) in normal *Drosophila* food media (Normal diet/food). The food was properly

mixed and poured in *Drosophila* culture vials and bottles and allowed to dry for further use.

2.5 Body weight determination

The body weight of flies fed on normal diet (without citric acid) and on citric acid mixed diet was measured by taking the weight (in grams) of flies. For this, first the weight of empty eppendorf tube (1.5mL) was measured and noted as initial weight (I_w) and the weight of eppendorf with 20 flies noted as final weight (F_w). The weight of empty eppendorf was subtracted from the weight of eppendorfs with flies ($F_w - I_w$). Assay was repeated 5 times for each group. The mean body weight of flies were calculated and it was divided by total number of flies used in the assay ($F_w - I_w/\text{No. of flies taken}$). Total 100 male and 100 female flies were taken from control and experimental group for weight analysis.

2.6 Locomotor Assay

The locomotor assay of *OregonR*⁺ and *GMRAB₄₂^{K52}*; *GMRAB₄₂^{K53}* flies fed on normal and citric acid mixed *Drosophila* diet were performed as described by [22] with little modification. For this, 20 flies of 10 and 20 days age group were chosen and flies were placed in vertical glass tube (30 cm long x 1.5 cm wide) and allowed to acclimatize for 2 min. Flies were tapped gently to the bottom of the vial. The number of the flies crosses 10cm/ 10seconds were counted. This was repeated five times. Total 100 flies from each group (control & experimental) were used for the locomotor assay. The assay was performed at 25°C under standard lighting condition.

2.7 Enzymatic Assay

This was done to see the effect of citric acid supplementation on antioxidant enzymes such as Superoxide Dismutase (SOD) & Catalase (CAT) activities in *Drosophila melanogaster*.

2.8 Preparation of protein sample

The protein samples were prepared by homogenizing 10-20 flies fed on normal diet and diet mixed with 0.15M, 0.3M, 0.45M and 0.6M citric acid monohydrate in 100 mM KPB (Potassium Phosphate Buffer). The sample was centrifuged at 8000 rpm at 4°C for 20 min. The supernatants were transferred into 1.5mL eppendorf tube and optical density (OD) was measured at 280nm by using biospectrophotometer (Eppendorf). Crude protein sample were further diluted (1:10) for SOD and CAT enzymatic assays and was stored at -20 °C till further use.

2.9 Superoxide Dismutase (SOD) enzymatic assay

The principle behind SOD (EC 1.15.1.1) activity assay was the inhibition of reduction of nitrobluetetrazolium (NBT) by super oxide. In the presence of O₂ riboflavin excitation occurs and methionine acts as a potent electron donor to generate superoxide anions. SOD assay is based on this phenomenon. The reduction of NBT by superoxide radicals gave purple color that was estimated

at 560 nm using spectrophotometer [23, 24]. "One unit of SOD activity is defined as the amount of enzyme causing half the maximum inhibition of reduction of NBT under the specified conditions".

SOD assay was performed as described by [24] with little modification. The reaction mixture contained 20 μ L of 250 mM Methionin (SRL, Cat# 19305), 5 μ L of 10mM NBT (SRL, Cat# 11207), 25 μ L of 1M KPB, 0.5 μ L of 100 mM EDTA (SRL, Cat# 54960), 25 μ L of 1M Na₂CO₃ and 380 μ L of distilled water with 40.5 μ L of desired diluted protein samples. 2 μ L of 1mM riboflavin (SRL, Cat# 34392) was added at last and the total volume of reaction mixture was 500 μ L. It was kept under white light for 8 min. The purple color was formed and the optical density (OD) was measured at 560nm using biospectrophotometer (Eppendorf).

2.10 Catalase (CAT) Assay

CAT enzymatic (EC 1.11.1.6) activity was measured according to the method described by [24]. The assay is based on the consumption of H₂O₂ in the presence of the enzyme source at 25°C. The reaction mixture contained 30% H₂O₂, 50mM KPB and 34 μ L of diluted protein samples from flies fed on normal diet and on 0.15M, 0.3M, 0.45M and 0.6M citric acid mixed diet. The OD (240nm) of reaction mixture was taken immediately at 0 min and at 5 min by using biospectrophotometer.

One unit of enzyme is the amount of enzyme required to convert 1 mol of H₂O₂ to product in 1 second. Enzyme activity was expressed as units per milligram of protein.

2.11 Oil Red O staining

This was performed to visualize the lipid droplets of fat body cells in *Drosophila* tissues. This was done according to the method described in [25]. For this, fat body cells from third instar *Drosophila* larvae were dissected out in 1X PBS (pH 7.4), fixed in 4% para-formaldehyde for 20 min at room temperature and washed twice with 1X PBS. The tissues were stained in freshly prepared 0.1% Oil Red O dye (Sigma Aldrich, USA, Cat# 0625-100G) in isopropanol (6 part of Oil Red O dye and 4 part of distilled water) for 30 min at room temperature. The tissues were washed first in distilled water followed by 60% isopropanol (to remove excess stain) and in 1X PBS. The tissues were mounted on slide using 2.5% DABCO mounting media and examined under Leica TCS SP5II laser scanning confocal microscope.

2.12 Analysis of transcript levels of *Brummer* & *FAS* in *Drosophila melanogaster*

This was performed to examine the fat anabolism/catabolism in citric acid monohydrate supplemented flies. For this, we examined the expression level of *Fatty acid synthase (FAS)* and *Brummer (Fatty acid lyase)* genes in citric acid (monohydrate) supplemented flies.

2.13 RNA isolation

Total RNA was isolated from 30 days old control and citric acid fed flies using TRIzol[®] reagent (Ambion/RNA, by Life

technology, Cat# 15596-018) following the manufacturer's instructions. Extracted RNAs were resuspended in Ultra pure[™] distilled water (DNase, RNase free) (Invitrogen Cat# 10977-015). The quality as well as the quantity (A₂₆₀/A₂₈₀ ratio) of the isolated RNA was verified through NanoDrop Spectrophotometer (Eppendorf).

Complementary DNA (cDNA) synthesis

The cDNA was synthesized from total RNA extracted from control and 0.15M and 0.3M citric acid monohydrate fed flies by using RevertAid first strand cDNA synthesis kit (Molecular Biology, Thermo scientific, Cat # K1622). Each reaction mixture consist of RNA template (2 μ g), Oligo (dT)₁₈ (1 μ L), 5X Reaction Buffer (4 μ L), RiboLockRNase Inhibitor (RI) (1 μ L), 10mM dNTP Mix (2 μ L), RevertAid[™] M-MuLV Reverse Transcriptase (RT) (1 μ L) and nucleases free water to make a final volume of 20 μ L. The cDNA was stored at -20°C till further use.

2.14 Polymerase chain reaction (PCR)

The synthesized cDNA (~1 μ L) from control and 0.15M & 0.3M citric acid fed flies were used for PCR amplification in a thermocycler (S1000[™] Thermal cycler, Bio-Rad, CA, USA) using specific primers for the *Drosophila Brummer* & *FAS* gene (Table 1). *Rp49* gene specific primers were used as an endogenous control. The amplicons were separated on a 2% agarose gel containing ethidium bromide at 5V cm⁻¹ and visualized under a gel doc imaging system SL 3500 X-Press (VilberLourmat, France).

Table 1: Primers sequence used for PCR.

<i>Brummer (F)</i>	5'-TCCCGAGTTTCTGTCCAAGT-3'
<i>Brummer (R)</i>	5'-GCGTCCTTCTGTGCTTCTT-3'
<i>FAS (F)</i>	5'-CGGAGAAGAGTTACATCCTG-3'
<i>FAS (R)</i>	5'-CAATCACCACCTTTACGC-3'
<i>Rp49 (F)</i>	5'-AATCTCCTTGGCGCTTCTT-3'
<i>Rp49 (R)</i>	5'-AGTATCTGATGCCCAACA-3'

2.15 Statistical Analysis

All the analyses were carried out in triplicate and expressed as mean \pm SEM. The analyses were carried out using the GraphPad Prism software, version 5.0. The difference among control and citric acid fed flies were compared by one way analysis of variance (ANOVA).

3. RESULTS AND DISCUSSION

3.1 Supplementation of citric acid monohydrate (0.15M & 0.30M) reduces body weight in *Drosophila melanogaster*

The effect of citric acid monohydrate supplementation was studied by feeding the flies on normal diet and diet mixed with citric acid monohydrate (0.15M & 0.3M). The mean body weight of *OregonR*⁺ male flies fed on normal diet was 0.0008g. While the mean body weight of *OregonR*⁺ flies fed on 0.15M and 0.3M citric acid monohydrate mixed diets were 0.0007g and 0.00065g, respectively (Fig. 1A) (P<0.05). Similarly, the mean body weight of *OregonR*⁺ female flies fed on normal diet was 0.0012g. While the mean body weight of *OregonR*⁺ flies fed on

0.15M and 0.3M citric acid monohydrate mixed diets were 0.0010g and 0.0009g, respectively (Fig. 1B) ($P < 0.05$). These results showed the significant reductions in body weight of citric acid monohydrate supplemented flies.

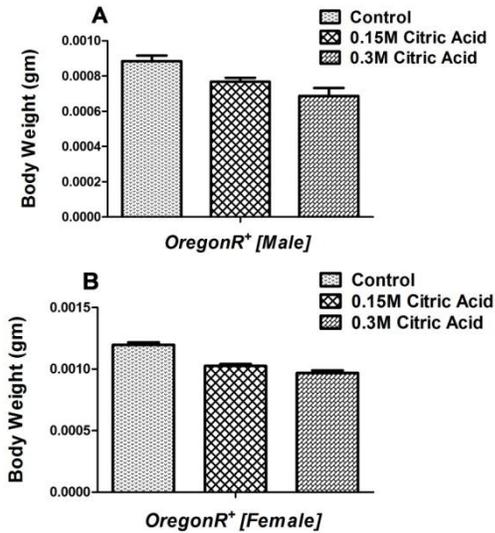


Fig.1: Citric acid supplementation results in reduction of body weight in *Drosophila melanogaster*. Graph showing the mean body weight of *OregonR*⁺ male (A) and female flies (B) fed on normal diet and diet mixed with 0.15M & 0.3M citric acid monohydrate. The flies (male & female) fed on 0.15M & 0.3M citric acid monohydrate showing a significant reduction in mean body weight as compared to the flies fed on normal diet. Total 100 flies were examined for each group. Error bars represents Mean \pm SEM (P value < 0.05).

Since, citric acid is an intermediate in the Krebs's cycle (TCA or Krebs's cycle) and it poured more energy (ATP) to the cell by accelerating the metabolism.^[5] Thus, the reduction in body weight of citric acid fed flies might be due to an increase in metabolism. This observation also supports a previous study by^[3]. supplementation of citric acid monohydrate reduces body mass in mice.

3.2 Supplementation of citric acid monohydrate improves locomotor activity in *Drosophila*

Since, locomotor activity is an integral component of most animal behaviors, and many human health problems are associated with locomotors deficits. In order to check the effect of citric acid on locomotor activity in *Drosophila*, locomotor assay was performed in 10 and 20 days flies fed on normal and 0.15M and 0.3M citric acid mixed diet. In locomotor test of *OregonR*⁺ flies (10 days) fed on normal diet, 77.5% flies crossed 10cm/10 sec while this fly % increased up to 87% and 97.5% when the flies were fed on 0.15M and 0.3M citric acid mixed food (Fig. 2A). In 20 days of locomotor activity test of *OregonR*⁺ flies fed on normal diet, 60% flies crossed 10cm/10sec while this fly % increased up to 72.5% and 77.5% when the flies were fed on 0.15M and 0.3M citric acid mixed food (Fig. 2A) ($P < 0.05$). This result suggest a significant increase in locomotor activity of *OregonR*⁺ flies fed on 0.15M and 0.3M citric acid mixed diet as compared to the flies fed on normal diet. Since, locomotor impairment is one of the key

feature of neurodegenerative disease in human as well as in animal models, thus, to further confirm our findings, we performed the locomotor activity test in Alzheimeric flies (*GMRA β ₄₂^{K52}*; *GMRA β ₄₂^{K53}*). The locomotor activity test of 10 days old *GMRA β ₄₂^{K52}*; *GMRA β ₄₂^{K53}* flies fed on normal diet, 70% flies crossed 10cm distance in 10 seconds while this fly % increased up to 85% and 90% when the flies were fed on 0.15M and 0.3M citric acid mixed food. Similarly, in 20 days test 52.5% *GMRA β ₄₂^{K52}*; *GMRA β ₄₂^{K53}* flies, fed on normal diet crossed 10cm/10sec while this fly % increased up to 67.5% and 70% when the flies were fed on 0.15M and 0.3M citric acid mixed food (Fig. 2B) ($P < 0.05$). This result further confirmed above observations. The increase in locomotor activity might be due to cumulative effect of reduction in body weight and an increase in cellular energy level.

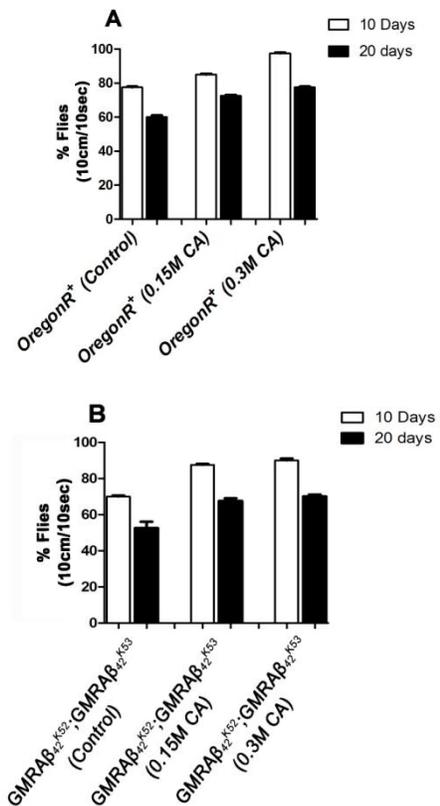


Fig. 2: Citric acid supplementation improves locomotor activity in *Drosophila melanogaster*. Above graphs shows locomotor activity of 10 and 20 days old *OregonR*⁺ & *GMRA β ₄₂^{K52}*; *GMRA β ₄₂^{K53}* flies. (A) Locomotor activity of *OregonR*⁺ flies, fed on normal diet and on 0.15M and 0.3M citric acid monohydrate mixed diet. Graph A showing an increase in locomotor activity in 0.15M and 0.3M citric acid monohydrate fed 10 & 20 days old flies as compared to the control. (B) Locomotor activity of Alzheimeric fly "*GMRA β ₄₂^{K52}*; *GMRA β ₄₂^{K53}*" fed on normal diet and on 0.15M and 0.3M citric acid monohydrate mixed diet. Graph showing an improved locomotor activity in 0.15M and 0.3M citric acid monohydrate fed 10 & 20 days old flies as compared to the control. Total 100 flies were taken per assay. Error bars represents Mean \pm SEM ($P < 0.05$). (CA= Citric Acid).

3.3 Supplementation of citric acid monohydrate reduces paraquat induced oxidative stress in *Drosophila*

As, it is well known that plants and plants based components possess great anti-oxidant properties, thus, to examine

the anti-oxidant activity associated with citric acid monohydrate, we performed SOD and CAT enzymatic assays in flies fed on normal and citric acid mixed diet. For this *OregonR*⁺ flies were fed on 1% sucrose (control), 20 mM paraquat and 0.15M, 0.3M, 0.45M and 0.6M citric acid monohydrate mixed diet. We used PQ a herbicides, commonly used to induce oxidative stress in flies [26]. For this *OregonR*⁺ flies were fed on filter paper soaked with 1% sucrose solution (control), 1% sucrose solution + 20mM PQ and 1% sucrose solution + 20mM PQ in combination of different concentration of citric acid monohydrate (0.15M, 0.3M, 0.45M and 0.6M). It was observed that flies fed on 20mM PQ had an induction of SOD and CAT enzymes activities while flies fed on PQ in combination of 0.15M, 0.3M, 0.45M and 0.6M citric acid showed a significant reduction ($P > 0.05$) in SOD (Fig. 3A) and CAT (Fig. 3B) enzymes activities. This result suggested that supplementation of citric acid reduces oxidative stress in *Drosophila melanogaster* and possess anti-oxidant property.

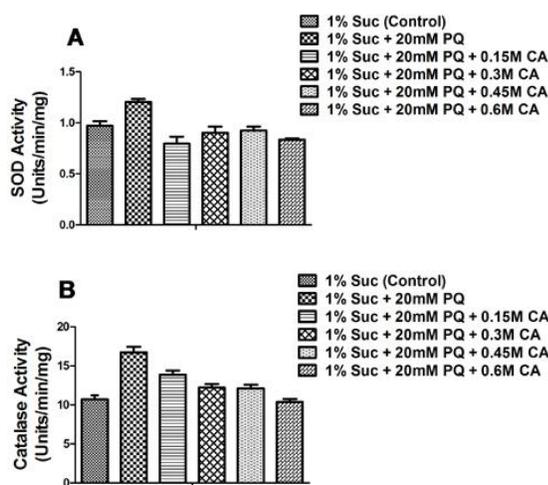


Fig. 3: Citric acid reduces paraquat (PQ) induced oxidative stress in *Drosophila melanogaster*. (A) Above graph showing an increase in SOD activity in PQ fed flies, as compared to the control (flies fed on 1% sucrose) while *OregonR*⁺ flies fed on PQ in combination of 0.15M, 0.3M, 0.45M and 0.6M citric acid monohydrate shows a significant reduction in SOD activity. (B) Graph showing an increase in CAT activity in PQ fed flies while a significant reduction in CAT activity in flies, fed on PQ in combination of 0.15M, 0.3M, 0.45M and 0.6M citric acid monohydrate as compared to the control (flies fed on 1% sucrose) flies. Error bars represents Mean \pm SEM (P value > 0.05).

3.4 Supplementation of citric acid monohydrate increases fat body size in *Drosophila*

As shown above supplementation of citric acid reduces body weight, improves locomotor activity and reduced oxidative stress in *Drosophila*. Thus, in order to find out the mechanism behind the same, the fat content in *OregonR*⁺ flies, fed on normal diet and on 0.15M & 0.3M citric acid monohydrate mixed diet were examined. For this, fat bodies were dissected out from the 3rd instar *Drosophila* larva and were stained with Oil Red O dye (a marker for lipid droplets) and the lipid droplets (LDs) size was measured. We observed 6 μ m average size of LDs in *OregonR*⁺ flies fed on normal diet (Fig. 4A & D) while this size increases up to 12 μ m (Fig. 4B & D) and 14 μ m (Fig. 4C & D) in

flies fed on 0.15M and 0.3M citric acid monohydrate mixed diet, respectively. This result clearly suggests an increase in fatty acid synthesis in fat body of flies fed on 0.15M and 0.3M citric acid monohydrate mixed diet.

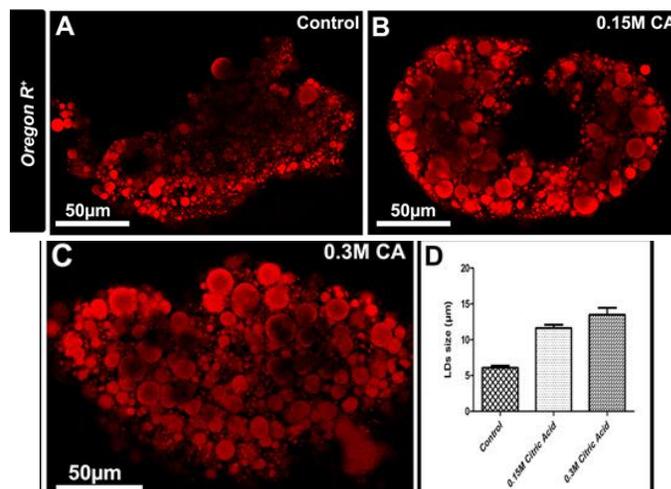


Fig. 4: Oil Red O staining in fat body tissues in *Drosophila melanogaster*. Oil Red O staining in fat body tissues of *OregonR*⁺ flies fed on normal diet (A), on 0.15M citric acid (B) and 0.3M citric acid (C). The histogram shows a significant increase in fat body size in citric acid supplemented flies as compared to the flies fed on normal diet. Error bars represents Mean \pm SEM (P value > 0.0001).

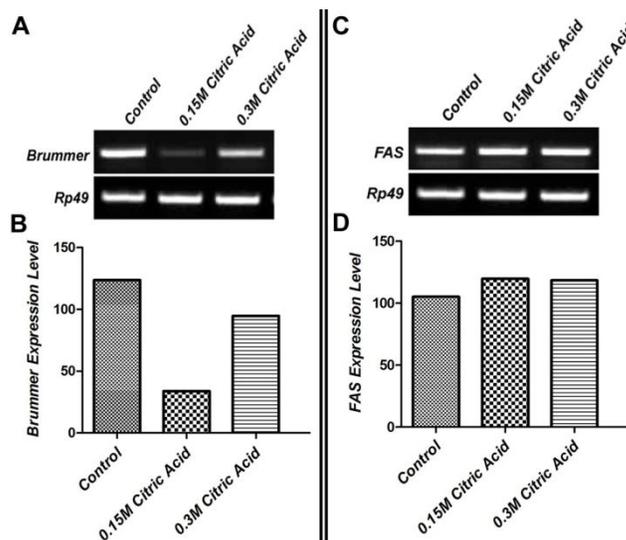


Fig. 5: RT-PCR analysis of *Brummer* (*bmm*) and *Fatty acid synthase* (*FAS*) genes in *OregonR*⁺ flies fed on normal and citric acid mixed diet. RT-PCR analysis showing *Brummer* (*bmm*) and *FAS* expression level in 30 days old *OregonR*⁺ flies fed on normal diet and on 0.15M and 0.3M citric acid mixed diet. *bmm* expression level was decreased in 0.15M & 0.3M citric acid monohydrate fed flies (A & B) while *FAS* expression level was increased significantly in 0.15M & 0.3M citric acid monohydrate fed flies (C & D).

In order to further confirm our findings, we examined the level of fatty acid synthesis by measuring the expression level of two genes associated with fat metabolism: *Fatty acid synthase* (*FAS*) and *Brummer* (*bmm*) (*Fatty acid Lyase*) in *OregonR*⁺ flies fed on normal diet and citric acid (0.15M and 0.3M) mixed diet. This was done by performing RT-PCR analysis using gene specific primers. We observed that *bmm* expression level was

significantly reduced (Fig. 5A & B) and *FAS* expression level was significantly increased (Fig. 5C & D) in flies fed on 0.15M and 0.3M citric acid monohydrate mixed diet as compared to the flies fed on normal diet. It is well known that *bmm* is fat mobilizing enzyme and plays a vital role in lipolysis. *bmm* gene is responsible for encoding fat body lipid droplet associated TAG-lipase. An earlier report by [27] suggested that loss of function of *bmm* gene in *Drosophila* results in obesity. The other enzyme *FAS* is responsible for fatty acid synthesis and its expression was increases in 0.15M and 0.3M citric acid supplemented flies. It might be possible that supplementation of 0.15M and 0.3M citric acid monohydrate inhibit lipid breakdown by decreasing *bmm* gene and an increase of fatty acid synthesis by increasing *FAS* enzymatic activity in *Drosophila*. Thus, the increase in lipid droplets size represents an increase in fatty acid synthesis hence an increase in fat storage in the form of Triglycerides [28]. The reason behind the increase in locomotor activity in citric acid fed flies might be due to the change in energy status of the cell by an increase of intracellular citrate level.

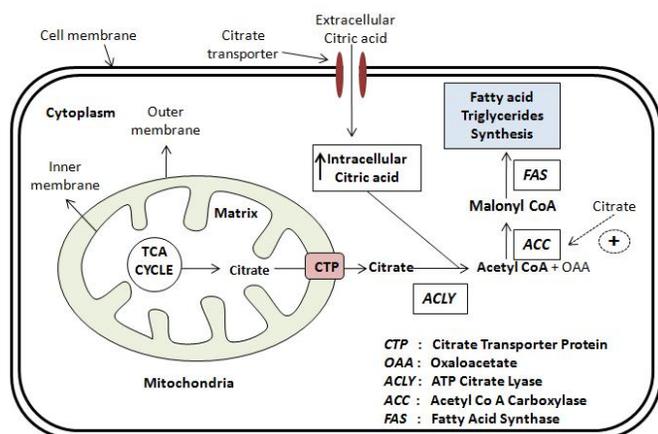


Fig.6: Schematic diagram representing the possible mechanism of fatty acid synthesis by supplementation of citric acid in *Drosophila melanogaster*. Supplementation of citric acid increases intracellular citric acid level and activates *ATP-Citratelase (ACLY)* enzyme. Activation of *ACLY* will produce Acetyl-CoA and oxaloacetate by utilizing citrate molecule. Acetyl-CoA will be converted into manonyl Co-A (FAs building block) by *Acetyl-CoA carboxylase (ACC)* enzyme. *ACC* will be also allosterically regulated by citrate. Manonyl Co-A will be converted into fatty acids and triglycerides by *Fatty acid synthase (FAS)* enzyme which will be ultimately stored in fat body tissue of *Drosophila*.

4. CONCLUSIONS

In conclusion, the present study suggested that supplementation of citric acid improves health span in *Drosophila melanogaster* and recommend the increase use of citrus fruit that is rich source of citric acid. The EFSA: European Food Safety Authority [29] has already recommended that use of citric acid monohydrate in food or food additives is safe for the consumer, it does not lead any environmental risk and can be used as preservative without restriction in feed for all animal species.

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