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The Effect of *Terminalia chebula* on Resistance to Hydrogen Peroxide Induced Oxidative Stress in *Drosophila melanogaster*

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Abstract

Oxidative stress induces to production of free radicals in all cells due to cellular metabolism reflected also by environmental factors. Accumulated free radicals has many negative effects on the cell including cellular aging, hence the present project is their nutritional impact on the stress resistance and production of free radicals using some phytochemicals. *Terminalia chebula* (one of the Triphala) was used in ancient medicine to cure many ailments, most of the disease are positively correlated with stress. *Drosophila melanogaster* flies exposed H2O2 and those treated with *T.chebula* exhibited more resistance to oxidative stress. The age of the flies enhanced to 10 hours, in a dose dependent manner.

Keywords: *Terminalia chebula, Drosophila melanogaster*, hydrogen peroxide, oxidative stress and Antioxidants

Introduction

Ayurveda is the oldest medical system known to man. It is the earliest natural healing systems of the Vedic Sciences it was gift given by the sages of India 5000 years ago and is often called the “Mother of All Healing”. Ayur (life) and Veda (science or knowledge) are two Sanskrit words that together translate to "The Science of Life." (Lad, 2003). Age-associated conditions such as memory loss, osteoporosis, diabetic sores, etc. have cures in Ayurvedic literature that are not effectively treated by modern medicine (Raj *et al*, 2011) [19]. Preparations made from plants are essential to the ayurvedic treatment system. Ninety percent of ayurvedic medications are made from plants. (Syal Kumar *et al*, 2016) [20].

There is a wealth of medicinal plants on the Indian subcontinent that are employed in conventional medical practices. There are around 15,000 documented medicinal plants in India. However, only 7,000-7,500 plants are utilized by traditional tribes to treat different ailments. Another estimate claims that 17,000 species of medicinal plants have been identified, with approximately 3,000 of those species currently being used in medical applications. Many compounds, including saponins, tannins, alkaloids, alkenyl phenols, glycol-alkaloids, flavonoids, sesquiterpenes, lactones, terpenoids, and phorbol esters, convey specific plant species with their beneficial therapeutic qualities. Ayurvedic medicines' main active components are plant alkaloids (Meena *et al*, 2009) [2].

Terminalia chebula commonly called ‘Haritaki’ is one of the medicinal plants which has great value in the field of medicine due to its variety of pharmacological properties. it is known as ‘king of medicine’ in Tibet. It is always listed at the top of the list of ‘Ayurvedic Materia Medica’ because of its extraordinary power of healing. He whole species has a high medicinal value and has historically been used to treat an extensive variety of human ailments. According to some folklore, this plant can be used to treat conditions like asthma, sore throats, vomiting, hiccups, diarrhea, dysentery, bleeding piles, ulcers, gout, heart problems, and bladder problems. (Anwesa Bag, 2013) [6]. Ingesting Haritaki has several health benefits such as It improve digestion strength, good for eyes, improves vision power, anti-aging, rejuvenative, improves life expectancy, nourishing, improves the body weight, helps in normalising bowel movements useful in asthma COPD, breathing difficulty, relieves cold and cough, Useful in diabetes and urinary tract disorders, useful in piles. Haritaki acts as an immunity booster and increases longevity if its powder is taken regularly fried in ghee. Haritaki helps in regulating blood sugar levels and decreases insulin sensitivity in the body, so it is a very useful remedy Diabetes patients. Haritaki is a natural blood purifier. It helps in remove toxins from the body. Haritaki oil is extremely helpful in healing of wound (Dodke and Pansare, 2017) [11]. *T. chebula* contains 33% of tannin. *T. chebula* they contain hydrolysable tannins such as gallic acid, chebulic acid, punicalagin, chebulanin, corilagin, neo chebulinic, ellagic acid, chebulagic acid, chebulinic acid, 1, 2, 3, 4, 6-penta-Ogalloyl-ß-D-glucose, 1,6,-di-O-galloyl-D-glucose, casuarinin, 3,4,6-tri-O-galloyl-D-glucose and terchebulin. The tannin content varies with the geological variation. Flavonol glycosides, triterpenoids,

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coumarin conjugated with gallic acid called chebulin, as well as phenolic compounds were also isolated (Chattopadhyay and Bhattacharyya, 2007). In addition, ethyl gallate and luteolin were isolated from the fruit of *T. chebula*. It also consists of nutrients, biomolecules and minerals. (Said Muhammad *et al*, 2012). *T. chebula* has so many pharmacological activities which have been observed. It has Antioxidant property, Anticarcinogenic activity, Hepatoprotective activity, Cardioprotective activity, neuroprotective activity, Cytoprotective activity, hyperglycemic activity, Antidiabetic activity, Antibacterial activity, Antifungal activity, Antiviral activity, Antiprotozoal activity, Anti-inflammatory and anti-arthritic activity, Anti-allergic activity, Anticaries activity, Wound healing activity, anti-aging, anti-psychiatric and Immunomodulatory activity and so on. (Sudhanshu Kumar Meher, 2018).

Oxidative Stress

One of the undesirable outcomes of aerobic respiration is development of reactive oxygen species (ROS) and other free radicals (Rambhadur Subedi, 2017) [17]. Oxidative stress occurs due to excess production of free radicals. The atoms, molecules or ions that have one or more unpaired electrons on the valence shell are called free radicals. These free radicals may be a product of cellular metabolism or an environment stressor such as pollution, ultraviolet radiation or ionizing radiation (Ndinawe Johnmark and Hellen Kinyi, 2021). These free radicals are unstable and because of their instability, they exhibit high reactivity to biological molecules such as protein, sugar, lipid or nucleotides. There are some other oxygen compounds which exhibit high reactivity, such as ozone, singlet oxygen or hydrogen peroxide. (Małgorzata Deska, 2020) [13]. Small amounts of ROS are required for the proper functioning of the organism as they are involved in the processes that protect the cell against oxidative stress and restore its redox balance. But higher concertation of these molecules causes toxic damages to cells and cell organelles. Presence of ROS in excessive amount creates imbalance in the body and this imbalance leads to oxidative stress. (Małgorzata Deska, 2020) [13]. Hence to maintain the balance in the body the cellular antioxidant system continuously reduces these into less toxic molecules. They maintain the balance either by preventing the production of ROS in the body or by removing them from the body. (Rambhadur Subedi, 2017) [17]. Apart from cell damage, oxidative damage plays major role in regulating the lifespan of the organism. Accumulation of free radicals indicate a reverse correlation with the potential life expectancy of an organism. This has been proven by studies on the production of ROS (such as superoxide anion radical, hydrogen peroxide or hydroxyl radical) and the protein oxidation process in insect mitochondria (Kasapoglu 2001; Korsloot 2003; Małgorzata Deska, 2020) [13].

Among the free radicals, Hydrogen peroxide (H2O2) is one of the major inducers of oxidative stress (Damilola A. Omoboyowa, 2022) [8]. It may be produced in a cell to serve as second messenger or signalling molecule (Hachiya and Akashi, 2005). In granulocytic leucocytes, H2O2 is produced for disposing the engulfed pathogens (Segal, 2005). Different types of enzymes (Cu-Zn or Mn based) localized in certain compartments in eukaryotic cell converts superoxide radicals into H2O2. A cell can tolerate extracellular H2O2 in the concentration range of 0.001-0.1mm with an appropriate physiological response (Jang and Imlay, 2007). The increase in extracellular concentration of H2O2, beyond these levels, exerts an oxidative stress that may cause an irreversible damage in the cell (Rambhadur Subedi, 2017) [17].

The ultimate aim of the present study is to reveal nutritional basis of resistance to hydrogen peroxide induced oxidative stress in *Drosophila melanogaster*. Because one of the simplest ways to strengthen the antioxidant capacity of cells and organisms is to supplement the diet with antioxidant compounds. These antioxidant food supplements may have a natural tendency to counter the free radicals either by directly participating into the reduction reaction or indirectly by inducing other parameters that could handle these free radicals (Rambhadur Subedi, 2017) [17]. Terminalia is one such medicinal plant which has several phytochemical constituents which can act as anti-oxidants. Hence in the present experiment *Drosophila* flies were administrated with *Terminalia chebula* and then hydrogen peroxide has been used to induce oxidative stress in the flies. *Drosophila* has been chosen as the model organism in the experiment because the experiment is mainly dependent on the longevity of the organism. The experiment on vertebrates is more difficult compared to insects, as their lifespan is relatively long

Materials and Methodologies

***Drosophila melanogaster* Stock Culture**

Experimental Oregon K strain of *Drosophila melanogaster* which is utilized in the experiment was extracted from, *Drosophila* stock centre, department of studies in zoology, University of Mysore, Mysuru. The Stock was cultured in the glass bottles consisting of yeast agar media. Yeast agar media was prepared by adding 100g of jaggery, 100g of Soji and 10g of agar into 1000ml of distilled water and boiled, then 7.5 ml propionic acid was added. After it got dried pinch of yeast was added. Flies were transferred to the media containing cultured bottle and maintained at optimum laboratory conditions such as 70% humidity, 12:12 dark light cycle and 22± 2ºC.

Diet Preparation: 3 different kinds of Medias were prepared. They are as follow,

i). **Control:** yeast agar media prepared as mentioned previously
ii). **Treatment 1:** 100ml of yeast agar media containing 250mg of powder of *Terminalia chebula* extract
iii). **Treatment 2:** 100ml of yeast agar media containing 300mg of powder of *Terminalia chebula* extract.

Establishment of Experimental Stock

10 pair of male and female flies were isolated form the stock and transferred to each bottles containing three different media and are tagged as control, treatment1 and treatment 2 respectively. Such that 10 replicates of each group were established. They are maintained in the laboratory conditions and were allowed to mate and lay egg. For every 2 days the flies were transferred to new culture bottles of respective media to avoid overcrowding of flies. After a week of transfer, overall, 40 bottles of flies from each group were obtained. These flies were used for the further experimentation.

Experimental Procedure

Flies were allowed to feed on control, 250mg and 300mg of *chebula* containing media for 5 days. 10 male flies and 10 female flies from control, treatment 1 and treatment 2 groups were transferred to new empty vials plugged with cotton. Piece of filter paper soaked in hydrogen peroxide was kept inside the vial. 5 replicates of males and 5 replicates of

females from each group were established and the vials were labelled with the name of their group, date, time and replicate number. These flies were maintained at 25ºC under 12 hours light and 12 hours dark condition. Their resistance to oxidative stress along with starvation was assessed by noting the number of flies dead for every 2 hours. The assessment was done till the death of the last fly. The data obtained from the assessment was statistically analysed to interpret the starvation resistance activity of *the Drosophila melanogaster.*

Statistical Analysis

The data obtained were analysed using IBM SPSS version 29.0. Mean, standard error, one way ANOVA, two-way ANOVA and Tukey's Post-Hoc test were carried out. Then a graph of concentration of *T. chebula* vs survival time of flies in hours was plotted.

Result

Fig.1 represents resistance to oxidative stress in female flies of *Drosophila melanogaster* raised in control and treated media. According to the data obtained, high resistance was exhibited by the flies cultured in treated media. The flies treated with 250mg and 300mg of *T.chebula* has exhibited equal resistance to hydrogen peroxide induced oxidative stress. Control flies survived for 24 hours whereas the flies treated with 250mg and 300mg of *Terminalia chebula* has survived upto 30 hours. Compared to the control flies, treated flies showed resistance for more than 6 hours.

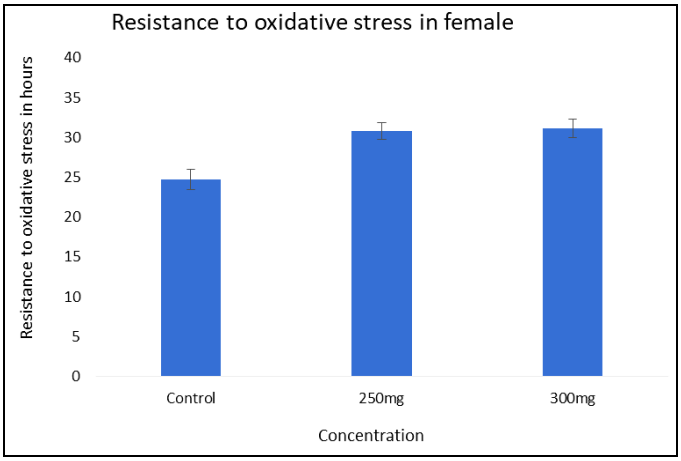

Fig 1: Resistance to hydrogen peroxide induced oxidative stress in Female *Drosophila melanogaster*

Fig.2 represents resistance to oxidative stress in male flies of *Drosophila melanogaster* raised in control and treated media. According to the data obtained, high resistance was exhibited by the flies cultured in treated media. The flies treated with 250mg and 300mg of *T. chebula* has exhibited equal resistance to hydrogen peroxide induced oxidative stress. Male flies show significant resistance to oxidative stress. Control flies survived upto 23 hours, whereas treated flies had survived upto 30 hours. The flies treated with 250mg and 300mg of *T. chebula* are both highly resistant to oxidative stress and has survival rate increased by 7 hours compared to control flies.

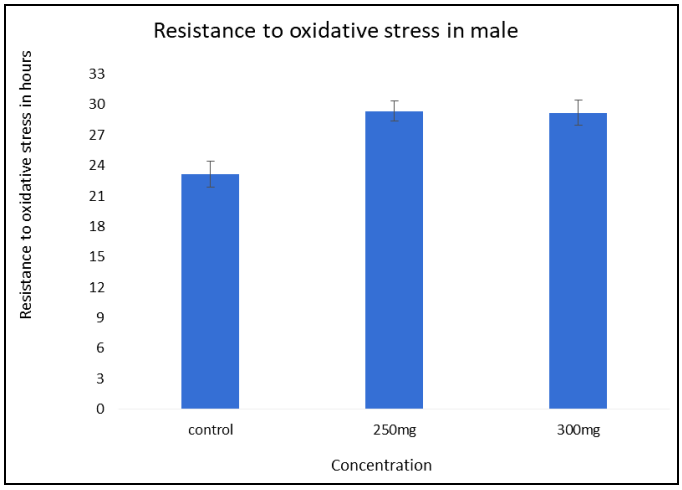

Fig 2: Resistance to hydrogen peroxide induced oxidative stress in male *Drosophila melanogaster*.

Fig 3 shows the compared means of starvation resistance in both male and female flies of *Drosophila melanogaster* raised in controlled and treated media. According to the data obtained, starvation resistance was high in both male and female flies cultured in treated media. Flies cultured in both 250mg and 300mg of *T. chebula* showed almost equal resistance to the oxidative stress induced by hydrogen peroxide. When stress resistant activity is compared between male and female flies, female had exhibited more resistance than female. Female flies treated with 250mg and 300mg of *T. chebula* has shown resistance stress upto 30 hours whereas survival rate of male flies had reduced 1 hour compared to female in both the treated media. Comparision between control and treated groups are significant

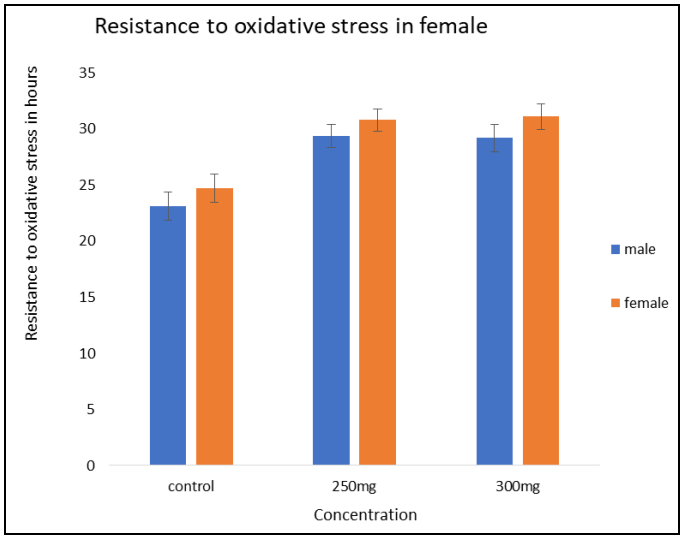

Fig 3: Resistance to hydrogen peroxide induced oxidative stress in both male and female *Drosophila melanogaster.*

Discussion

Numerous serious illnesses have been connected to the production of oxidative stress. Thus, one of the busiest study fields in the last ten years has been the creation of techniques and plans to lessen oxidative stress (Ichiishi *et al*., 2016). It has been said that by controlling oxidative stress, a number of conventional formulations can reduce the symptoms of a certain illness (Modak *et al*., 2007). Nonetheless, the scientific confirmation of these items using contemporary tools is required. Thus, the goal of the current experiment is to investigate *Terminalia chebula's* antioxidant properties (Małgorzata Deska, 2020) [13]

As a by-product of oxygen metabolism, hydrogen peroxide is produced in every aerobic organism's cell and takes part in a number of metabolic and signaling cascades. Maintaining the intracellular concentration of normal H2O2 is essential for proper cell function and viability. This is tightly regulated by cellular redox systems and is referred to as "oxidative distress." The antioxidant defense system, which includes cellular enzymes for H2O2 elimination (peroxiredoxins, glutathione peroxidases, and catalase) and reduction of oxidized proteins (thioredoxins and glutaredoxins), counteracts the harmful effects of stress under conditions of externally induced oxidative stress (Valeri Zenin *et al*, 2022) [22]. Insects have a set of antioxidative enzymes and low molecular weight antioxidants that protect against both endogenous and environmental origin oxidants. The main antioxidant enzymes that occur in insects are superoxide dismutase, catalase, glutathione transferase, glutathione reductase, as well as a number of antioxidants such as ascorbic acid, glutathione, vitamin E and carotenoids (Małgorzata Deska, 2020) [13]. But At high H2O2 doses, the oxidation/reduction cycle can collapse that causes the drop in H2O2 detoxification capacity in a cell which eventually leads to death. Hence in the present experiment analysing the Longevity of the *Drosophila* has been selected to study the oxidative stress resistance in it.

The aim of the experiment was to study the antioxidant property of *T.chebula* using *Drosophila melanogaster* as a model organism. In the current experiment *Drosophila* flies were allowed to feed on the media containing different concentration such as 250mg/100ml and 300mg/100ml water of *T.chebula*. After 5 days of feeding the flies were kept in empty vial without any access to water or food. Then a piece of filter paper soaked in hydrogen peroxide were kept in the vials to induce oxidative stress in the flies. Then their stress resistance activity was measured by assessing how many hours they were alive without food. The data was taken till the death of the last fly.

According to the data obtained, the male and female flies fed on control media did not show any significant resistance to oxidative stress. But the male flies fed on treated media exhibited significant resistance. Flies treated with 250mg of *T. chebula* and flies treated with 300mg of *T. chebula* both exhibited equal resistance to the oxidative stress. Between the group it is significant with $P<0.05$, df=2 and F=9.151. Similarly female flies treated with 250mg and 300mg of *T. chebula* also shown equal resistance to oxidative stress. It is significant with $P<0.05$, df=2 and F=9.746.

This shows that the *T.chebula* was the prime constituent which allowed the flies to resist for much longer time than the control flies. *T. chebula* contains several components which allowed it to have great antioxidant property. According to a comparative study by Bhatt *et al., T.chebula* fruit contained a maximum value for total phenols, total flavonoids, gallic acid, catechin, chlorogenic acid, and coumaric acid among the ten wild edible fruits specimens tested (Rakibul Hassan Bulbul, 2022). High performance liquid chromatography (HPLC) analysis confirmed that the fruit of Terminalia extract contains phenolic compounds and these compounds are good scavengers of free radicals (Said Muhammad, 2012). So, it can be concluded that the anti-oxidant system of *Drosophila* was enhanced by supplementing *T. chebula* in their diet. However, more investigations are still required to comprehend the specific pathways of this action.

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