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Research Article/Araştırma Makalesi

Effects of Herbal Safflower Oil on Longevity and Oxidative Stress

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Abstract: Safflower (*Carthamus tinctorius* L.) is one of the oldest cultivated plants. Safflower oil, separated from its seeds, has superior properties than many vegetable oils. In this study, it was aimed to determine the toxic, antitoxic or antioxidant effects of safflower oil. For this purpose, 72±4-hour old larvae of the *Drosophila melanogaster* (fruit fly) model organism Oregon R wild strain were used. According to the preliminary studies, application doses were determined as 0.3125%; 0.625%; 1.25%, and 2.5%. In addition, distilled water, ascorbic acid, and H_2O_2 control groups were formed and the toxic or antitoxic effects of using them separately or together on the larvae were investigated. In addition, the lifespan of individuals that matured from larvae were studied and antioxidant parameters (TAS/TOS/OSI) were examined in male individuals fed at the doses with the best results. All experimental sets were repeated three times. As a result, it was determined that Safflower oil does not cause any toxic effect on the larvae at the concentrations used; on the contrary, when used with H_2O_2, it has a reduced toxic effect. As a result of the longevity studies of safflower oil, it was observed that the longest average life was in the %1.25 Safflower Oil + H_2O_2 application group with 65±1.09 days. It was determined from the data obtained from antioxidant studies that the antioxidant capacity of safflower oil was high, but this result was not statistically significant compared to the control group Ascorbic acid ($p>0.05$).

Keywords: Antioxidant effect, *Carthamus tinctorius*, *Drosophila melanogaster*, larval mortality, life span.

Bitkisel Aspir Yağının Ömür Uzunluğu ve Oksidatif Stres Üzerine Etkileri

Öz: Aspir (*Carthamus tinctorius* L.) en eski kültür bitkilerindendir. Tohumlarından ayrıştırılan aspir yağı, birçok bitkisel yağa göre üstün özelliklere sahiptir. Bu çalışma ile aspir yağının toksik, antitoksik veya antioksidan etkilerinin belirlenmesi amaçlanmıştır. Bu amaçla *Drosophila melanogaster* (meyve sineği) model organizması Oregon R yabanıl soyunun 72±4 saatlik larvaları kullanılmıştır. Yapılan ön çalışmalarla uygulama dozları %0.3125; %0.625; %1.25 ve %2.5 olarak belirlenmiştir. Ayrıca Distile su, Askorbik asit ve H_2O_2 kontrol grupları oluşturularak ayrı ayrı ve birlikte kullanımın larvalar üzerindeki toksik ya da antitoksik etkinliği araştırılmıştır. Ayrıca larvadan erginleşen bireylerin ömür uzunlukları çalışılmış ve en iyi sonuçların alındığı dozlarda beslenen erkek bireylerde antioksidan (TAS/TOS/OSİ) parametrelere bakılmıştır. Tüm deney setleri üç kez tekrar edilmiştir. Sonuç olarak aspir yağının kullanılan konsantrasyonlarda larvalar üzerinde herhangi bir toksik etki yaratmadığı aksine H_2O_2 ile kullanıldığında toksik etkiyi azaltıcı etkinlik gösterdiği tespit edilmiştir. Aspir yağının ömür uzunluğu çalışmaları sonucunda da en uzun ortalama ömrün 65±1.09 gün ile %1.25 Aspir Yağı+H_2O_2 uygulama grubunda olduğu gözlenmiştir. Antioksidan çalışmalardan elde edilen verilerden de aspir yağının antioksidan kapasitesinin yüksek olduğu ancak istatistiksel anlamda kontrol grubu Askorbik asite göre bu sonucun anlamlı olmadığı belirlenmiştir ($p>0.05$).

Anahtar kelimeler: Antioksidan etki, *Carthamus tinctorius*, *Drosophila melanogaster*, larval mortalite, uzun yaşam.

1. Introduction

Nutrition is the ability of a human being to meet the nutrients her body needs in an adequate and balanced manner in order to live a long life (Orbay, 2019). It is also defined as the eating and drinking pattern of a person throughout her life.

Our conclusion, based on research, is that our diet has a strong impact on the development of diseases. Nowadays, the food ingredient most associated with diseases is fat. It is thought that the more fat in the diet, the more fat will accumulate in people's bodies, which will pave the way for multiple diseases along with obesity (Çakmakçı & Kahyaoğlu, 2012).

New studies have shown that healthy fats are necessary for our body and have numerous benefits for our health. The real issue is what type of fat we consume.

Animal fats are less popular for health reasons due to their high saturated fat content and varying amounts of cholesterol. People who adopt a natural and healthy diet are more likely to turn to vegetable oil sources (Yurtvermez & Gıdık, 2021). The majority of the oils produced worldwide currently consist of vegetable oils (86%) (Soylu Erşahin, 2018). List of the plants from which oil is produced from their seeds around the world, from those with the largest share in production to the least, is as follows: soybean, sunflower, cotton (cottonseed), rapeseed, peanut, sesame, safflower, castor oil, poppy, flax, hemp, jojoba, corn (corn extract), olive, palm (fruit and seed), and coconut. In our country, the plants that produce oil include sunflower, olive, safflower, poppy, sesame, rapeseed, cottonseed, soybean, peanut, and corn (Gulluoglu et al., 2017).

Safflower, with its Latin name *Carthamus tinctorius* L., belongs to the Astraceae family. It is one of the oldest plants cultivated by humans (Şeker, 2019). There are 25 species of safflower discovered in the world (Soylu Erşahin, 2018). Safflower oil is obtained from the seeds of

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the safflower plant (Genç, 2019).

Safflower oil is superior and of higher quality than many vegetable oils (Taşlıgil & Şahin, 2016) containing linoleic acid, one of the unsaturated fatty acids (Öztürk et al., 2007). Recently, it has attracted attention with its conjugated linoleic acid content, which reduces fat content and provides weight loss in a healthy way (Baydar & Erbaş, 2020).

Safflower species grown in Turkey are divided into two types as oleic and linoleic types and they have many species among themselves. BAY-ER, Linas, Olas, ASOL, Balcı, Remzibey-05, Dinçer, Yenice, Yekta, Gelendost 1 and 2. Olas, and ASOL are oleic type safflower. Linas, Balcı, Gelendost, Dinçer, Remzibey-05, Yenice, and Yekta are linoleic type safflowers. BAY-ER, on the other hand, has multiple lines and fatty acid ratios depending on the lines (Demirci, 2020).

In healthy individuals, reactive oxygen species and antioxidant enzyme activity are always kept in balance. Otherwise, various diseases and oxidative damage occur. Oxidant and antioxidant molecules can be measured with different analytical methods (Güneş, 2016a). A faster and more functional method, TAS and TOS measurement, is attracting attention (Scandalios, 2002). Erel (2005) discovered an easy and economical method for measuring TAS and TOS and calculated the oxidative index (Çobanoğlu, 2011; Güneş, 2015). The ratio of total oxidant status to total antioxidant status as a percentage gives the oxidative stress index (Kosecik et al., 2005).

Aging and long life have been a subject of people's curiosity for years. This biological process is quite complex and intricate. Free Radical Theory is the most accepted and most researched theory among aging theories (Harman, 1956). In our study, oxidative stress and longevity were investigated based on this theory.

Insect species have an important place in studies in the field of nutrition and are frequently preferred due to reasons such as the fact that insects have an important place as balance keepers in the existing ecosystem, direct interventions to nutrients can be easily realized and controlled, and also the substances to be added to foods can be easily processed (Güneş, 2016b). *Drosophila melanogaster*, colloquially known as the 'fruit fly', was first used in genetic studies in 1910 by American Thomas Hunt Morgan (1866-1945), who worked in the fields of zoology, embryology, developmental biology, and genetics (Topçu & Duran, 2021). It has continued to be used as a model organism for more than 100 years due to reasons such as being easy to raise, comfortable feeding conditions and low cost, easy reproduction to produce a large number of offspring in a short period of time, and thus increasing the reliability of the data obtained, short life cycles, and ease of observation (Hales et al., 2015; Tamtürk, 2019; Yi et al., 2021).

In this study, it was aimed to determine the toxic, antitoxic or antioxidant effects of safflower oil obtained from the safflower plant, which is not on our tables despite its frequent production in our country, regarding the importance of herbal nutrition, which is shown as the secret of a healthy and long life, and to investigate its effect on longevity.

2. Material and Methods

2.1. Material

2.1.1. Safflower oil

Safflower oil (*Carthamus tinctorius*), which is used as a food supplement in our experimental study, prepared by cold pressing without the use of any solvent and has a high value in terms of monounsaturated fatty acid (oleic acid), was obtained from a health products company named Zade Vital Pharmaceutical Inc. (Konya, Türkiye). It was stored at +4 degrees until used in the study.

2.1.2. *Drosophila melanogaster*

D. melanogaster used in our study has been propagated by hybridization at the Biological Research Laboratory of Amasya University, Faculty of Science and Letters, for years. In our experimental study, the Oregon (R) (wild type) strain of *D. melanogaster*, which has normal round, red eyes and no mutant characters, was used to determine larval mortality. *D. melanogaster* environment is at 40%-60% relative humidity, at 25±1 °C temperature and under constant dark conditions.

2.2. Method

2.2.1. Larval mortality and longevity studies

Safflower oil concentrations used in our study were determined by taking other studies using similar vegetable oil sources as an example (Ayar et al., 2021; Güneş, 2016b; Güneş & Danacıoğlu, 2018; Heinrichsen et al., 2014). Based on previous studies, the concentrations of Ascorbic Acid (ASC) and Hydrogen Peroxide (H_2O_2) control groups were determined as 20 mM and 0.02%, respectively (Bahadorani et al., 2008; Vitorovic et al., 2021). The rates of Safflower Oil (SFO) application groups were determined as 0.3125%, 0.625%, 1.25%, and 2.5% by preliminary tests.

D. melanogaster larval mortality and substance application studies were carried out in ready-made Drosophila medium (Instant *D. melanogaster* Medium, Carolina Biological Supply Company) and lifespan studies were carried out in standard Drosophila medium.

To obtain the larvae to be used in our study, ♀25 crossed adult individuals were removed from the bottles after 1 day. After mating of the adults, 3rd instar larvae, approximately 72±4 hours old, were collected (Fig. 1).

Three separate experimental groups were created to determine larval mortality. There are four separate control groups in our study. These are distilled water, ASC (ascorbic acid), H_2O_2, and ASC+ H_2O_2. Other groups consisted of our active ingredient, safflower oil-distilled water solution at different doses and safflower oil+H_2O_2 groups, which we used to compare toxic-antitoxic effects. To induce oxidative stress, 0.02% H_2O_2 was added to the nutritional medium of *D. melanogaster*. The other control group, antioxidant ascorbic acid (ASC), was used as 20 mM. The collected 3rd stage larvae were added to safflower oil-water solution dissolved in 5 ml of distilled water at the determined concentrations (0.3125%; 0.625%; 1.25%, and 2.5%), and 5 ml of control group (distilled water, ASC, H_2O_2, and H_2O_2+ASC) bottles, other groups (0.3125%SFO+ H_2O_2; 0.625%SFO+ H_2O_2; 1.25%SFO+ H_2O_2, and 2.5%SFO+ H_2O_2) were transferred to glass bottles containing *D. melanogaster* ready medium. 100 larvae were

placed in each bottle and waited to develop into adults. Adult individuals formed after approximately 1 week were transferred to small cylinder bottles and stunned with ether. The number of stunned adults was counted and recorded in a table of larval survival and mortality rates.

Figure 1. Images of individual selection and larval collection for standard cross.

Both control groups and treatment groups were kept in constant darkness, 40%-60% relative humidity, and 25±1°C oven during the experiment.

Fresh medium was prepared for the adult individuals obtained as a result of the larval mortality experiment and they were transferred to sterilized 200 ml bottles. When the media left to rest reached the appropriate consistency, these adult individuals were transferred to bottles determined according to the concentrations they were treated with. Thus, individuals were provided with nutrition throughout the lifespan experiment. The medium was renewed twice a week and the number of living adults and the number of dead adults were noted during each renewal. Dead individuals were removed from the environment. This counting continued until the last individual remained in all experimental groups and was recorded in the tables (Piper et al., 2005; Wongchum et al., 2022).

2.2.2. Biochemical analyzes (TAS/TOS)

An additional study including biochemical analyses was planned to determine the effect of safflower oil on oxidative stress markers. According to the data obtained from larval mortality and life span studies, the doses (distilled water, ASC, H_2O_2, ASC+ H_2O_2, 0.3125%, and 1.25%+ H_2O_2) that gave the best results were applied to the larvae. Adult individuals were collected approximately 1 week later. Male and female separation was done under light microscope and 10 males individuals were selected from each concentration. The collected adults were extracted by mixing with cold homogenisation buffer (1.15% KCl, 1.15% potassium chloride, 25 mM dipotassium hydrogen phosphate, 5 mM ethylene diamine tetra acetic acid, 2 mM phenylmethylsulfonyl fluoride, 2 mM dithiothreitol, pH 7, 4, +4 °C) in an ultrasonic homogeniser. Sample supernatants were stored in the freezer (-18°C) until biochemical analyses were performed.

Total antioxidant status (TAS) measurement is a measurement method based on the antioxidants in the samples converting the dark blue green ABST radical into a colorless form. Commercial kits (Baran medical, Rel Assay Diagnostics) were used in the measurements and sample absorbance was measured at 660 nm (spectrophotometer Biochrom Libra S22) as specified in the kit procedure (Erel, 2004; Güneş 2016a). TAS levels (µmol Trolox Eq/L) of the samples were calculated according to the generally used standard formula (Erel, 2004). Total oxidant status (TOS) measurement is based on the color reaction of ferric ions, which are formed by the oxidants in the samples oxidizing the ferrous ion-chelator complex to ferric ions, with the chromogen substance in an acidic environment. In the measurements, a commercial kit (Baran medical, Rel Assay Diagnostics) and kit procedure were used (spectrophotometer Biochrom Libra S22). The absorbance of the samples was measured at 530 nm (Erel, 2005; Güneş 2016a). TOS levels (µmol H_2O_2 Eq/L) of the samples were calculated according to the generally used standard formula. The procedures were repeated 3 times for all samples and TOS/TAS levels and Oxidative stress index OSI were determined (Erel, 2005). The ratio of TOS to TAS was considered as the oxidative stress index (OSI). For calculation, the resulting TAS unit was converted to µmol/L and the OSI value was calculated according to the formula:

OSI (arbitrary unit) = TOS (µmol H_2O_2 equivalent/L) / TAS (µmol Trolox equivalent/L)

2.2.3. Statistical analysis of data

For the analysis of the data we obtained as a result of our study, SPSS version 27.0 (Statistical Package for the Social Sciences) program was used. For this purpose, the "One-way Analysis of Variance" (One-way ANOVA) method was applied. Duncan test was evaluated at 0.05 probability level for the data obtained from survival rates, lifespan, and biochemical studies ($p<0.05$). Larval mortality graphs of adult individuals, lifespan survival curves, and other graphs were drawn using the Microsoft Windows Office Excel program.

3. Results

3.1. Results from Larval Mortality and Lifespan Studies

First of all, from the results obtained from larval mortality studies, it was determined that the highest larval mortality rate was in the H_2O_2 control group (0.02%) and the 2.5% SFO+H_2O_2 application group (24%) (Table 1). The best survival was observed in the 1.25% SFO+ H_2O_2 application group (100%) (Fig. 2 and Table 1).

In the second stage of our study, larvae were collected with a new experimental setup and substances were applied at determined doses from larvae to adult. 100 male adults obtained from these larvae were fed on standard medium and their mortality rates were monitored throughout their lifespan. All studies were repeated 3 times and averages were taken Then, the differences between the averages obtained as a result of pairwise comparisons between the study groups and the control groups were evaluated statistically (Table 2).

When Table 2 is examined, it is seen that the maximum lifespan of distilled water, ascorbic acid (20mM), H_2O_2 (0.02%), and H_2O_2+ASC control groups was 77±1.26 and 47±0.75, respectively. It was determined as 59±1.11 and

69±1.12 days. The most interesting result here was determined in the Ascorbic acid control group. Even though the experiment was repeated many times, the result did not change and the maximum lifespan was very short in this group. Based on the appearance of the medium, it was thought that the reason for this was that ascorbic acid created a suitable environment for microorganisms and the lifespan was short due to excessive contamination; and thus, this group was not included in the statistical calculations. The highest maximum lifespan detected was 78±0.91 days in the 1.25% SFO+H_2O_2 application group and the lowest maximum lifespan was 51±1.2 days in the 2.5% SFO application group (Table 2 and Fig. 3).

Table 1. Survival and mortality rates of larvae chronically fed with different concentrations of safflower oil.

Experiment Sets	N	Mortality Rate (%)	Survival Rate (%)
Distilled Water	100	9	91
Ascorbic Acid (ASC) (20mM)	100	8	92
H_2O_2 (%0.02)	100	25	75
H_2O_2+ASC	100	6	94
%0.3125 Safflower Oil (SFO)	100	8	92
%0.625 Safflower Oil (SFO)	100	9	91
%1.25 Safflower Oil (SFO)	100	8	92
%2.5 Safflower Oil (SFO)	100	16	84
%0.3125 Safflower Oil (SFO) +H_2O_2	100	17	83
%0.625 Safflower Oil (SFO) +H_2O_2	100	7	93
%1.25 Safflower Oil (SFO) +H_2O_2	100	0	100
%2.5 Safflower Oil (SFO) +H_2O_2	100	24	76

Table 2. Lifespan data of individuals fed with different concentrations of safflower oil.

Experiment Sets	N	Max. Lifespan (days) ± S.E.	Average Lifespan (days) ± S.E.
Distilled Water	100	77±1.26^{d}	62±1.13^{d}
ASC (20mM)	100	47±0.75*	39±0.05*
H_2O_2 (%0.02)	100	59±1.11^{a}	45±1.13^{b}
H_2O_2+ASC	100	69±1.12^{c}	52±1.13^{c}
%0.3125 SFO	100	76±1.12^{d}	61±1.11^{d}
%0.625 SFO	100	57±1.25^{a}	37±0.09^{a}
%1.25 SFO	100	68±1.26^{c}	51±0.92^{c}
%2.5 SFO	100	51±1.26^{a}	35±0.46^{a}
%0.3125 SFO+H_2O_2	100	59±0.88^{a}	42±0.88^{b}
%0.625 SFO+H_2O_2	100	61±0.91^{ab}	47±0.85^{bc}
%1.25 SFO+H_2O_2	100	78±0.91^{d}	65±1.09^{d}
%2.5 SFO+H_2O_2	100	66±1.16^{d}	52±1.16^{c}

N: Total number of individuals; Max: Maximum; ASC: Ascorbic acid; SFO: Safflower Oil; SE: Standart error; $^{a\text{-}d}$The values of the experimental groups shown with different letters in the same column are significant at the $p<0.05$ level. Groups were compared among themselves. *Due to contamination, the lifespan was thought to be short and was not included in the statistical calculations.

When looking at the average lifespans, the longest average lifespan is again in the 1.25% SFO+H_2O_2 application group and the lowest average lifespan is 65±1.09 and 35±1.09%, respectively, in the 2.5% SFO application group. It was determined as 0.46 days (Table 2, Figs. 3-6).

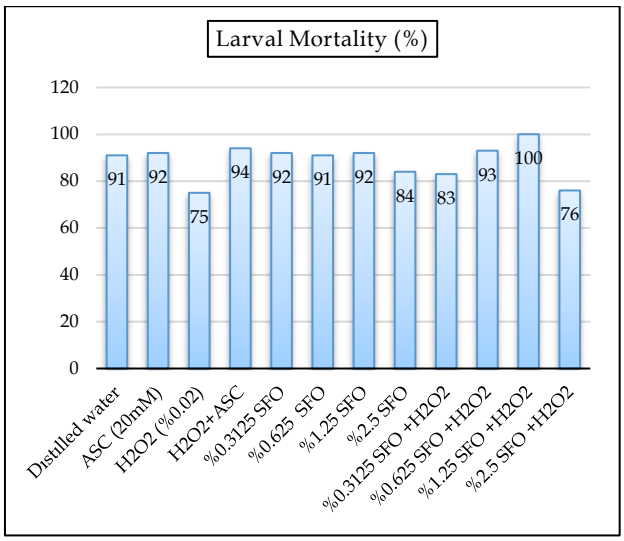

Figure 2. Survival rates of larvae fed with safflower oil.

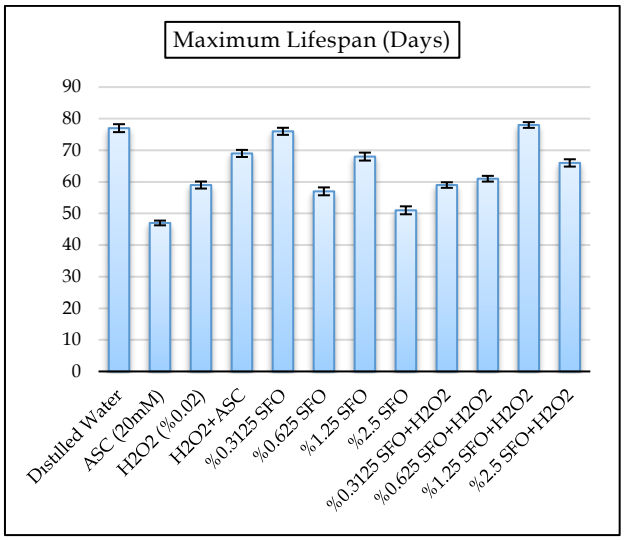

Figure 3. Maximum lifespan data of individuals fed with different concentrations of safflower oil.

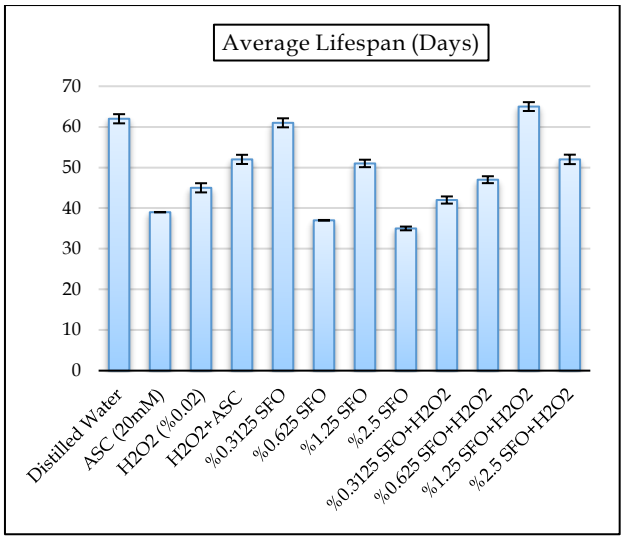

Figure 4. Average lifespan data of individuals fed with different concentrations of safflower oil.

It was determined that safflower oil has an antitoxic effect by inhibiting the toxic effect of H_2O_2 and has a life-extending effect, although not at all concentrations. In fact,

the results obtained in the 1.25% SFO+H_2O_2 application group were found to be more effective than the distilled water control group and ASC+H_2O_2 application groups. The differences obtained were also found to be statistically significant ($p<0.05$) (Table 2).

However, at higher safflower oil concentration (2.5% SFO+H_2O_2), distilled water showed a life-shortening effect, not a life-extending effect, compared to the control group.

In summary, from all the data obtained, we can say that safflower oil generally showed inhibitory activity on the toxic effect induced by H_2O_2 (Table 2, Figs. 3-6).

3.2. Results Obtained from Biochemical Analyzes

In order to determine the biochemical activity of safflower oil, the doses that gave the best results according to the larval mortality and life span data obtained as a result of our study were used (0.3125% SFO and 1.25% SFO+H_2O_2). Biochemical measurements were made on male individuals that matured from larvae fed with safflower oil and control substances at specified doses.

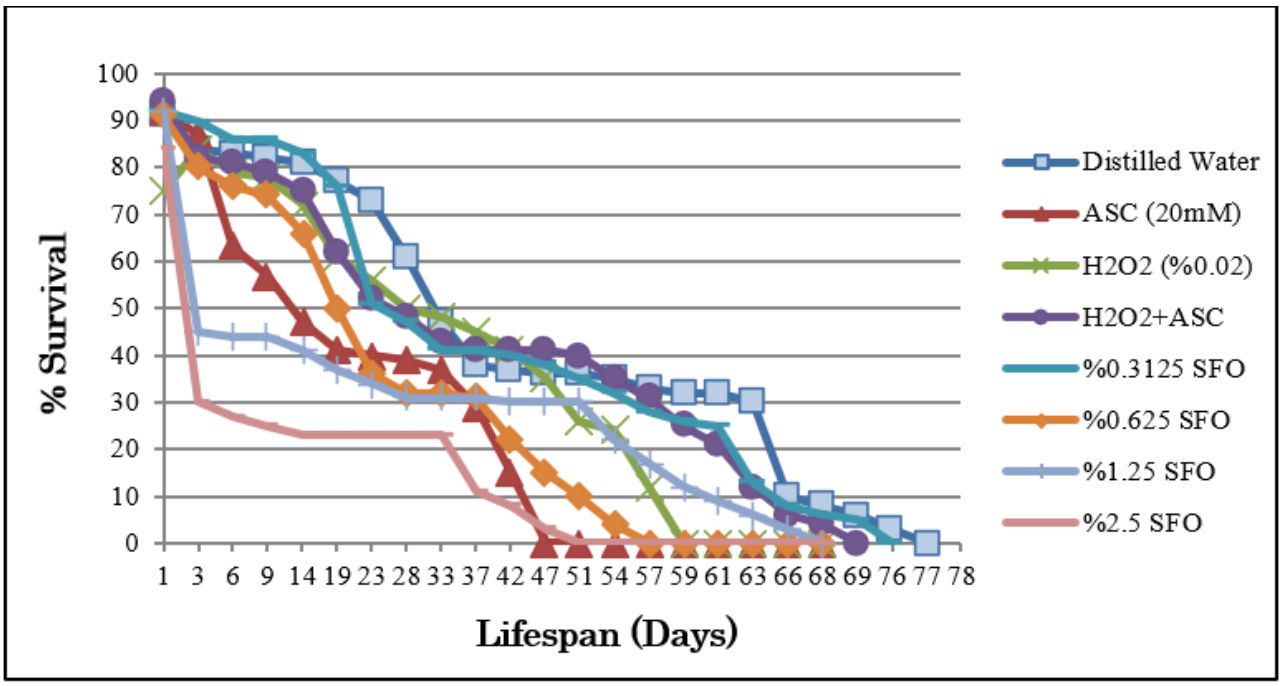

Figure 5. Lifespan curves of individuals fed and not fed with different concentrations of safflower oil.

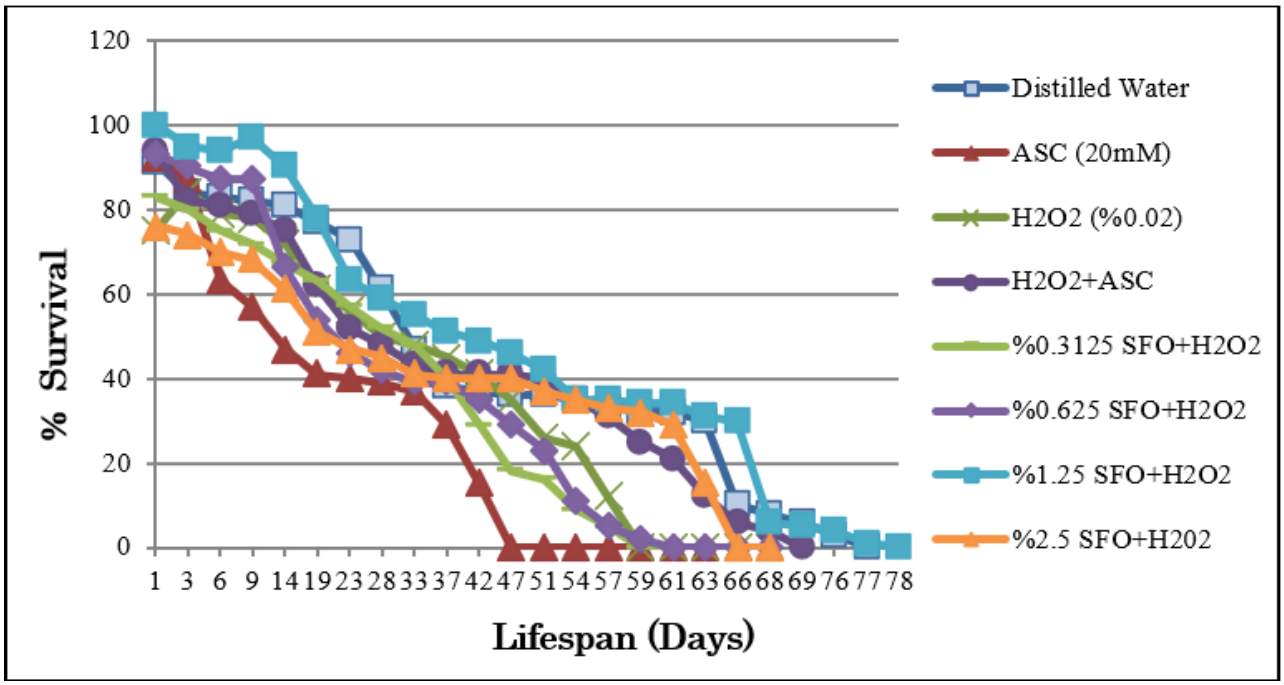

Figure 6. Lifespan curves of adult individuals chronically fed with different concentrations of safflower oil and H_2O_2.

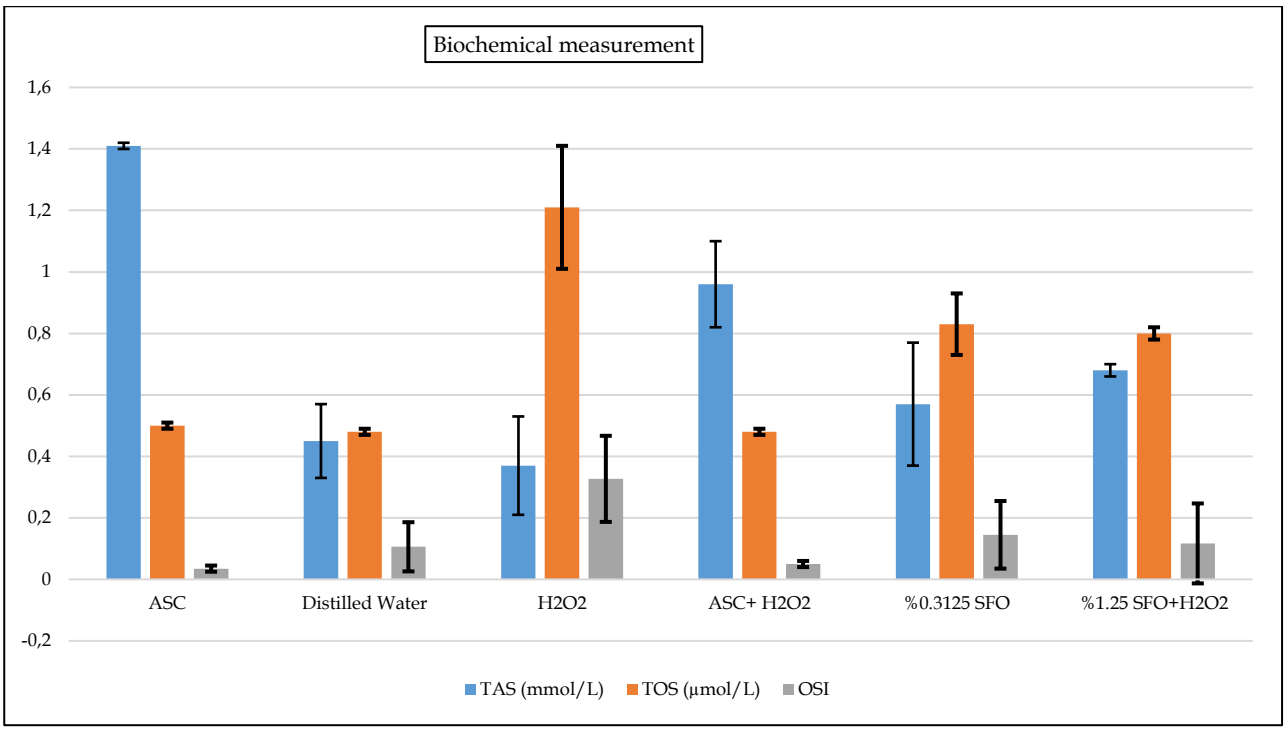

Figure 7. Biochemical data obtained from adult individuals fed with safflower oil (SFO)

When the biochemical analysis results were examined, it was observed that the highest antioxidant status (TAS) was in the ascorbic acid group (1.41 mmol/L) and the lowest was in the H_2O_2 group (0.37 mmol/L) (Table 3 and Fig. 7). However, although an increase in TAS values was observed in the safflower oil application groups compared to the negative control group, this increase remained quite low compared to Ascorbic acid and was not statistically significant ($p>0.05$).

Table 3. Comparison of biochemical data obtained from adult individuals fed with safflower oil (SFO).

Experiment Set	TAS (mmol/L)	TOS (µmol/L)	OSI
ASC	1.41±0.01^{d}	0.50±0.01^{a}	0.035±0.01^{a}
Distilled Water	0.45±0.12^{ab}	0.48±0.01^{a}	0.106±0.08^{b}
H_2O_2	0.37±0.16^{a}	1.21±0.20^{c}	0.327±0.14^{d}
ASC+ H_2O_2	0.96±0.14^{b}	0.48±0.01^{a}	0.050±0.01^{a}
%0.3125 SFO	0.57±0.20^{c}	0.83±0.10^{b}	0.145±0.11^{c}
%1.25 AY+H_2O_2	0.68±0.02^{c}	0.80±0.02^{b}	0.117±0.13^{b}

TAS: Total Antioxidant Status; TOS: Total Oxidant Status; OSI: Oxidative Stress Index; ASC: Ascorbic acid; SFO: Safflower Oil; $^{a\text{-}c}$The values of the experimental groups shown with different letters in the same column are significant at the $p<0.05$ level. Groups were compared among themselves.

When total oxidant status (TOS) was examined, the highest level was determined in the H_2O_2 group and the lowest level was determined as 1.21 µmol/L and 0.50 µmol/L in the ascorbic acid group, respectively (Table 3 and Fig. 7).

When the OSI values were examined, it was observed that the highest stress level was in the H_2O_2 application group and the lowest stress level was in the ASC and ASC+ H_2O_2 application groups (Table 3 and Fig. 7).

4. Discussion

The aim of our study is to investigate in vivo the effects of safflower oil, which we think may have high antioxidant potential, against inflammation caused by animal and saturated fat sources, which are consumed extensively in our country. It is aimed to popularise safflower oil that is very easy to grow and produce in our country but not widely used as cooking oil in our kitchen.

While there are necessary conditions for the supply of many different oil crops in our country, oilseed production cannot meet consumption (Küçük et al., 2022). The most important import products after petroleum products are oilseeds and vegetable oils. For this reason, the need for alternative oil sources is increasing. Considering the conditions of our country, safflower plant gains importance in this regard (Aşçı et al., 2022).

Turkiye currently ranks 8th in the world in safflower production and can meet approximately 7% of the world safflower production. Although safflower production increased in our country until 2014, a decline was observed in the following years. Efficiency also decreased at this rate. As of 2022, an increase of 2-3% is expected in production (Aşçı et al., 2022).

Literature review results regarding studies conducted on various vegetable oils and many different organisms, especially safflower oil, which is an alternative vegetable oil source, and the safflower plant from which it is obtained, are given below. In the literature research, it was determined that safflower plant and its oil are the subject of studies in many research areas (Amer et al., 2021; de Souza et al., 2022; Rahimi et al., 2014; Higa et al., 2010). In line with this information, no study has been found in the literature on the effect of safflower oil on longevity; therefore, our study is unique. At the same time, other vegetable oil sources have been shown to be effective in studies on longevity and antioxidant activity (Zhang et al., 2010; Zhang et al., 2017; Geçioğlu, 2016). Studies conducted with the expectation that alternative vegetable oil sources will increase the quality of life due to their positive effects

on health parameters and that the increased quality of life will also reduce disease risk factors have occupied an important place in the literature for years. In addition, the need for alternative oil sources in production supports these studies.

In their study, Zemour et al. examined extractable methanol obtained from safflower oil grown in a semi-arid climate in terms of antioxidant activity and anti-aging effect. As a result of the study, they thought that it had these effects to a significant extent; thus, it could be a valuable source of polyphenols and have a great place in the field of cosmetics (Zemour et al., 2019).

As a result of our lifespan experiments, Safflower oil gave better results than the ascorbic acid (ASC) control group. This shows how much antioxidant effect Safflower oil has compared to ASC, which is a natural antioxidant source. It is thought that the reason for its positive effect on lifespan is due to the high unsaturated fatty acid it contains and the high level of linoleic acid.

In a study on broiler chickens, the organisms were fed a combination of safflower oil and vitamin C. As a result of the study, it was observed that this diet increased the growth rate, immunity and antioxidant capacity of chickens (Amer et al., 2021).

In a study on the maternal period, safflower oil was given to mother rats. Its effect on reflex maturation, memory, and offspring hippocampal oxidative stress was examined. Safflower oil reduced lipid peroxidation as measured by MDA levels and increased antioxidant defense through SOD, CAT, GST, and GSH levels. The use of safflower oil supplement by the mother is effective on the baby's reflexes, cognitive development in the adult period, and also improves antioxidant mechanisms in the hippocampus (de Souza et al., 2022).

As observed in the studies mentioned above and in many other studies, including ours, safflower oil had an antioxidant effect on *D. melanogaster*, positively increasing lifespan.

Due to its high-quality content, many benefits of safflower oil have been observed by other studies. These benefits include antidiabetic, anticarcinogenic, antiatherogenic, and antiobesity effects (Nazir et al., 2021).

In another study, the hepatoprotective and hypolipidemic effects of safflower seed oil on diabetic rats were examined. Diabetes in rats was achieved with the help of 120 mg alloxan monohydrate per kg and safflower seed oil was given to diabetic rats as a single dose of 200 mg per kg for 28 days. As a result of blood measurements in rats at the end of the experiment, it was observed that safflower oil decreased blood sugar levels, TC, LDL, ALT, ALP, AST, and TGs levels and increased HDL cholesterol levels (Rahimi et al., 2014).

In a study conducted by Higa et al. (2010) to determine the capacity of dietary supplementation with 6% olive or 6% safflower oil, it was stated that safflower supplementation reduced malformation rates in maternal diabetes.

In a study of 40 3-week-old C57BL/6 mice, the mice were divided into 3 groups: control group (5% lard + 5% SFO), high lard group (45% lard + 5% SFO), and high safflower oil group (45% SFO + 5% lard). As a result of 10 weeks of application, it was observed that the safflower oil-supplemented diet strongly changed gene expression related to adipocytic adiposity and prevented diet-induced obesity (Zhang et al., 2010).

In addition to the positive effects of the studies, whether the safflower plant and its oil have any toxic effects has been a matter of curiosity and has been addressed by various studies.

In Zhang et al.'s (2017) study, flavonoids from the safflower plant were extracted and tested on rats for 4 weeks. The extract was used in 3 different doses (100 mg/kg, 300 mg/kg, and 500 mg/kg). As a result of the study, no significant toxicity was found (Zhang et al., 2017).

In a study conducted in Türkiye, the level of erucic acid contained in safflower seeds and their current properties were examined. The data obtained were found to be in accordance with the reference values. It was reported that the amount of erucic acid in safflower oil obtained from safflower seeds grown in Turkey would not have any negative effects on health (Geçioğlu, 2016).

Many vegetable oils have positive effects on *D. melanogaster* in many ways (antimutagenic, antitoxic, antioxidant) (Campos-Sánchez et al., 2007; Öz & Arıca, 2019). However, it has been observed that oil sources such as palm oil (Güneş et al., 2019), *Artemisia absinthium* L. essential oil (Mihajilov-Krstev et al., 2014), *Cymbocarpum erythraeum* (Apiaceae) essential oil (Aksakal et al., 2019), sunflower and soybean oil (Demir, 2011), and coconut oil (Heinrichsen & Haddad, 2012; Heinrichsen et al., 2014) have a toxic effect on *D. melanogaster*. Contrary to these studies, the safflower oil we used in our study showed a positive result by showing an antioxidant effect on *D. melanogaster*.

Many studies have shown that many food products and supplements extend the lifespan of *D. melanogaster* such as olive leaf (Güneş & Danacıoğlu, 2018), açai (Sun et al., 2010), blueberry (Peng et al., 2012), lutein (Zhang et al., 2014), turmeric (Abolaji et al., 2020), white tea (Ayar et al., 2021), perga (Fidan and Ayar, 2023), pineapple (Vicente-Crespo et al., 2021), apple (Wang et al., 2019), ursolic acid (Staats et al., 2019), and royal jelly (Kunugi and Mohammed Ali, 2019). Safflower oil is also one of the foods that extends life. In our study, survival rates were determined on our model organism fed during the larval period and the highest larval mortality rate was observed in the H_2O_2 application group and the best survival was observed at 1.25% SFO+ H_2O_2 concentration. Adult individuals were fed in standard medium and mortality rates were monitored throughout their lifespan and the longest maximum lifespan was found in the 1.25% SFO+H_2O_2 application group (78 days) and the longest average lifespan detected was 65 days in the same group.

Many nutrients used in studies such as chitosan (Güneş & Nizamlıoğlu, 2023), quinoa (Güneş, 2016a), and *Lupinus albus* L. (Güneş et al., 2020) have a positive effect on *D. melanogaster* and TAS and TOS levels. In our study, safflower oil showed a similar effect. When our biochemical analysis results were examined, the highest antioxidant level (TAS) was in the ascorbic acid group (1.41

mmol/L) and the lowest was in the H_2O_2 group (0.37 µmol/L). However, although an increase in TAS values was observed in the safflower oil application groups compared to the negative control group, this increase remained quite low compared to Ascorbic acid and was not statistically significant ($p>0.05$).

Again, many food components such as ellagic acid (Kharat et al., 2020), amaranth (Ndinawe & Kinyi, 2021), *Origanum compactum* (thyme) essential oil (Başer, 2022), and *Cyperus rotundus* (Wongchum et al., 2022) inhibited oxidative stress and inflammation induced by H_2O_2. Safflower oil, which we used in our study, also showed antitoxic effect by inhibiting the toxic effect of H_2O_2 at many concentrations.

As a result, although the basic mechanisms of aging and longevity are not yet fully understood, it is suggested that this process may be delayed. Today, studies conducted on humans on this subject are not sufficient. It is thought that the effectiveness of safflower oil, which has rich nutritional content, on *D. melanogaster* lifespan will make a significant contribution to the literature.

Ethics committee approval: Ethics committee approval is not required for this study

Conflict of interest: The authors declare that there is no conflict of interest.

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