



Toxicity and *in vitro* antioxidant potential of *Curcuma longa* Linn and *Zingiber officinale* Rosc rhizomes in *Drosophila melanogaster*

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Abstract

Medicinal plants like *Curcuma longa* and *Zingiber officinale* contain phytochemicals which have antioxidant properties. The study evaluated the antioxidant effect of *Curcuma longa* and *Zingiber officinale* on the survival of *Drosophila melanogaster* administered isoniazid. *Drosophila melanogaster* aged 3-5 days old were exposed to different concentrations (5-1000 mg/ 5 g diet) of isoniazid, and extract for 7 days to determine the lethal concentration (LC₅₀). A 14-day survival assay was performed to evaluate the effect of isoniazid and the herbal extract on the survival rate of *Drosophila melanogaster*. The antioxidant activity of the herbal extract on the stable 2,2-diphenyl-1-picrylhydrazyl (DPPH) was also evaluated. The result showed an antioxidant activity greater than that of a standard, silymarin. An increase in survival rate of the fruit flies was observed at the lowest dose of the extract, when compared to those fed diet only. In conclusion, this study provided valuable insights into the antioxidant properties of the herbal extract, highlighting its potential benefits, while further research to elucidate the effects of phytochemicals especially at high doses is necessary. The results also underscore the importance of considering various factors in toxicity assessments as they affect fly survival.

Keywords: Antioxidant; Fruit fly; Ginger; Oxidative stress; Turmeric

INTRODUCTION

Plants produce a variety of antioxidant phytochemicals like alkaloids, flavonoids, terpenoids and saponins that have been found to possess many biological activities [1]. *Curcuma longa* is a member of the Zingiberaceae family [2], utilized in Ayurvedic and traditional medicine for a variety of maladies, including gastric, hepatic, gynecological, and infectious diseases [3].

Curcumin (diferuloylmethane), the main yellow bioactive component of turmeric has been shown to have a wide spectrum of biological actions, including anti-inflammatory and antioxidant activities [4]. *Zingiber officinale* has reported antioxidant activity and is crucial in the fight against oxidative stress-related drugs [5], such as that caused by isoniazid [6]. Polyphenol compounds, such as 6-gingerol and its

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derivatives, are present in the rhizome of this plant and are known for their significant antioxidant properties [7, 8]. Antioxidants are nutritional substances which could prevent or slow down oxidative damage and act as free radical scavengers, preventing and repairing cellular and molecular damage. In recent years, much attention has been devoted to natural antioxidant-containing plants and their association with health benefits. In response to elevated levels of Reactive Oxygen Species (ROS), antioxidant defense mechanisms are activated to reduce oxidative damage and enhance survival prospects.

Drosophila melanogaster or fruit fly, is used as a model organism to research everything from basic genetics through tissue and organ development. The *drosophila* genome is 60 % identical to that of humans, with less redundant genes, and roughly 75 % of human illness genes have homologs in *Drosophila melanogaster* [8]. These characteristics, combined with a short generation period, low care costs, and the availability of sophisticated genetic tools, make the fruit fly suitable for studying complex biological pathways [9].

Isoniazid (INH) is an anti-tubercular medication of choice, alongside rifampicin, pyrazinamide and ethambutol, used for the treatment and prophylaxis of active tuberculosis. It has, nevertheless, been linked to major side effects such as hepatitis, peripheral neurotoxicity, lupus-like syndrome, hypersensitivity reactions, and uncommon but serious central nervous system issues [10].

Drosophila exposed to various environmental stressors, such as high temperatures or exposure to toxins such as that produced by isoniazid, experience an increase in ROS production. Reactive Oxygen Species can act as signaling molecules in *Drosophila* and other organisms. They play a role in immunity, stress response, and lifespan regulation, where they activate signaling pathways such as the insulin/insulin-like

growth factor signaling (IIS) pathway, which influences lifespan of *Drosophila melanogaster* [11].

Reactive Oxygen Species (ROS) are chemically reactive molecules containing oxygen, such as superoxide radicals ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2), and hydroxyl radicals ($\bullet OH$). Extensive research has been conducted on the involvement of reactive oxygen species (ROS) in the aging process of *Drosophila* [12]. Elevated levels of ROS are associated with age-related physiological decline and decreased lifespan [13]. Specifically, the induction of oxidative stress via free radical formation and reactive oxygen species (ROS) has been associated with the use of this drug, isoniazid [14], ultimately affecting the life-span of the fruit flies. When reactive oxygen species (ROS) levels become excessively high, they can cause oxidative stress and damage biomolecules like DNA, proteins, and lipids, which can have detrimental effects on an organism's health. Studies have shown that antioxidant supplementation can extend the lifespan of *drosophila* under certain stressful conditions [15].

The aim of this study was to assess how the antioxidant properties of *Curcuma longa* and *Zingiber officinale* impact on the survival of fruit *Drosophila melanogaster* exposed to isoniazid, a well-documented toxic.

EXPERIMENTAL METHODS

Chemicals. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) was purchased from Sigma Aldrich Co., St Louis, USA. All other chemicals used in this study were of analytical grade, and the assay kits for toxicological studies were purchased from Human Diagnostics, Faridabad, India.

Collection of plant materials and preparation of extracts. The whole plants of *Curcuma longa* and *Zingiber officinale* comprising the leaves and roots were collected from a farm in Zangon Kataf, Kaduna State in

the month of November. They were then identified by Mr. Joseph Azila of the Federal College of Forestry, Jos and authenticated at the herbarium of the college. Samples were subsequently deposited at the herbarium. The rhizomes of *Curcuma longa* and *Zingiber officinale* were carefully washed separately, sun-dried for 2 days and further oven dried at 45°C until a constant weight was attained. They were then cut into pieces at the Technology Incubation Centre, National Board for Technology Incubation, Bukuru, Plateau state, Nigeria. The two dried plant parts were pulverized into powder with an electric blender and mixed together in a ratio of 1:1 (100 g each).

The extraction process involved hot aqueous (water-based solution) maceration. In this method, two litres of hot water was added to 200 g of the herbal mixture and left to stand undisturbed in the refrigerator set at 2.8°C for three days. Afterward, the extract was filtered, first using a Whatman No.1 filter paper then a muslin cloth, and the resulting filtrate was concentrated to dryness on a water bath set at 30°C. The concentrated extract was then placed in a drying cabinet to remove any remaining moisture. The weight of the extract was measured, and carefully stored in a tightly sealed container. To maintain its quality, the extract was stored in a refrigerator at 2.8°C until it was needed for future use.

***Drosophila melanogaster* stock and media.**

The Harwich strain of *Drosophila melanogaster* was cultured at the Africa Centre of Excellence in Phytomedicine Research and Development (ACEPRD) Fly Laboratory, University of Jos, Jos, Nigeria. *Drosophila melanogaster* were fed with standard yellow corn meal medium mixed with brewer's yeast (1 % w/v), agar (1 % w/v), and methylparaben (0.08 % w/v) and maintained under the prescribed temperature (23±1°C), relative humidity (60 %) and 12 hours dark/light conditions.

Determination of acute toxicity (LC₅₀)

LC₅₀ of the mixture of *Curcuma longa* and *Zingiber officinale* extract in *Drosophila melanogaster*. The acute toxicity of the mixture of *Curcuma longa* and *Zingiber officinale* was determined as described by Iorjiim et al. [17], with a slight modification. Briefly, the fruit flies were divided into eight groups each receiving different concentrations of the extract (i.e., 1 mg, 5 mg, 10 mg, 50 mg, 100 mg, 250 mg, 500 mg and 1000 mg) in replicates of three vials per group, with each vial containing sixty (60) fruit flies of both sexes. The different concentrations of the extract were incorporated into 5 g each of the flies' diet and then transferred into empty sterilized vials, with group one containing only 5 g of the diet as negative control. *Drosophila melanogaster* were then transferred into the vials containing the diets and extract. Mortality was recorded daily for seven (7) days and used to calculate the lethal concentration (LC₅₀).

LC₅₀ of standard toxicant (Isoniazid) in *Drosophila melanogaster*.

The fruit flies were divided into eight groups in replicates of three vials per each group with each vial containing 60 flies of both sexes. Different concentrations of isoniazid (5 mg, 12.5 mg, 25 mg, 50 mg, 125 mg, 250 mg, 500 mg and 1000 mg) were incorporated into 5 g of each of the fruit flies' diet and then transferred into sterilized empty vials with group one containing only 5 g of the diet as negative control. The flies were then transferred into the vials containing the diets and test drug. Total mortality was recorded daily for seven days.

Survival assay. Four groups of 1-4 day old *Drosophila melanogaster* were obtained, with each group containing five replicate vials consisting of 60 *Drosophila melanogaster* of both sexes. The fruit flies were then exposed to 25, 50, 75 and 100 % (1.20, 2.40, 3.60 and 4.81 mg respectively) of the LC₅₀ of INH per 5 g of fly diet for 14 days. A negative control group containing only 5 g of the fly diet was also set up. Mortality was recorded daily for a period

of 14 days, and the diet changed at 3-day intervals with the same dose of INH incorporated in each new diet. A Kaplan-Meier survival curve was then plotted. The same set up was performed for the extract.

In vitro antioxidant assay. The antioxidant activity (free radical scavenging activity) of the extract on the stable radical 2,2-Diphenyl-1-picrylhydrazyl (DPPH) was determined according to the method described by Ojerinde et al. [18]. Briefly, 12.5 mg of the extract was dissolved in methanol using a 25 mL volumetric flask. Different concentrations of the extract (500, 250, 125, 62.50, 31.25, 15.62, 7.8125, 3.91, 1.95 and 0.98 $\mu\text{g/mL}$) and the standard (Silymarin) were prepared. 2 mL of each prepared concentration was mixed with 4 mL of 50 μM DPPH solution in methanol. The experiment was performed in triplicate.

The absorbance was measured at 515 nm on a UV-Vis spectrophotometer (Shimadzu, UV-1620PC, Japan). The difference in absorbance between the test and the control (DPPH in methanol) was calculated and expressed as a percentage (%) scavenging of DPPH radical.

The IC_{50} value, defined as the concentration of the sample leading to 50 % reduction of the initial DPPH concentration, was calculated from the separate linear regression of the mean of the antioxidant

activity against concentration of the test extract ($\mu\text{g/mL}$).

RESULTS

Acute toxicity determination. LC_{50} of the extract of *Curcuma longa* and *Zingiber officinale* was estimated to be 512.6 mg/5g diet while that of Isoniazid was 4.813 mg/5g diet (Figures 1 and 2).

Survival assay. Results of the Survival Assay showed a survival rate of 78.2 % in *Drosophila melanogaster* at the lowest dose of isoniazid (0.0012 g/5 g diet. At 0.0024 g/5g diet, survival rate was 66.5 %, 58.1 % at dose of 0.0036 g/ 5g diet; survival rate was 25 % at the highest dose (0.0048 g/5 g diet) (Figure 3).

Survival rate in *Drosophila melanogaster* administered the extract was 90 % at the lowest dose (0.0641 g/5 g diet). Survival rate was observed to be 71.6 % at the dose of 0.128 g/5 g diet, 44.5 % at the dose of 0.1922 g/5 g diet and 25 % at the highest dose of extract (0.2563 g/5 g diet) (Figure 4).

Determination of antioxidant activity. Antioxidant Activity of Silymarin Using 2,2-Diphenyl-1-picrylhydrazyl (DPPH) was determined to be IC_{50} = 121.4 $\mu\text{g/mL}$. IC_{50} of extract was determined to be 103.5 $\mu\text{g/mL}$ (Figures 5 and 6).

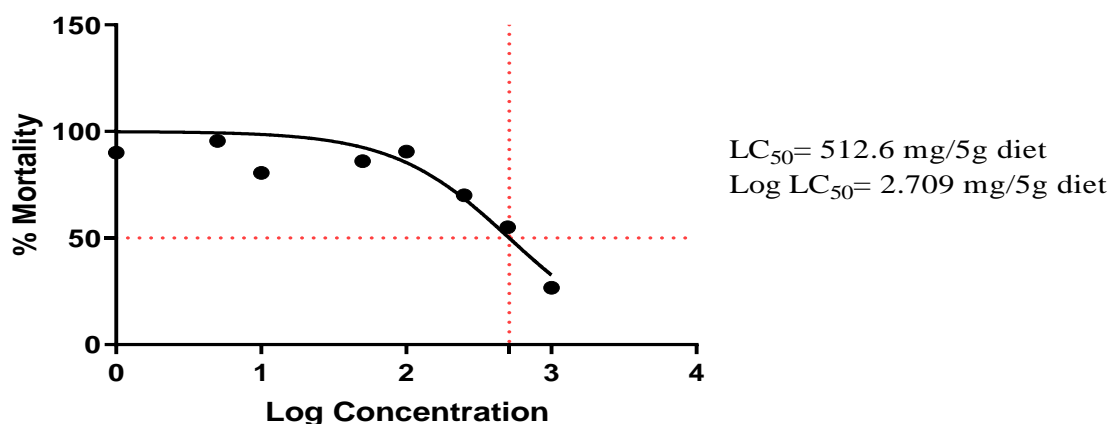


Figure 1. LC_{50} of aqueous extract of the rhizomes of *Curcuma longa* and *Zingiber officinale* in *Drosophila melanogaster*

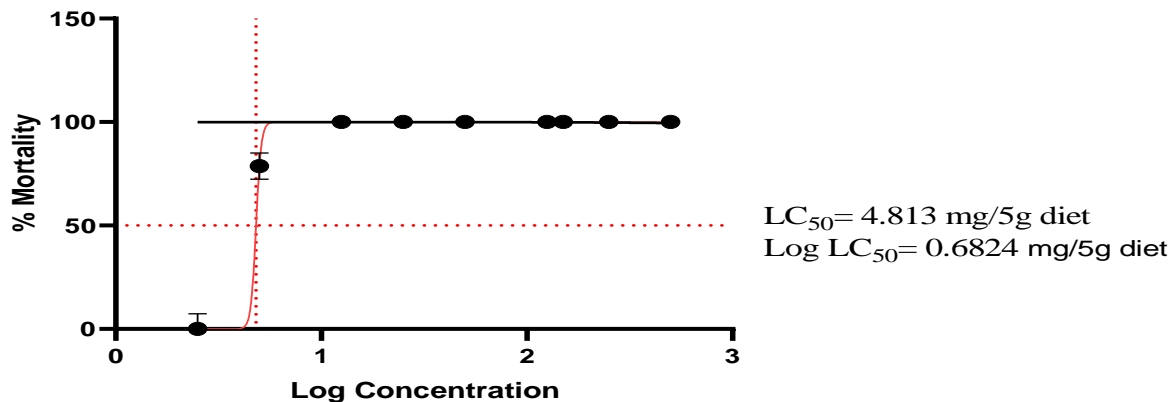


Figure 2. LC₅₀ of Isoniazid Using *Drosophila melanogaster*

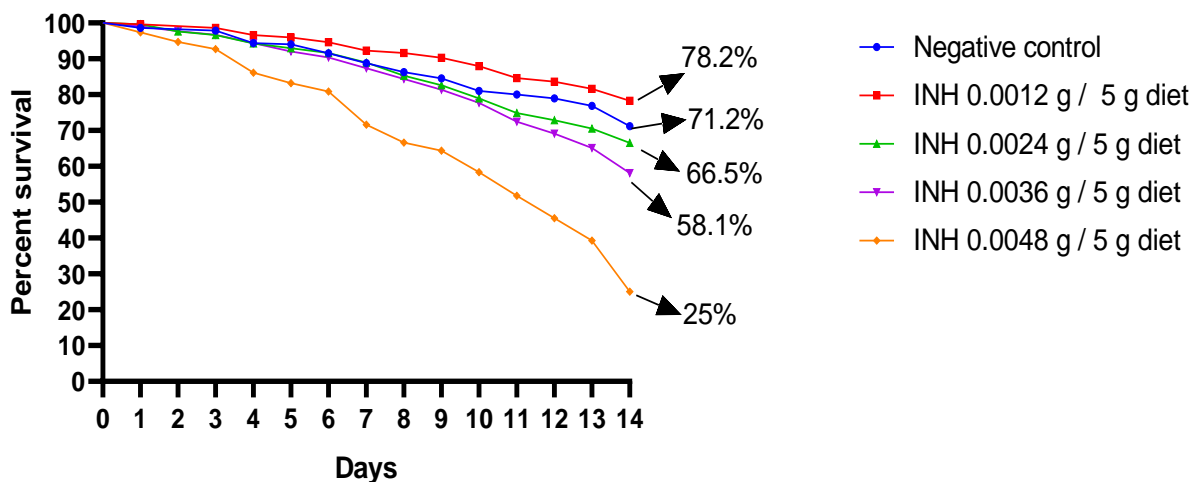


Figure 3. 14-day Kaplan-Meier Survival Curve of Isoniazid-triggered Lethality in *Drosophila melanogaster*

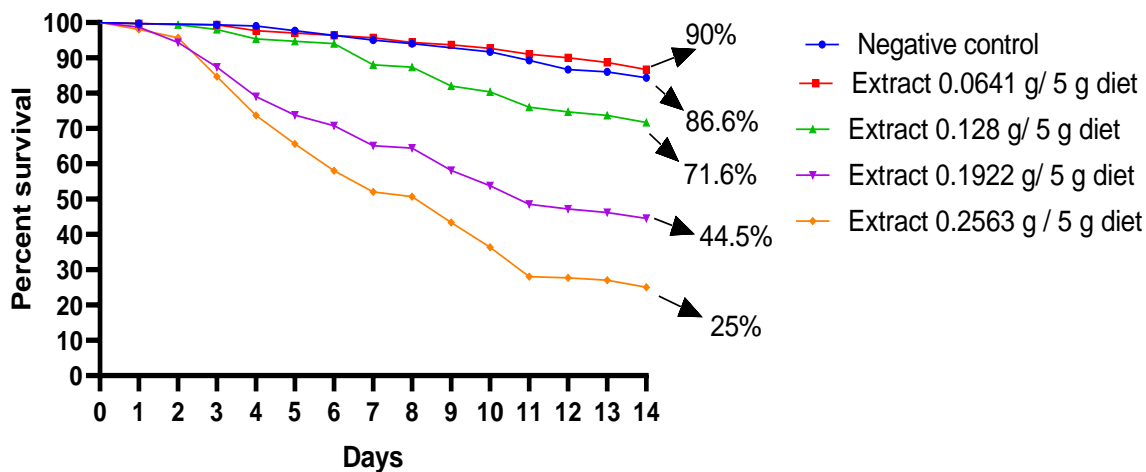


Figure 4. A 14-day Kaplan-Meier Survival curve of *D. melanogaster* exposed to different concentrations of *Curcuma longa* and *Zingiber officinale* extract.

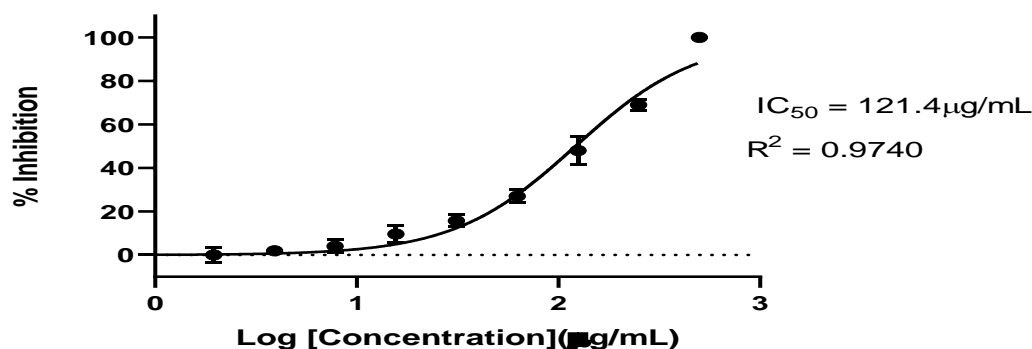


Figure 5. Antioxidant activity of silymarin using 2,2-Diphenyl-1-picrylhydrazyl (DPPH)

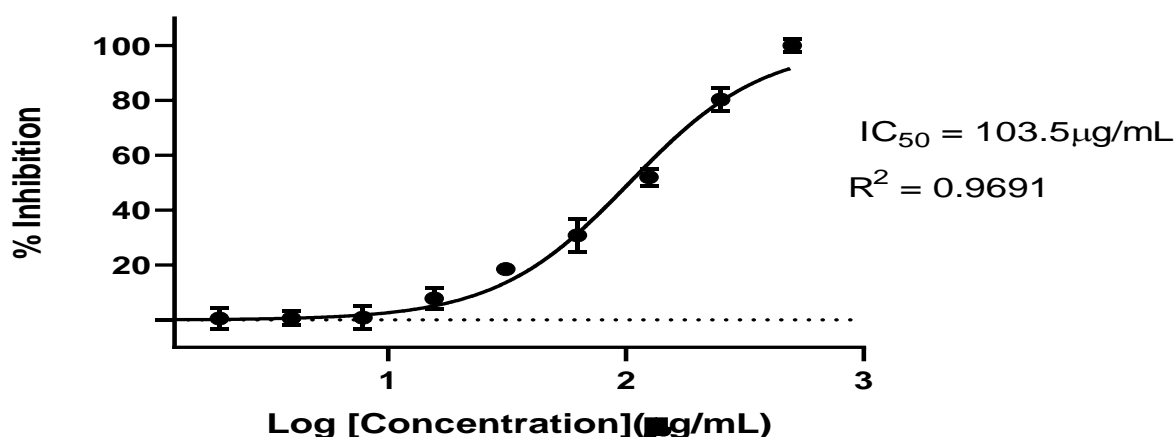


Figure 6. Antioxidant activity of *Curcuma longa* and *Zingiber officinale* extract using 2,2-Diphenyl-1-picrylhydrazyl (DPPH)

DISCUSSION

The determination of free radical scavenging activity of silymarin and the extract on the stable 2,2-Diphenyl-1-picrylhydrazyl (DPPH) showed they both possessed antioxidant activity; however, the extract showed even better antioxidant activity as seen by its lower IC_{50} . Koksai et al. [19] and Maizura et al. [20] reported similar findings for silymarin, a standard antioxidant, and a combination of *Curcuma longa* and *Zingiber officinale* extract respectively. This could be attributed to the presence of different phytochemicals in the extract such as alkaloids, tannins, saponins, flavonoids, anthraquinones, steroids, terpenoids, phenols, and glycosides which have different reported activities, including antioxidant activity as

previously reported by Suresh and Nagarajan [21] and Hossain and Nagooru [22]. While there are similarities to previous studies, our study evaluated the *in vitro* antioxidant effect of a mixture of *Curcuma longa* and *Zingiber officinale* extract and effect of the same extract on survival of *Drosophila melanogaster* exposed to isoniazid. Flavonoids, alkaloids and tannins found in *Curcuma longa* and *Zingiber officinale* have been reported to have an ability to effectively neutralize reactive oxygen species by scavenging hydroxyl radicals, superoxide anions, and lipid peroxyl radicals [23-26]. Consequently, it was reasonable to anticipate a similar antioxidant effect of these rhizomes in our study. Alkaloids, as detected in our study, have

always attracted scientific interest due to their effects.

An increased antioxidant defense system is usually correlated decrease in oxidative stress [27]. *Drosophila melanogaster* administered the extract showed higher survival at the lowest dose when compared to *Drosophila melanogaster* administered diet only. This apparent adaptive response was possibly induced by the mild increase of ROS at the lower concentrations of extract, leading to an increase in the activities of these biomarkers in the *Drosophila melanogaster*, thus improving its antioxidant capacity. Pinho et al reported a similar finding in an earlier study [28].

As seen with INH, survival of the *Drosophila melanogaster* exposed to higher doses (compared to control) of the extract had decreased percentage survival over the treatment period. This observation is similar to a previous report on the effect of the inclusion of bitter kola on the levels of oxidative stress markers in *Drosophila melanogaster* where a lower concentration of bitter kola increased the survival rate of the fruit *Drosophila melanogaster* [29]. It is important to note that the effects of phytochemicals on fly survival can vary widely depending on the specific phytochemical, the concentration, the type of fly species, and the exposure method. This could possibly explain the observation at higher doses. Alkaloids, for example, found in some plants can be highly toxic to insects. Interestingly, many alkaloids can behave both as anti-oxidants and pro-oxidants, depending on conditions [30]. Certain phytochemicals can deter *Drosophila melanogaster* from feeding on plant tissues. To counter attacks, plants have been reported to produce secondary metabolites and proteins that have toxic, repellent, and/or antinutritional effects on the herbivores [31].

Acute toxicity test for both herbal extract and the drug was performed, with isoniazid showing an LC₅₀ less than 5 mg/kg and the

herbal extract more than 500 mg/kg. According to Teke and Kuete [32], substances with LC₅₀ below 5 mg/kg are classified to be highly toxic, while substances/extracts with LC₅₀ between 500 and 5000 mg/kg are classified as slightly toxic. *Curcuma longa* and *Zingiber officinale* which are components of the mixture investigated have been reported to be safe in animals [33, 34], though long-term chronic use of *Zingiber officinale* has been found to induce oxidative stress [34]. Acute toxicity testing can be affected by many factors, such as animal species and strain, age and sex, diet, food deprivation prior to dosing, temperature, season and experimental procedures [32]. As a matter of fact, it has been shown that life expectancy in *Drosophila melanogaster* can be easily altered by varying the ambient temperature — for instance, *Drosophila melanogaster* are significantly longer lived when raised at 18°C compared with 25°C [35]. A major challenge faced in the course of our study was unstable power supply which made it difficult to maintain the flies at 18°C throughout the period of the research.

Conclusion. In conclusion, this study has yielded valuable insights into the antioxidant properties of the herbal extract, accentuating its potential advantages. However, it underscores the necessity for additional research to fully understand the effects of specific phytochemicals and the factors that impact their activity, particularly at higher dosage levels. Furthermore, the findings highlight the significance of taking various variables into account when conducting toxicity assessments and recognizing the intricate relationship between phytochemicals and the survival of *Drosophila melanogaster*.

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