# Amaranth Leaf Extract Protects Against Hydrogen Peroxide Induced Oxidative Stress in Drosophila Melanogaster

**Johnmark Ndinawe**
Kampala International University
**Hellen W. Kinyi** ( hkinyi@kab.ac.ug )
Kabale University https://orcid.org/0000-0002-8007-3618

**Research note**

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# Abstract

### Objective

Amaranths leaves are rich in ascorbic acid and polyphenol compounds which have antioxidant activity. The aim of this study was to evaluate their *in vivo* antioxidant activity. The effect of consumption of Amaranth leaf extract on *in vivo* antioxidant activity, catalase enzyme activity and $H_2O_2$ induced oxidative stress in *Drosophila melanogaster* flies was assessed.

### Results

Consumption of Amaranth leaf extract was associated with increased survival on exposure to $H_20_2$ in a dose dependent manner in *Drosophila melanogaste*r flies.


# Introduction

Oxidative stress refers to an imbalance arising due to excess production of free radicals and reduced activity of antioxidants. Free radicals are atoms or molecules that contain unpaired electrons and react with other molecules by taking or giving electrons (1). They can be products of normal cellular metabolism or environmental stressors and have been implicated in the pathogenesis of many diseases (2, 3).

Antioxidant compounds neutralize harmful effects of free radicals by either preventing their formation or removing them before they can cause damage to cellular structures (4). Consumption of fruits and vegetables rich in antioxidants has been linked to reduction in the incidence of oxidative-stress related diseases such as; cancer, diabetes, neurodegenerative diseases, inflammation as well as cell and cutaneous aging issues (5).

Amaranths, the generic name by which the vegetables of the genus *Amaranthus* are referred to are widely distributed short-lived herbs that occur in temperate and tropical regions. The *Amaranthus* species is one of the few plants from which leaves are eaten as a vegetable while the seeds are eaten as cereals(6). The *in vitro* antioxidant activity of Amaranths leaves has been well documented and attributed to both vitamin C and polyphenol compounds found in extracts (7–9) The specific objective of this work was to evaluate the *in vivo* antioxidant protective activity of Amaranths in a *Drosophila melanogaster* model of oxidative stress.

# Main Text

# Methods

The model used for the study was the $w^{1118}$ (*white*) strain of *Drosophila melanogaster*, a gift from Dr. Isabel Palacios (UK) and maintained at the Institute of Biomedical Research, Kampala International

University Western Campus, Ishaka-Bushenyi, Uganda. They were fed on cornmeal-yeast-agar medium (10) and maintained at $25^0$C under a 12/12 hour light and dark cycle in a digital fly incubator. From the stock population, virgin females and young males were placed on fresh food and allowed to mate. Fresh eggs were collected, and dated to ensure that the dates of birth are synchronized for all flies. From these, 360 male adult flies were collected and divided into 3 groups to be fed on: normal food (NF), Amaranth extract, and ascorbic acid (ASA) groups. The NF (180 flies), was fed on the standard cornmeal diet, the Amaranth group (180 flies) fed on the Amaranth-fly food mixture and ASA group on ASA-fly food mixture. To determine the *in vivo* antioxidant activity, catalase enzyme activity and resistance to oxidative stress, groups of *Drosophila* flies were fed on the respective diets for 5 days (11).

# Preparation of experimental food

Amaranth leaves used in the study were purchased from a vendor in the Central market of Ishaka town of Bushenyi Municipality of Western Uganda. Extraction was done as previously described (12). To prepare the fly food with Amaranth and ascorbic acid, 0.25mg and 0.5mg of Amaranth extract and ascorbic acid were each dissolved in 10mls of distilled water to obtain a concentration of 2.5mg/ml and 5mg/ml respectively. Each was separately added to 490mls of molten fly food and mixed thoroughly to obtain a mixture with Amaranth extract and Ascorbic acid each at a concentration of 0.05mg/ml and 0.1mg/ml. These mixtures were then poured in labeled vials and left to cool. The food was covered with cotton balls and stored at $4^0$C.

**Determination of**in vivo**antioxidant activity**

Ten flies each from the NF, Amaranth and ASA groups were separately anaesthetized on ice and homogenized in 100µl of cold 0.05% phosphate buffered saline tween (PBST) solution (pH 7.4). The homogenate was centrifuged at 4000g for 10 minutes and the supernatant collected and used for the determination of 2, 2-diphenyl-1-picrylhydrazyl radical (DPPH) scavenging activity. DPPH scavenging activity was determined as previously described (13). Briefly, 50µl of the fly homogenate was added to 5mls of 0.004% methanol solution of DPPH. This mixture was incubated in the dark at room temperature for 30 minutes. The absorbance of the mixture was then read at 517nm against a DPPH blank. The assay was carried out in triplicate and percentage of inhibition was calculated using the formula:

% Inhibition = [(AB-AA)/AB] *100

Where AB = Absorbance of blank; AA = Absorbance of test.

# Determination of resistance to hydrogen peroxide induced oxidative stress

The resistance to hydrogen peroxide-induced oxidative stress was conducted as previously described with minor modifications (14). Briefly, ten fruit flies from each of the NF, Amaranth and ASA groups were transferred to empty vials after 5days of feeding. The flies were starved for 6 hours to stimulate the

uptake of hydrogen peroxide. Afterwards, the flies were transferred to vials containing only filter paper soaked with 1% hydrogen peroxide in 5% sucrose solution. Flies alive/dead were recorded every day until the last one died.

# Catalase enzyme activity

The assay was carried out on *Drosophila* flies fed on the NF, Amaranth and ASA food for five days. Ten flies per group anaesthetized by chilling on ice were homogenized in ice cold phosphate buffer saline (pH 7.4) and centrifuged at 2,500rpm for 10 minutes. The resulting supernatant was collected and used for the catalase assay. The catalase activity was measured as previously described (15).

# Statistical Analysis

Data was analyzed using the free software Paleontological statistics software (PAST 3), expressed as mean and standard deviation and presented as graphs.

Data of DPPH scavenging and catalase activities were analyzed using factorial analysis of variance (ANOVA) followed by a Tukey's multiple comparison test.
Oxidative stress resistance was determined using Kaplan-Meier survival analysis with significance set at $P < 0.05$.

## Results

Homogenates from flies fed on food containing Amaranth or ascorbic acid at 0.05mg/ml respectively had significantly higher DPPH scavenging activity compared to homogenates from flies fed on normal food as shown in Fig. 1. However, the increase was not significant at 0.1mg/ml of Amaranth extract. The increase was similar to that observed when supplementing food with ascorbic acid.

There was no significant increase in catalase activity in *Drosophil*a flies fed on food supplemented with Amaranth leaf extract.

Supplementing food with Amaranth extract improved survival of flies exposed to $H_20_2$ in a dose dependent manner. The improvement at 0.1mg/ml was statistically significant and similar to the protection offered by the antioxidant ascorbic acid.

## Discussion

Green leafy vegetables such as Amaranths contain antioxidant vitamins including ascorbic acid, α-tocopherol and β-carotene. However, most of their antioxidant activity is from polyphenol compounds such as flavonoids, isoflavones, flavones, anthocyanins, catechin and isocatechin (16–18). Intake of compounds containing exogenous antioxidants is associated with an enhancement of endogenous antioxidant defenses (9, 11). This has also been seen in this study with the increase in the *in vivo* DPPH free radical scavenging activity seen in *Drosophila* flies fed on Amaranth leaf extracts. Exogenous

antioxidants can also modulate endogenous enzyme activity (5). For example, catalase activity is increased in *Drosophila* flies fed on mango tree leaf extracts (11). However, in this study the increase in catalase activity in flies fed on Amaranth extract was not significant.

Oxidative stress is an important factor in the pathogenesis of many diseases (4). Several plant extracts with antioxidant activity have been shown to ameliorate effects of oxidative stress induced by compounds such as paraquat and hydrogen peroxide (19–23). The concentration of Amaranths that provides *in vivo* protection to oxidative stress does not significantly increase general *in vivo* antioxidant activity (DPPH scavenging activity) or the catalase enzyme activity. The protection, therefore, may be due to the interaction of the extract with other endogenous biochemical pathways which further studies could address.

In conclusion, our study demonstrates that the ethanolic extract of Amaranth leaves offer protection against hydrogen peroxide-induced oxidative stress *in vivo* in a dose dependent manner.

# Limitations

These results are limited to male flies. Studies on female flies would provide more information on any gender related variations. Although Amaranths are widely consumed by humans, this study has been done in a lower invertebrate model and there is need for further testing in a vertebrate model before the results can be extrapolated to humans. The results are also limited to hydrogen peroxide induced oxidative stress and catalase enzyme. They cannot be used to draw conclusions on other antioxidant pathways.

# Abbreviations

$H_20_2$
Hydrogen peroxide; ASA:Ascorbic acid; NF:Normal Food; DPPH:2, 2, Diphenyl 1picrylhydrazyl

# Declarations

### Ethical approval and consent to participate

This was acquired from the Kampala International University Ethics and Scientific review board. Consent to participate was not applicable for this study.

### Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on request.

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Not applicable

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### Consent to publish

All authors have consented

### Competing interests

The authors declare that they have no competing interests.

### Author's contribution

HWK conceptualized the research, HWK and NJM designed the study, NJM collected the data; HWK and NJM conducted data analysis and interpretation.

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# Figures

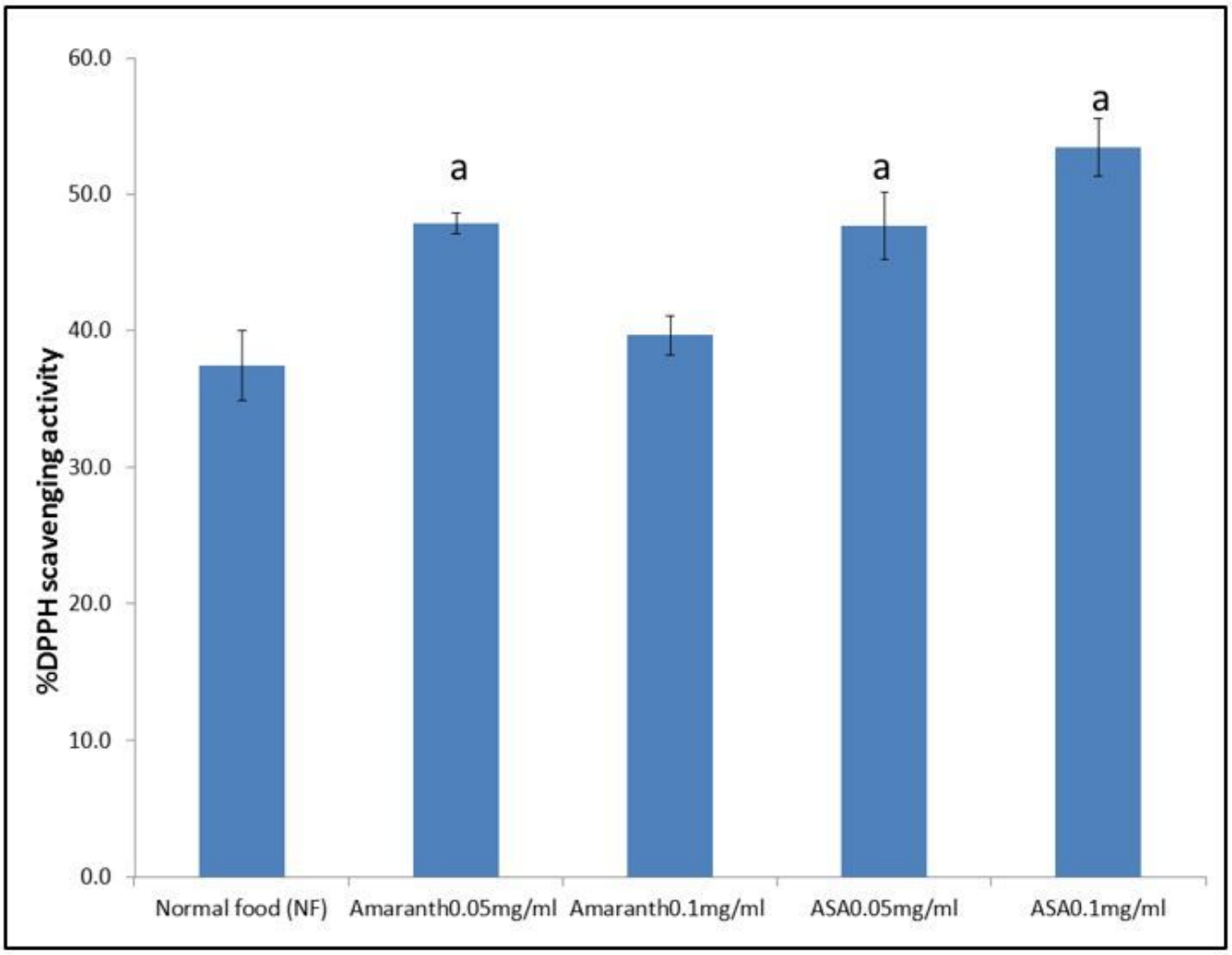
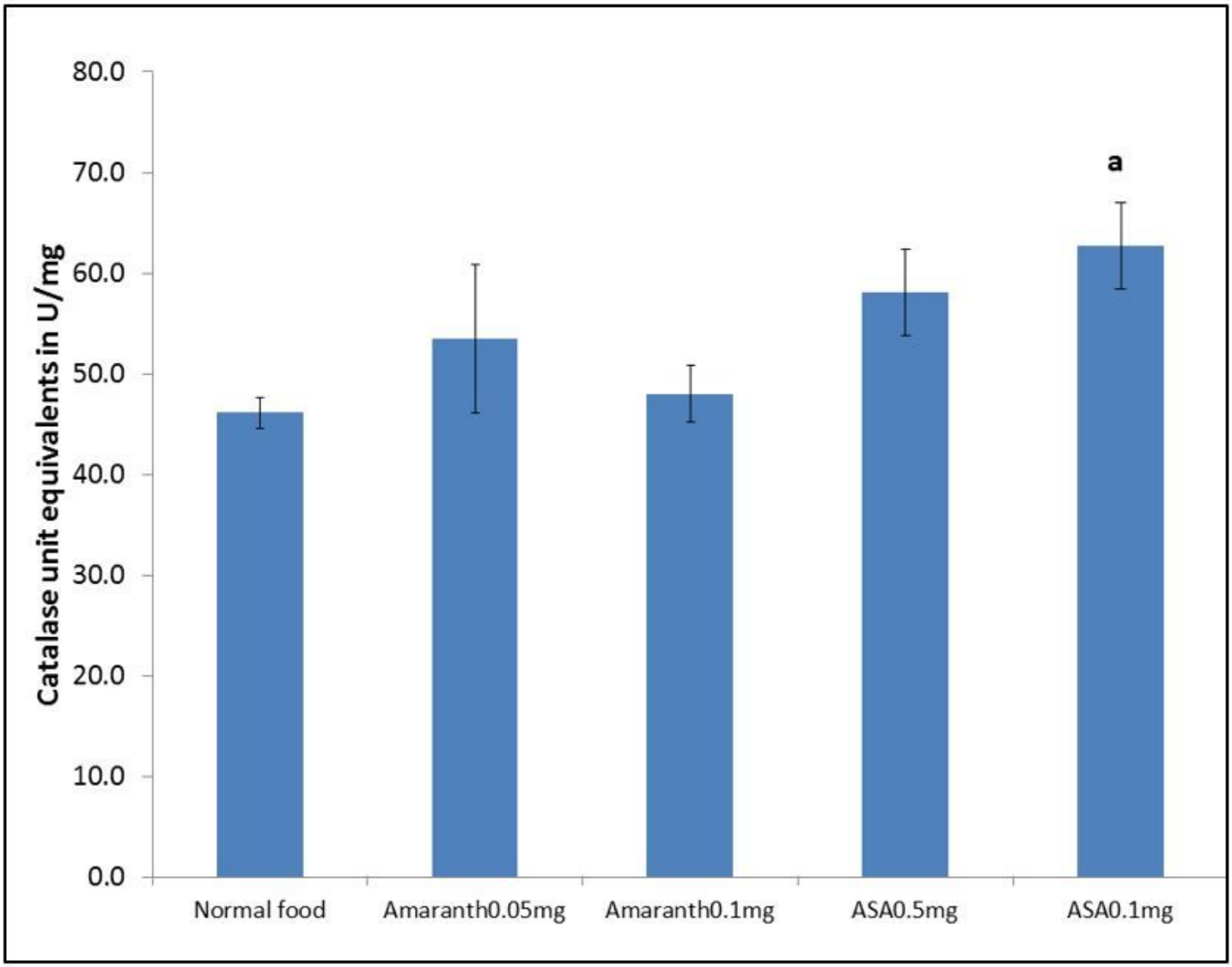


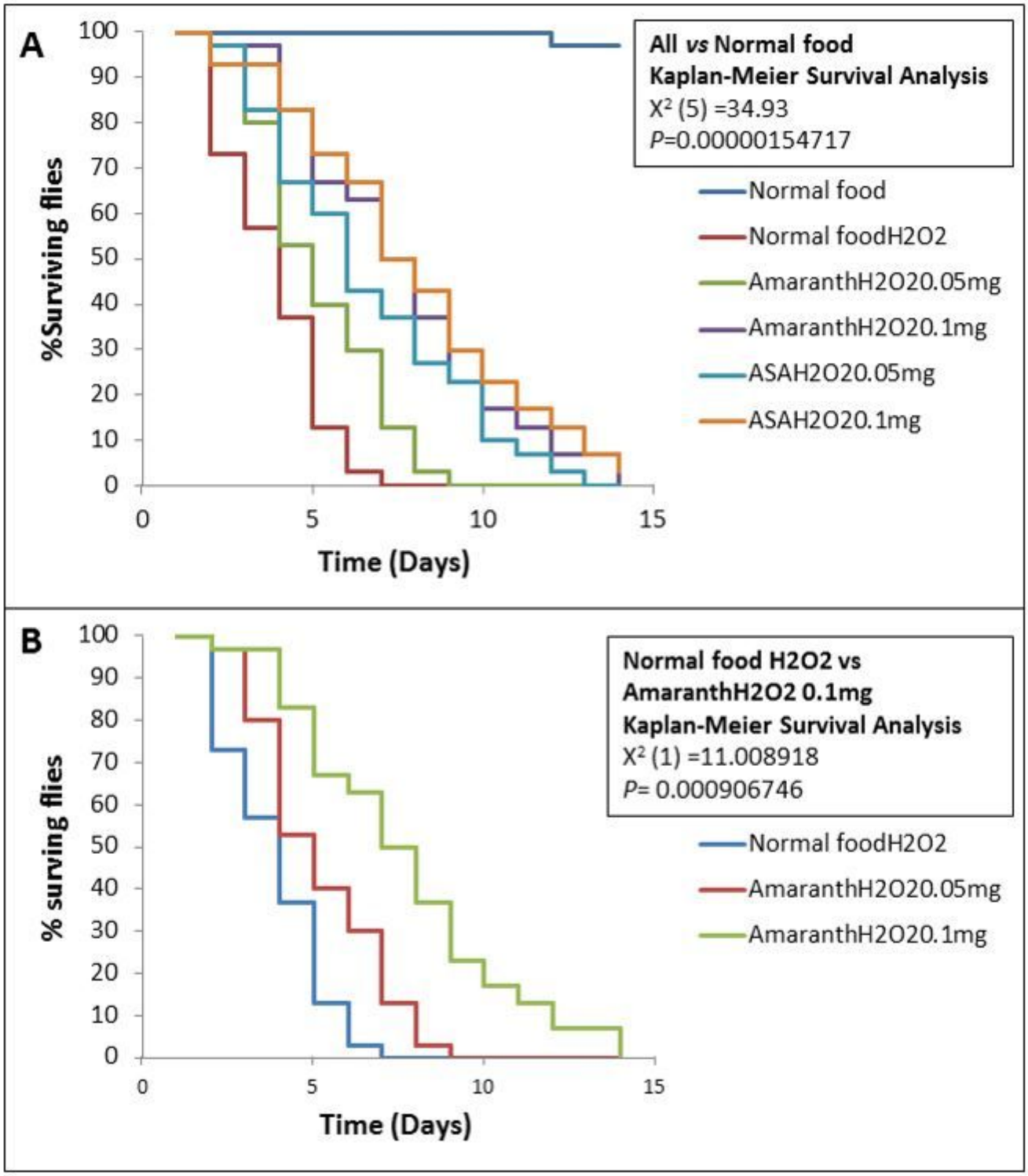

**Figure 1**

In vivo DPPH scavenging activity. Percentage DPPH scavenged by fly homogenates after feeding in experimental or control food for 5days. a P< 0.05 (Tukey's vs normal food)




## Figure 2

In vivo catalase activity. Catalase unit equivalents of fly homogenates after feeding in experimental or control food for 5days. a P< 0.05 (Tukey's vs normal food)




## Figure 3

Survival of flies exposed to hydrogen peroxide. A) All flies exposed to hydrogen peroxide showed reduced survival. B) Supplementing food with 0.1mg/ml of Amaranths for 5days improved survival rate.