Short communication

The *In vivo* Antioxidant Protective Activity of *Mangifera indica* Cold Aqueous Leaf Extract in *Drosophila melanogaster*.

ABSTRACT

Objective: To evaluate *in vivo* antioxidant activity of *Mangifera indica* cold aqueous leaf extract

Methods: a number of 50 adult flies were exposed to graded concentrations of *Mangifera indca* cold aqueous leaf extract, 2.5 mg/10 g diet, 5 mg/10 g diet and 10 mg/10 g diet for 5 days.Each concentration was prepared in 200µl of distilled water and replicated five times. 10g diet with 200µl distilled water served as control. Mortality reading was taken at 24 hours interval. The flies were homogenized, centrifuged and the supernatant was used to assay for Glutathione-S-transferase (GST), Catalase (CAT) and Total thiol content.

Results: The % mortality of flies after 5 days showed 32.5 %, 0 %, 15.5 % and 37 % in the control (10g diet with 200µl of distilled water), 2.5 mg/10 g diet, 5 mg/10 g diet and 10 mg/ 10g diet respectively. There was elevation in total thiol content and high GST and CAT activity in 2.5 mg/10 g diet and 5 mg/10 g diet treated flies.

Conclusion: the 100% and 85% survival of 2.5 mg/10 g and 5 mg/10 g diet-treated flies respectively and increase of fly' s antioxidant system after 5days exposure at these concentrations may suggest protective activity of *Mangifera indica* in *D. melanogaster*.

Keywords

In vivo, Antioxidant activity, Cold aqueous extract, *Drosophila melanogaste, Mangifera indica,* Catalase ,Total thiol, Glutathione-S-transferase

1 Introduction

Antioxidants act as a defense mechanism that protect against deleterious effects of oxidative reaction produced by reactive oxygen species (ROS) in a biological system [1]. Overproduction of ROS and/or inadequate antioxidants has been implicated in the pathogenesis and complications of some disease conditions like diabetes, Alzheimer□s disease, cancer, atherosclerosis, arthritis, neurodegenerative disease, and aging process [2, 3]. Antioxidants have been reported to prevent oxidative damage caused by ROS by reacting with

free radicals, chelating, and catalytic metals and also by acting as oxygen scavengers [4,5] .Oxidative stress is characterized by imbalance between oxidant-producing systems and antioxidant defense mechanisms, resulting in excessive formation of reactive oxygen species (ROS). Excessive accumulation of ROS can damage bio-molecules, including lipids, proteins and nucleic acids [6].Thiol groups are important members of the antioxidant team and have been shown to destroy ROS and other free radicals by enzymatic and non-enzymatic mechanisms [7]. Total thiol groups of proteins are mainly responsible for their antioxidant response, and they can serve as a sensitive indicator of oxidative stress [8.]

Mango (*Mangifera indica L.*) is a juicy stone fruit belonging to the family of Anacardiaceae in the order of Sapindales and is grown in many parts of the world, particularly in tropical countries; Mango is now commercially grown in more than 87 countries [9]. It has been well documented that mango fruits are an important source of micronutrients, vitamins and other phytochemicals. Moreover mango fruits provide energy, dietary fiber, carbohydrates, proteins, fats and phenolic compounds [9], which are vital to normal human growth, development and health [10]. Various parts of mango are used for more than thousands of years as wide variety of ethno medicinal use [11]. Mango extracts from leaves, fruit, seed kernel, fruit pulp, roots, bark and stem bark have been used extensively for medicinal purposes in many countries[12]. The ethno-medical use of mango stem bark aqueous extract in Cuba has been documented widely [5]. It has been extensively used in cancer, diabetes, asthma, infertility, lupus, prostatisis, prostatic hyperplasia, gastric disorders, arthralgies, mouth sores and tooth pain [12]

Drosophila melanogaster, known colloquially as the fruit fly, remains one of the most commonly used model organisms for biomedical science. For more than one hundred years, the low cost, rapid generation time, and excellent genetic tools have made the fly indispensable for basic research. The addition of numerous molecular tools has allowed the model system to keep up with the latest advances. In this issue, various authors provide examples of how Drosophila is currently being used, and what directions they think the system is moving in. From human disease modeling to the dissection of cellular morphogenesis and to behavior and aging.[13]

The *in vitro* antioxidant activity of *Mangifera indica* plant extract has been established but with no or little information on its *in vivo* antioxidant activities. The specific objective of this work was to evaluate the *in vivo* antioxidant protective activities of *Mangifera indica* in *Drosophila melanogaster*.

2 Material and methods

2.1 Chemicals

All chemicals used were of analytical grade. Distilled water purchased from Africa Centre of Excellence in Phytomedicine Research and Development, Jos, plateau State. Randox Protein kit was purchased from Medicom,Jos Plateau State. 1-chloro-2,4-dinitrobenzene,(CDNB) and 5,5'-dithiobis(2-nitro-benzoic acid) (DTNB) were purchased from Sigma Aldrich (St Louis, MO).

2.2 Plant Collections

Mangifera indica leaf was collected from University of Jos Senior staff quarter, Jos North, Plateau State, Nigeria. The leaves were air dried using room temperature for 7 days, and then pulverized to powder using a commercial grinding machine. It was kept in an air tight container before extraction. The extraction was carried out by maceration method using 1:10 of plant material to distilled water for 72hrs. Filtered and the filtrate was concentrated to dryness using freeze dryer.

2.3 *In vivo* Antioxidant Assay

In vivo antioxidant assay was carried out by exposing (ingestion) 50 flies to graded concentration (2.5mg, 5mg, and 10mg) of cold aqueous leave extracts for 5 days.Each concentration was prepared in 200µl of distilled water and replicated 5 times. 10g diet with 200µl of distilled water served as control. At the end of the exposure period (5 days), the flies (50) from each group of control and cold leaves extract-treated flies were anaesthetized in ice, weighed, and homogenized in 0.1 M phosphate buffer, pH 7.0 (1 mg: 10 µL), and centrifuge for 10 min at 4000 rpm (temperature, 4 °C). The supernatants obtained were used to determine the activities of Catalase (CAT), Glutathione-S-transferase (GST) and Total thiol content.

2.3.1 Total thiol determination: Total thiol content was determined using the method of Ellman [14]. The reaction mixture contained 510uL potassium phosphate buffer (0.1 M, PH 7.4), 25 uL of sample as well as 30uL of DTNB (10 mM). After incubation for 30 min at room temperature, the absorbance was measured at 412 nm and used to calculate the sample total thiol levels (in mmol/mg protein) using 35ul of GSH as standard.

2.3.2 Glutathione-S-transferase (GST) activity: The activity of glutathione-S-transferase (GST; EC 2.5.1.18) was determined by the method of Habig and Jacoby [15] using 1-chloro-2,4-dinitrobenzene (CDNB) as substrate. The assay reaction mixture contained 600uL of solution A (20 uL of 0.25 M potassium phosphate buffer, pH 7.0 with of 2.5 mM EDTA, and 510 uL of 0.1 M GSH at 25 °C), 60 uL of sample (1:5 dilution) and 30uL of 25 mM CDNB. An increase in absorbance was measured at 340 nm for 2min at 10 s interval using spectrophotometer (Jenway).

The data were expressed in mmol/min/mg of protein using the molar extinction coefficient (ε) of 9.6 mM1 cm1 of the coloured GS−DNB conjugate formed by GST.

2.3.3 Catalase (CAT) activity: The measurement of catalase (CAT; EC 1.11.1.6) activity was followed by a procedure described by Aebi [16]. The reaction mixture containing 100 mL of potassium phosphate buffer, pH 7.0, 194mL of 300 mM H2O2 to form solution A. 10 uL of sample was reacted with 590ul of solution A and monitoring the clearance of H2O2 at 240 nm at 25 °C. The decrease in H2O2 was monitored for 2 min (10 s intervals), at 240 nm using a UV– visible spectrophotometer (Jenway) and expressed as mmol of H2O2 consumed/min/mg of protein.

2.4 Statistical analysis

The data was expressed as mean±SEM (standard error of mean), and the statistical analysis was carried out using one-way analysis of variance (ANOVA) followed by Turkey's post-hoc test. The results was considered statistically significant at $p < 0.05$.

3. Results and discussion

3.1 Five (5) Days Mortality of *Mangifera indica* cold aqueous leaf extract-treated Flies

Mortality result of Flies exposed to *Mangifera indica* cold aqueous leaf extract was high at 10 mg / 10 g diet while the least mortality was recorded in 2.5 mg /10 g diet. 32.5 % mortality was recorded in the control while 0 %, 15.5 % and 37 % was recorded in 2.5 mg /10 g diet, 5 mg /10 g diet and 10 mg/10 g diet of *Mangifera indica* cold aqueous leaf extract respectively. There was significant difference ($p<0.05$) comparing 2.5 mg/10 g diet of *Mangifera indica* cold aqueous leaf extract to the control group. This suggests that 2.5 mg/10 g diet and 5 mg /10 g diet of *M. indica* may have protective activity in *D. melanogaster.* The % mortality result is presented in figure 1

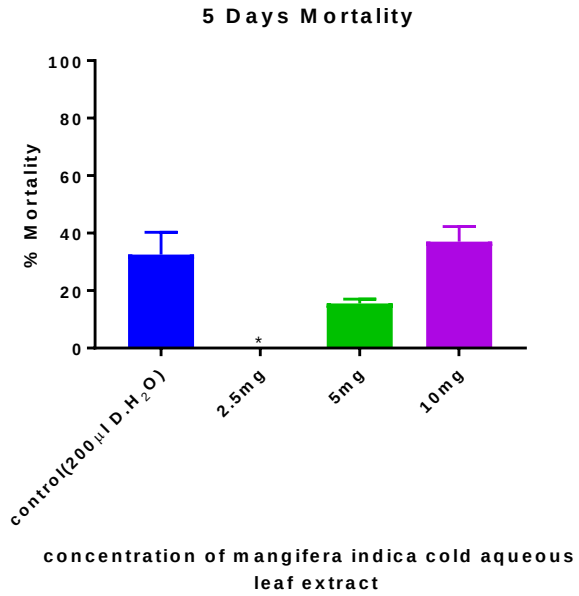

Fig 1: 5 Days %Mortality of *Mangifera indica* cold aqueous leaf extract-treated Flies

3.2 Total Thiol contents and Glutathione-S-transferase(GST) Activity

The Thiol groups are important members of the antioxidant team and have been shown to destroy ROS and other free radicals by enzymatic and non-enzymatic mechanism[17]. Total thiol groups of proteins are mainly responsible for their antioxidant response, and they can serve as a sensitive indicator of oxidative stress [8]. Total thiols are composed of both intracellular and extracellular thiols. Intracellular thiols such as glutathione and thioredoxin play an important role in maintaining the highly reduced environment inside the cell[17]. Extracellular thiols are protein bound and are mainly disulfide proteins due to the oxidative environment. Total thiol status in the body, especially thiol groups present on protein are considered as major plasma antioxidants *in vivo* and most of them are present over albumin, and they are the major reducing groups present in our body fluids [18]. Total thiol groups are very susceptible to oxidation and considered as one of the most important plasma sacrificial antioxidants. When the cells are exposed to oxidative stress, thiol groups are the first antioxidants that are consumed [8]. We found decreased plasma total thiol levels in oxidative or nitro oxidative condition. The total thiol contents of flies treated with *Mangifera indica* cold aqueous leaf extract for 5 days ranged from 9.25±1.8- 24±2.3 Mmol/mgprotein. The highest total thiol contents was recorded in the 5mg-treated flies while the lowest total thiol contents was recorded in the 10mg-treated flies. There was significant difference ($p<0.05$) comparing only 5 mg-treated flies to the control flies. The total thiol result is presented in figure 2. The GST activity of *Mangifera indica* leaf cold aqueous extract-treated flies ranged from 0.49±0.11-1.08±0.27 µmol/min/mgprotein. The highest activity was recorded in 2.5 mg-treated flies while

the lowest activity was recorded in the controlled flies. There was elevation of GST activities in all the extract-treated flies but no significant difference ($p>0.05$) comparing to the controlled flies. The result is presented in figure 3. The result suggests that *Mangifera indica* leaf cold aqueous extract could improve the total thiol content and GST activity of flies.

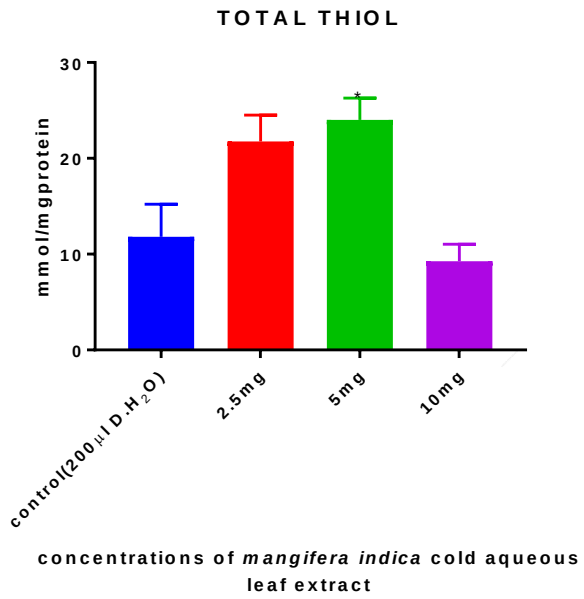

Fig2: Total Thiol contents of *Mangifera indica* cold aqueous leaf extract-treated Flies

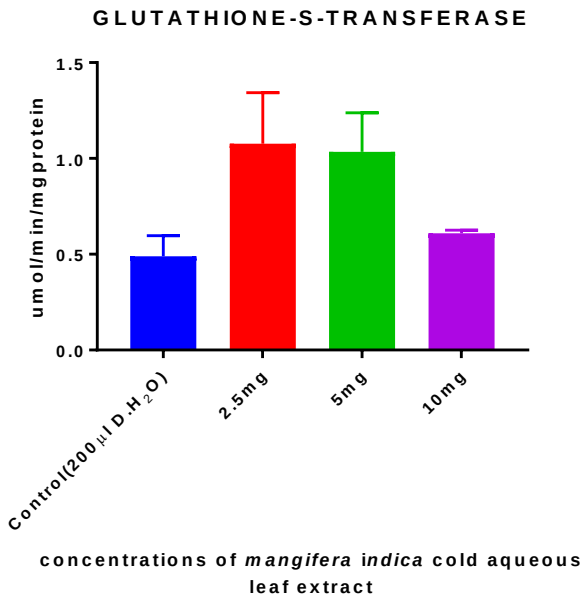

Fig3: GST activity of *Mangifera indica* cold aqueous leaf extract-treated Flies

3.2 Catalase (CAT) activity

To scavenge ROS (Reactive Oxygen Species), SOD (superoxide dismutase) is the first and most important enzyme of the antioxidant system, catalyzing the dismutation of superoxide anions to hydrogen peroxide (H_2O_2) and water. In the second step, CAT catalyzes the decomposition of H_2O_2 to water and oxygen [19]

The CAT activity of *Mangifera indica* leaf cold aqueous extract-treated flies ranged from 0.36±0.13-0.97±0.19 µmol/min/mgprotein. The highest activity was recorded in 2.5 mg-treated flies while the lowest activity was recorded in the controlled flies. There was elevation of CAT activities in all the extract-treated flies but no significant difference ($p>0.05$) comparing to the controlled flies. The result is presented in figure 3. This result suggests that *Mangifera indica* leaf cold aqueous extract may improve the production of CAT to scavenge free radicals. Similar result was reported by Karuppanan *et al*., [20], who evaluated the *in vivo* antioxidant properties of *Mangifera indica* leaf in mice. Their result showed similar high level of CAT activity against free radical. This high activity of catalase in both *Drosophila melanogaster and* Mice may indicate high antioxidant activity of *Mangifera indica* leaf extract. Zhang, *et al*.[21], carried out a work on the effects of Rosemary extract on the lifespan and Antioxidant System of *Drosophila melanogaster*; their result showed that the CAT activity in each dosage group is higher than that of control group compared with control group. They concluded that a certain amount of extract of Rosemary can increase endogenous antioxidant activity(SOD, CAT) in Drosophila. Lu and Yeap Foo [22] studied *Salvia officinalis* (L.) for its antioxidant activity and polyphenol content and reported that rosmarinic acid and various catechols were responsible for the radical scavenging activity and caffeic acid was responsible for the xanthine oxidase (EC 1.17.3.2) inhibition . Zhao *et al*. [23] investigated the

antioxidant activity of *Salvia miltiorrhiza* and *Panax notogensing* . The results showed that *Salvia miltiorrhiza* had a higher reducing power and scavenging activities against free radicals, including superoxide and hydroxyl radicals, although it showed weak hydrogen peroxide scavenging . Furthermore, Javanmardi *et al*.[24] tested the Iranian *Ocimum sp.* accessions to determine the antioxidant activities and total phenolic contents and demonstrated that the antioxidant activity increased in parallel with the total phenolic content .Evaluation of the pomegranate peel extracts to discover its antioxidant and antimutagenic activities using different solvents such as ethyl acetate, acetone, methanol and water has been carried out [24] . The results showed the highest anti-mutagenic and the lowest antioxidant activity in the water extract. Catalase activity result of *Mangifera indica* cold aqueous leaf extract-treated flies is presented in figure 4.

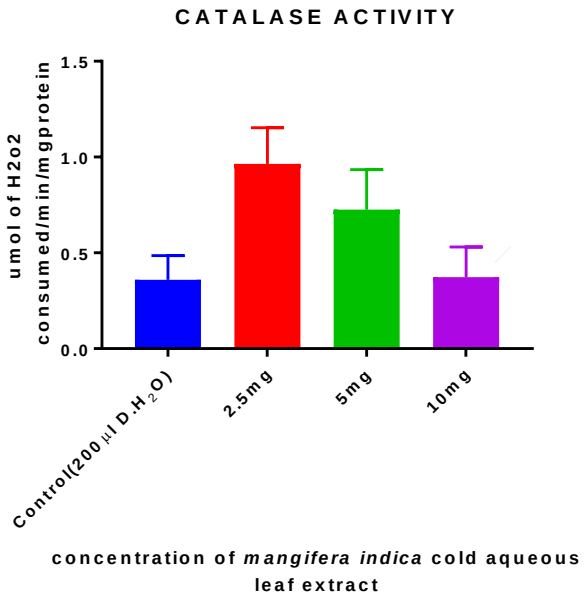

Fig 4: CAT activity of *Mangifera indica* cold aqueous leaf extract-treated Flies

Conclusion and Recommendations

Conclusion

From the results, 2.5 mg/ 10 g diet and 5 mg/10 g diet of *Mangifera indica* aqueous leaf extract-treated flies showed low % mortality and high level of total thiol content, GST activity and Catalase activity compared to the control and this may be due to its scavenging power. Therefore, it can be concluded that *Mangifera indica* cold aqueous leaf extract at these concentrations may have high activity against free radicals in *D. melanogaster*.

Recommendation

Characterization and *In vivo* antioxidant screening of *Mangifera indica* cold aqueous leaf extract Fractions should be carried out in *Drosophila melanogaster* to determine the phyto-chemicals and bioactive components of the extract responsible for its antioxidant activity.

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