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Ethanolic Extract of *Luffa Cylindrica* L. Sponge Ameliorates 4-Vinyl Cyclohexene Monoepoxide (VCM) – Induced Oxidative Stress in *Drosophila Melanogaster*

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Abstract

*Luffa cylindrica (Curcurbitaceae), is a plant whose leaves and fruits (sponge) are employed for the management of pain, inflammation as well as diabetes mellitus. This study investigated the ameliorative effects of Luffa cylindrica sponge on 4-**Vinyl Cyclohexene Monoepoxide** (VCM)–induced oxidative stress using Drosophila melanogaster model. The Luffa cylindrica sponge was crushed and extracted with 50% ethanol to facilitate ethanolic extract, Luffa cylindrica (LUC). The antioxidant activity of the extract was investigated via 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay. Both genders of Drosophila melanogaster aged between 1 and 3 days old were treated with vehicle (ethanol, negative control), gallic acid (0.1 mM), LUC (5 mg/10 g diet), LUC (10 mg/10 g diet), VCM (1 mM), (1 mM VCM + 0.1 mM gallic acid), (1 mM VCM + 5 mg/10 g diet) and (1 mM VCM + 10 mg/10 g diet) in the diet for 5 days of treatment. Afterwards, the survival and negative geotaxis assays were determined followed by the determination of the activities of total thiol, catalase, glutathione S-transferase and acetylcholinesterase. The results showed that LUC demonstrated significant antioxidant activity with 50 % Inhibitory Concentration (IC_{50}) of 155.20 µg/mL compared with gallic acid (IC_{50} = 23.2 µg/mL). Furthermore, our results suggest that LUC significantly ($p<0.05$) regenerated VCM–induced depletion of total thiol and inhibition of catalase, glutathione S-transferase and acetylcholinesterase activities. Our findings revealed that Luffa cylindrica sponge extract possessed ameliorative effects on VCM–induced Drosophila melanogaster by increasing the activities of in vivo antioxidant enzymes. This study upheld the ethnomedical uses of Luffa cylindrica for the management of oxidative stress-related diseases.*

Keywords: Catalase, *Drosophila melanogaster*, Glutathione, *Luffa cylindrica*, and Oxidative stress.

INTRODUCTION

Inflammation and oxidative stress have been reported to associate with the development of chronic diseases including cancer, diabetes, cardiovascular diseases, and neurodegenerative diseases (Alzheimer's and Parkinson's diseases) (Biswas, 2016). Both inflammation and oxidative stress are strongly connected pathophysiological occurrences that are closely linked with one another (Biswas, 2016). Oxidative stress occurs as a result of an imbalance between

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the reactive species, Reactive Oxygen Species and Reactive Nitrogen Species (ROS & RNS) and antioxidant systems. (Ramos-Gonadeza *et al*., 2021). An increase in oxidative stress induces damage to the cellular structure and destroys tissues (Soomro, 2019). An injured tissue results in the production of inflammatory cytokines such as TNF-α and IL-6 which stimulate the production of free radicals (Velagapudi *et al*., 2018). Free radicals are highly reactive species that contain oxygen, and nitrogen that have the capacity to damage cells, proteins, and DNA in the body (Soomro, 2019). An uncontrolled production of these free radicals results in oxidative stress due to the imbalance between oxidants and antioxidants (Ramos-Gonadeza *et al*., 2021). Thereby, an increase in oxidative stress results in oxidative stress–derived inflammation which is adjudged a major mechanism in the pathogenesis and progression of chronic diseases (Soomro, 2019). However, the harmful effects of these free radicals on the protein, human DNA, and cells are prevented or inhibited by antioxidant agents via the donation of electrons, thereby reducing their activities (Biswas, 2016). Natural products have been reported by Si *et al*., (2021) to be a reservoir of antioxidant molecules that have the capability to remove the free radicals produced in the cells. Various classes of phytocompounds in plants including alkaloids, tannins, flavonoids, phenols, and terpenoids have been investigated to demonstrate good antioxidant activities, one of such plants is *Luffa cylindrica.*

Luffa cylindrica belongs to the family, Curcurbitaceae, a unique vegetable that belongs to a family of cucumber, known as a vegetable sponge or sponge gourd (Azeez *et al*., 2013). *Luffa cylindrica* is widely distributed in the tropics and sub-tropics, as a cultivated and naturalized plant (Al-snafi, 2019). Different parts of this plant have been found useful by the traditional medicinal practitioners for the treatment of inflammation, asthma, intestinal worms, chronic bronchitis, bladder haemorrhagia, jaundice, leprosy and menorrhagia (Khan *et al*., 2013). Various parts of *Luffa cylindrica* (leaves, fruits, pulp, seeds and flowers) have been reported to demonstrate significant anti-inflammatory activity (Muthumani *et al*., 2010; Kanwal *et al*., 2013). Antidiabetic activities of the seeds and fruits of *Luffa cylindrica* have been reported (El-Fiky *et al*., 1996; Akther *et al*., 2014), while the antimicrobial activities of the whole plant, seeds, leaves and fruits have been extensively studied with significant activities (Oyetayo *et al*., 2007; Indumathy *et al*., 2011). The seeds of *Luffa cylindrica* demonstrated anti-HIV activity in HIV-1 infected C8166 T-cell lines (Ng *et al*., 2011). The leaves of *Luffa cylindrica* showed anthelmintic activity against the earthworm (*Pheretima posthuman*) with good activity (Partap *et al*., 2012). Moreover, the leaves extract of *Luffa cylindrica* showed significant antioxidant activity when compared with the standard, ascorbic acid (Tripathi *et al*., 2016). Hepatoprotective effects of the fruits and leaves extracts of *Luffa cylindrica* were reported to protect rats against both paracetamol and erythromycin toxicity (Shete *et al*., 2011; Sharma *et al*., 2014). The extracts of the whole plant, leaves and seeds of *Luffa cylindrica* demonstrated significant anticancer activity against *Brine Shrimp* and various cancer cell lines (Ng *et al*., 1992; Nassr-Allah *et al*., 2009; Sharma *et al*., 2015; Garai *et al*., 2018), while the fruit peels of the plant demonstrated significant antiemetic activity (Kanwal *et al*., 2013).

In addition, the immunomodulatory activity of the fruits, leaves and stems of *Luffa cylindrica* was reported with significant activity (Khajuria *et al*., 2007), while the wound healing effect of the whole plant was investigated and studied using excision wound model in rats (Abirami *et al*., 2011). The oxytoxic effect of the aqueous leaves extracts of *Luffa cylindrica* corroborated the ethnomedical application of the plant to hasten contraction during delivery and expulsion of retained placenta (Kamatenesi-Mugisha *et al*., 2007). Also, the seeds extract of *Luffa cylindrica* showed significant bronchodilator activity in Guinea pig trachea when compared with the standard, aminophylline (Muthumani *et al*., 2010).

Moreso, Four glycoproteins including luffin-a, luffin-b, luffin-PI and luffacyclin were isolated from the seeds of *Luffa cylindrica* with cytotoxic, abortifacient and antifungal activities (Ng *et al*., 1992; Zhang *et al*., 1998; Parkash *et al*., 2002; Ng *et al*., 2011). p-coumaric acid, 1-O-feruloyl-β-D-glucose, 1-O-p-coumaroyl-β-D-glucose; 1-O-caffeoyl-β-D-glucose; 1-O-(4-hydroxybenzoyl)glucose; diometin-7-O-β-D-glucuronide methyl ester; apigenin-7-O-β-D-glucuronide methyl ester; and luteolin-7-O-β-D-glucuronide methyl ester were isolated as antioxidant constituents from the fruits of *Luffa cylindrica* (Du *et al*., 2006). In addition, saponins of gypsogenin, olaeanolic acid, gypsogenin lactone, aegyptinin A, aegyptinin B, ginsenosides – Re, ginsenosides – Rg 1, lucyoside 1, 3-O-β-D-glucopyranosyl hederagenin, 3-O-β-D-glucopyranosyl oleanolic and lucycoside A to P were isolated from *Luffa cylindrica* extracts (Yoshikawa *et al*.,1991; Xiong *et al*., 1994). Furthermore, two triterpenoids, Oleanolic acid and Echinocstic acid were isolated from the seeds of *Luffa cylindrica* with significant immunomodulatory activity (Khajuria *et al*., 2007).

Unfortunately, little is known about the ameliorative potentials of the *Luffa cylindrica* sponge on 4-Vinylcyclohexene monoexpoxide-induced oxidative stress in *Drosophila Melanogaster*. 4-VinylcyclohexenMonoepoxide (4-VCM) has been reported to induce oxidative stress in *Drosophila melanogaster* by altering the activities of antioxidant enzymes such as catalase, GST as well as the level of total thiol in these flies (Abolaji *et al*., 2014). The versatility and diversity of *Drosophila melanogaster* as a model in different biological assay have been associated with the similarities in genetic codes in humans (Bourg, 2001). The antioxidant enzymes such as superoxide dismutase, catalase, glutathione reductase and glutathione S-transferase present in humans for defending itself against oxidative stress are also found in *Drosophila melanogaster* (Bourg, 2001). This makes the flies useful in the study of different classes of diseases in which oxidative stress is implicated (Balogun *et al*., 2021). This study therefore, aimed to induce oxidative stress in *Drosophila melanogaster* using 4-Vinylcyclohexenemonoepoxide (VCM) and seeking to understand if the *Luffa cylindrica* sponge would ameliorate the toxic effects of the ovotoxic VCM.

MATERIALS AND METHODS

Source of the Chemicals Employed

Gallic acid, reduced glutathione (GSH), 1-chloro-2,4-dinitrobenzene (CDNB), acetylcholine iodide, 5'5'-dithiobis (2-nitrobenzoic acid (DTNB), were purchased from Sigma Chemical Company, St Louis, USA.

Drosophila melanogaster Stock and Culture

Drosophila melanogaster (Harwich strain) fruit flies were obtained from the fly laboratory of Africa Centre of Excellence in Phytomedicine Research and Development (ACEPRD), University of Jos, Nigeria. The flies were reared and maintained on cornmeal medium mixed with brewer's yeast (1% w/v), agar (1% w/v) and methyl paraben (preservative, 0.01% w/v) at 25° ± 1 ˚C and 60–70% relative humidity and were kept with a 12 hours dark/light cycle.

Collection, Preparation and Extraction of Plant Materials

Dried fruits of the *Luffa cylindrica* plant were purchased from Traditional medicine practitioners at a local market (Jos Terminus Market), in March 2021 and identified at the Department of Pharmacognosy and Traditional Medicinal, University of Jos. The sponges were obtained manually by peeling off the skin of the dried fruit and beating off the seeds. Thereafter, the sponges were reduced in size and extracted with 50% ethanol for 72 hours. The extract was filtered and dried *in vacuo* to afford dried extract of *Luffa cylindrca* (LUC).

1,1-Diphenyl-2-picrylhydrazyl Radical (DPPH) Assay
The antioxidant activity (free radical scavenging activity) of extract on the stable radical 1,1-diphenyl-2-picrylhydrazyl (DPPH) was determined according to the method described in (Odumosu *et al*., 2015; Ojerinde *et al*., 2022;).

***Luffa cylindrica* Exposure and Survival Rate Analyses**
Survival assay and toxicity were investigated using the method described by Balogun *et al*. (2021). Briefly, *D. melanogaster* (both genders) of 1 to 3 days old were divided into five groups of 30 flies each, each containing five replicates/group, treated with LUC (10, 25, 50 and 100 mg/10 g diet) and 2.5% ethanol was used as the control. Mortality was recorded daily which was employed to establish the lifespan and 7-day survival rate. This assay was employed in order to determine the appropriate dose to be used and the extent of exposure during the experiment.

Negative Geotaxis Assay
The locomotor performance of the LUC-treated *Drosophila melanogaster* and control flies was evaluated using the negative geotaxis behaviour assay as described by Abolaji *et al*. (2014). After a 5-day exposure to the control, the different doses (5 and 10 mg/10 g diet) of LUC extract and 0.1 mM gallic acid as a positive control were used for the assay. Ten LUC-treated and control flies were immobilized under mild ice anaesthesia placed separately in labeled vertical glass columns (length, 15 cm; diameter, 1.5 cm). After about 20 minutes of recovery from ice exposure, the flies were gently tapped to the bottom of the column, and the number of flies that climbed up to the 6 cm mark of the column in 6 s as well as those that remained below this mark after this time were recorded. The scores represent the mean of the number of flies at the top (n_{top}) expressed as a percentage of the total number of flies (n_{tot}). This process was repeated three times at 1-min interval

Samples for Biochemical Assays
For the determination of biochemical assays, 60 flies (of both gender) were exposed to final concentrations of LUC (5 and 10 mg/10 g diet), negative control and positive control (0.1 mM gallic acid) in media for 5 days as described earlier to mitigate the toxic effects of VCM (1 mM) after 5 days of treatment. Subsequently, flies were sedated in ice, weighed, homogenized in 0.1 M phosphate buffer (pH 7.4, a ratio of 1 mg:10 µL), and centrifuged at 4000 *g* for 10 minutes at 4 ^{o}C in a refrigerated centrifuge (Thermo fisher Sorvall Legend Micro 17R (Fresco)). Thereafter, the supernatants obtained were transferred into new labelled Eppendorf tubes and used for the determination of total plasma protein, total thiol content (T-SH), as well as catalase (CAT), glutathione S-transferase (GST), and acetylcholinesterase (AChE) activities. All the assays were carried out in triplicates.

Total Thiol Determination
The total thiol content was estimated by the method previously illustrated by Balogun et al. (2021). The reaction mixture contained 170 µL potassium phosphate buffer (0.1M, pH 7.4), 20 µL of the sample as well as 10 µL of 5,5-dithiobis(2-nitrobenzoic acid) (DTNB) (10 mM). After incubation for 30 min at room temperature, the absorbance was measured at 412 nm. Standard curve was plotted for each measurement using GSH as standard (expressed as µMol/mg protein).

Determination of Glutathione-S-transferase Activity
The activity of the glutathione-S-transferase was evaluated by the method of Habig and Jacoby (1998) using 1-chloro-2,4-dinitrobenzene (CDNB) as substrate. The assay reaction

mixture was made up of 270 µL of the solution containing (20 mL of 0.25 M potassium phosphate buffer pH 7.0, with 2.5 mM EDTA, 10.5 mL of distilled water and 500 µL of 0.1 M GSH at 25 ˚C), 20 µL of sample (1:5 dilution) and 10 µL of 25 mM CDNB. An increase in absorbance was measured at 340 nm using Jenway 7315 UV Spectrophotometer for 120 seconds at 10 seconds interval. The data were expressed as millimole per minute per milligram protein using the molar extinction coefficient (ε) of 9.6 mM^{-1} cm^{-1} for the coloured Glutathione-S -2,4-dinitrobenzene (GS-DNB) conjugate formed GST.

Determination of Catalase Activity

The catalase (CAT) activity was determined according to the method described by Aebi (1984). The reaction mixture containing 1.8 mL of 50 mM potassium phosphate buffer, pH 7.0, 180 µL of 300 mM H_2O_2, and 20 µL of sample (1:50 dilution) was carried out by monitoring the clearance of H_2O_2 at 240 nm at 25 ^{0}C. The decrease in H_2O_2 was monitored for 120 seconds at 10 seconds intervals at wavelength of 240 nm using a Jenway 7315 spectrophotometer and expressed as µmol of H_2O_2 consumed/min/mg of protein.

Determination of Acetylcholinesterase Activity

The Acetylcholinesterase activity was evaluated according to the method of Ellman *et al*.(1961). The reaction mixture contained 285 µL of distilled water, 180 µL of 0.1 M phosphate buffer, pH 7.4, 60 µL of DTNB, 15 µL of a sample, and 60 µL of acetylcholine. Change in absorbance was monitored at 423 nm for 3 minutes, 15 seconds interval using a Jenway 7315 spectrophotometer, the data were calculated against blank and sample blank and the results were corrected with the protein content.

Statistical Analysis.

Data were expressed as Mean ± Standard deviation using Graph Pad prism 7 for the statistical analysis. Statistically significant differences were determined using One Way ANOVA with $p < 0.05$ taken to be significant.

RESULTS

The antioxidant activity of the extract, LUC was evaluated using DPPH assay. As shown in Figure 1, LUC showed significant antioxidant activity with IC_{50} values of 155.20 ± 0.03 µg/mL compared with gallic acid (23.20 ± 0.01 µg/mL) standard.

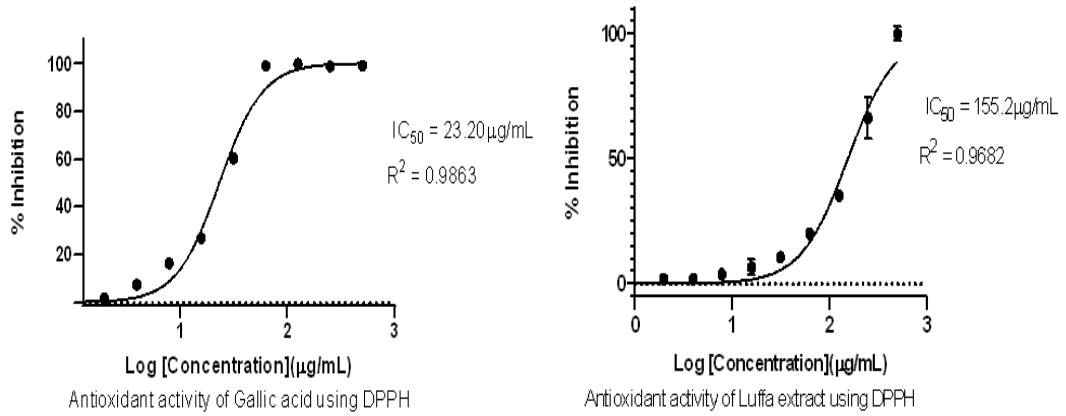


Figure 1 **Antioxidant activities of Gallic acid and aqueous ethanolic sponge extract of *Luffa cylindrica* using DPPH**

The effect of *Luffa cylindrica* on the lifespan of *Drosophila melanogaster* and the 14-day survival assay is depicted in Figure 2. The LUC extract at doses of 10, 25, 50 and 100 mg/10 g diet decreased the lifespan of flies by 29, 41, 48 and 50% respectively after 14-days of exposure. Furthermore, the survival assay showed significant mortality of flies with increase in the concentration of LUC extract (Figure 2).

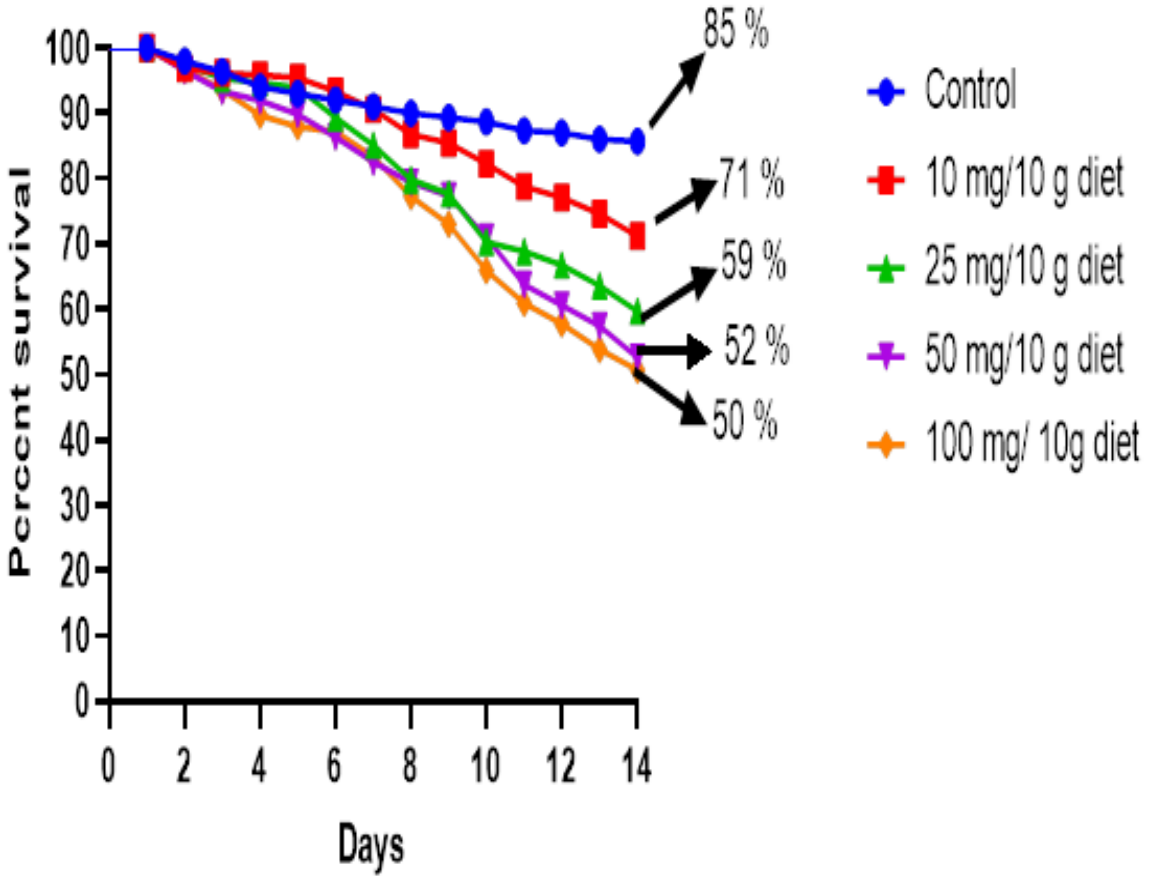


Figure 2: 14-days Survival Study of Fruit Flies Administered Aqueous Ethanolic *Luffa cylindrical* Sponge Extract (10-100 mg/10 g diet significantly declined($p<0.05$) the survival proportion of fruit flies compared to control).

The effect of *Luffa cylindrica* sponge extract on VCM-induced behavioural shortfall investigating the negative geotaxis and inhibition of acetylcholinesterase activity are shown in Figures 3 & 4 respectively. *Luffa cylindrica* sponge extract restored both VCM-induced diminution of negative geotaxis and inhibition of acetylcholinesterase in flies treated for 5 days ($p< 0.05$).

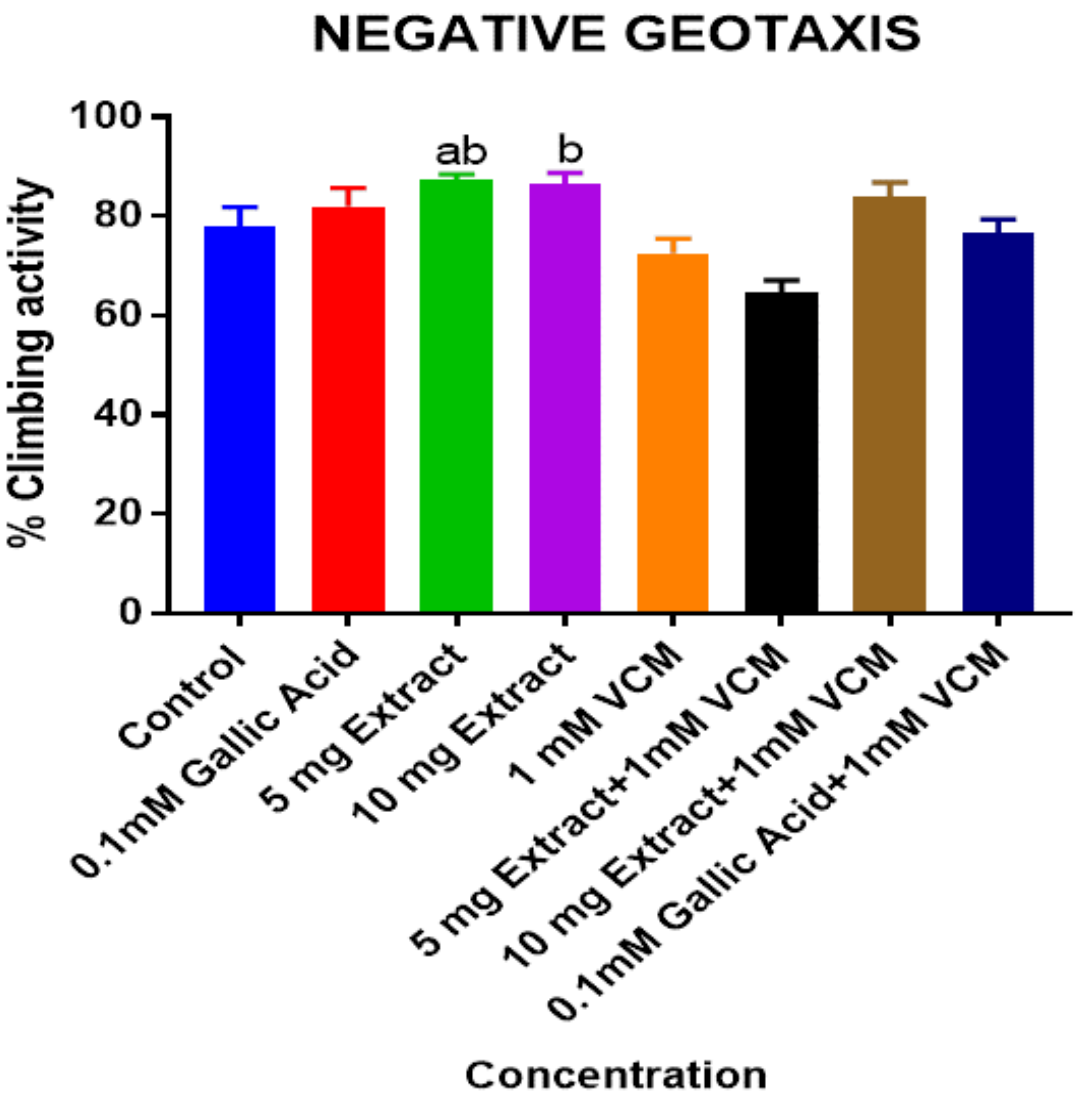


Figure 3: Climbing Activities of Fruit Flies Administered Aqueous Ethanolic *Luffa cylindrical* Sponge Extract (5 mg Aqueous ethanolic *Luffa cylindrica* Sponge extract significantly elevated($p<0.05$) the climbing activities compared to control[a]. 1mM VCM diet significantly declined($p<0.05$) the climbing activities of fruit flies compared to 5 mg and 10 mg aqueous ethanolic *Luffa cylindrica* Sponge Extract[b]).

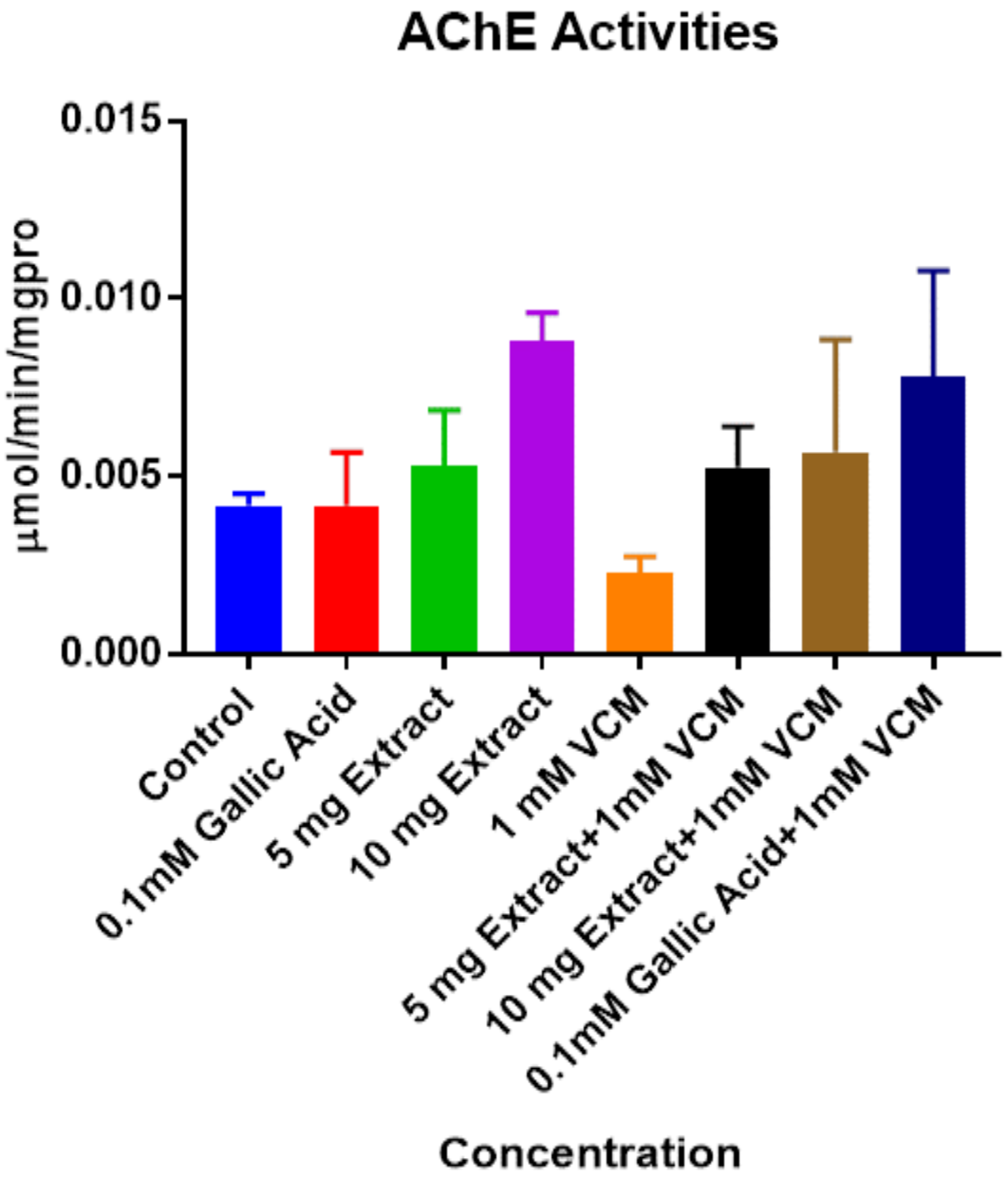


Figure 4 **AChE Activities of Fruit Flies Administered Aqueous Ethanolic *L. cylindrica* Sponge Extract.1 mM VCM, non-significantly declined (p>0.05) AChE activities compared to any other group. There was a non-significant elevation (p>0.05) of AChE activities of 5 mg extract + 1mM VCM, 10 mg extract + 1mM VCM, and 0.1mM gallic acid + 1mM VCM fed fruit flies compared to 1 mM VCM-fed fruit flies**

The effects of *Luffa cylindrica* sponge extract on total thiol content and glutathione as well as catalase activity in VCM-induced toxicity are shown in Figures 5, 6 and 7 respectively. Flies treated with VCM had a significant inhibition of GST (Figure. 6) and CAT (Figure 7) activities ($p<0.05$). Moreso, there was a significant depletion of total thiol content in flies treated with VCM (Figure 5). However, the co-treatment of *Drosophila melanogaster* with *Luffa cylindrica* sponge extract restored VCM-induced reduction of both GST and CAT activities as well as the total thiol content was restored in extract-treated *Drosophila melanogaster.* Furthermore, the mechanism of VCM-induced oxidative stress and the ameliorative role of *Luffa*

cylindrica Sponge was depicted in the scheme 1 which shows the ameliorative effects of *Luffa cylindrica.*

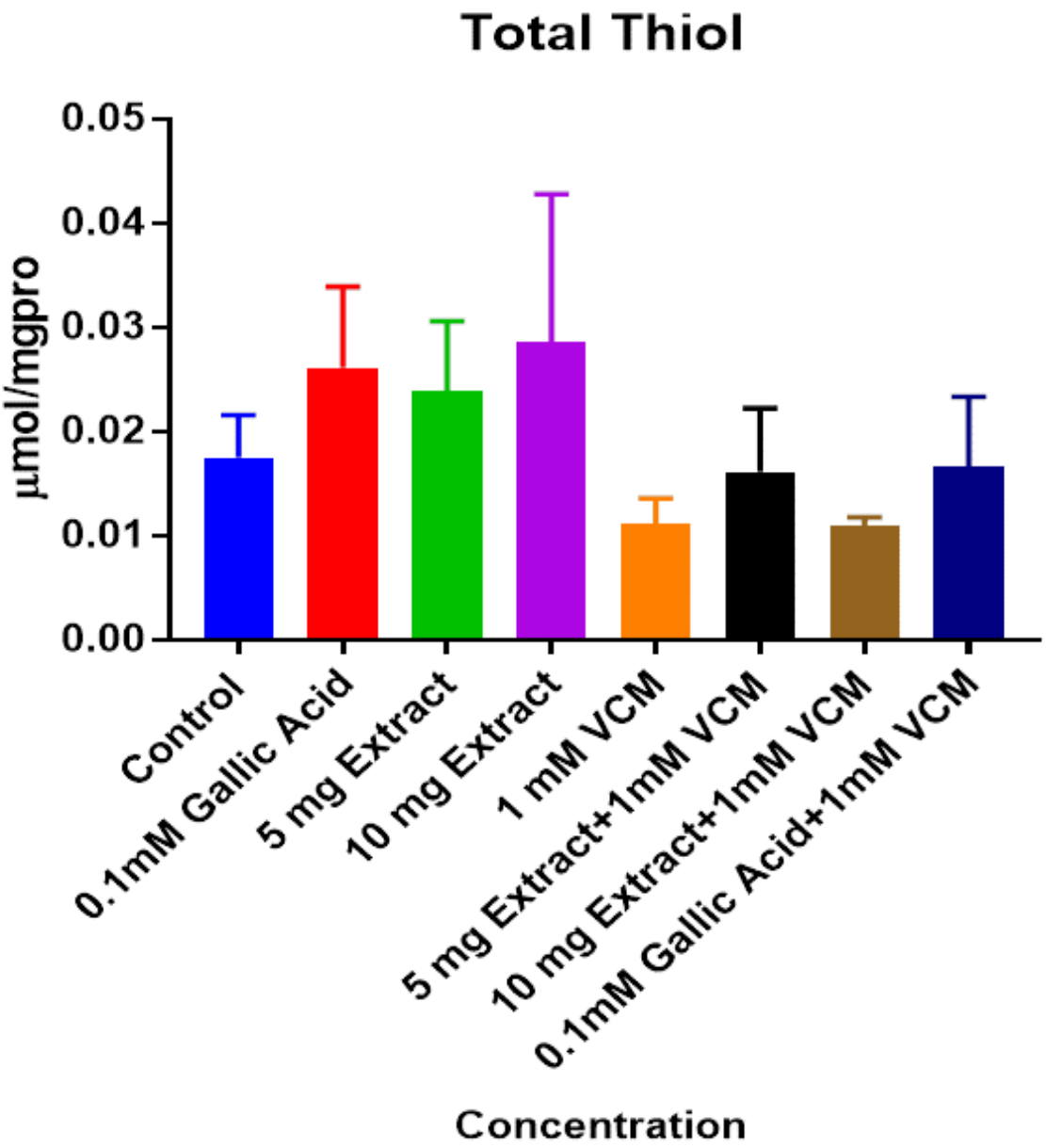


Figure 5**: Total Thiol Content of Fruit Flies Administered Aqueous Ethanolic *Luffa cylindrica* Sponge Extract. There is non-significant elevation(p>0.05) of the thiol content in 0.1mM gallic acid, 5, and 10 mg aqueous ethanolic *Luffa cylindrical* Sponge extract and compared to control. 1 mM VCM, non-significantly declined (p>0.05) the total thiol content compared to any other group.**

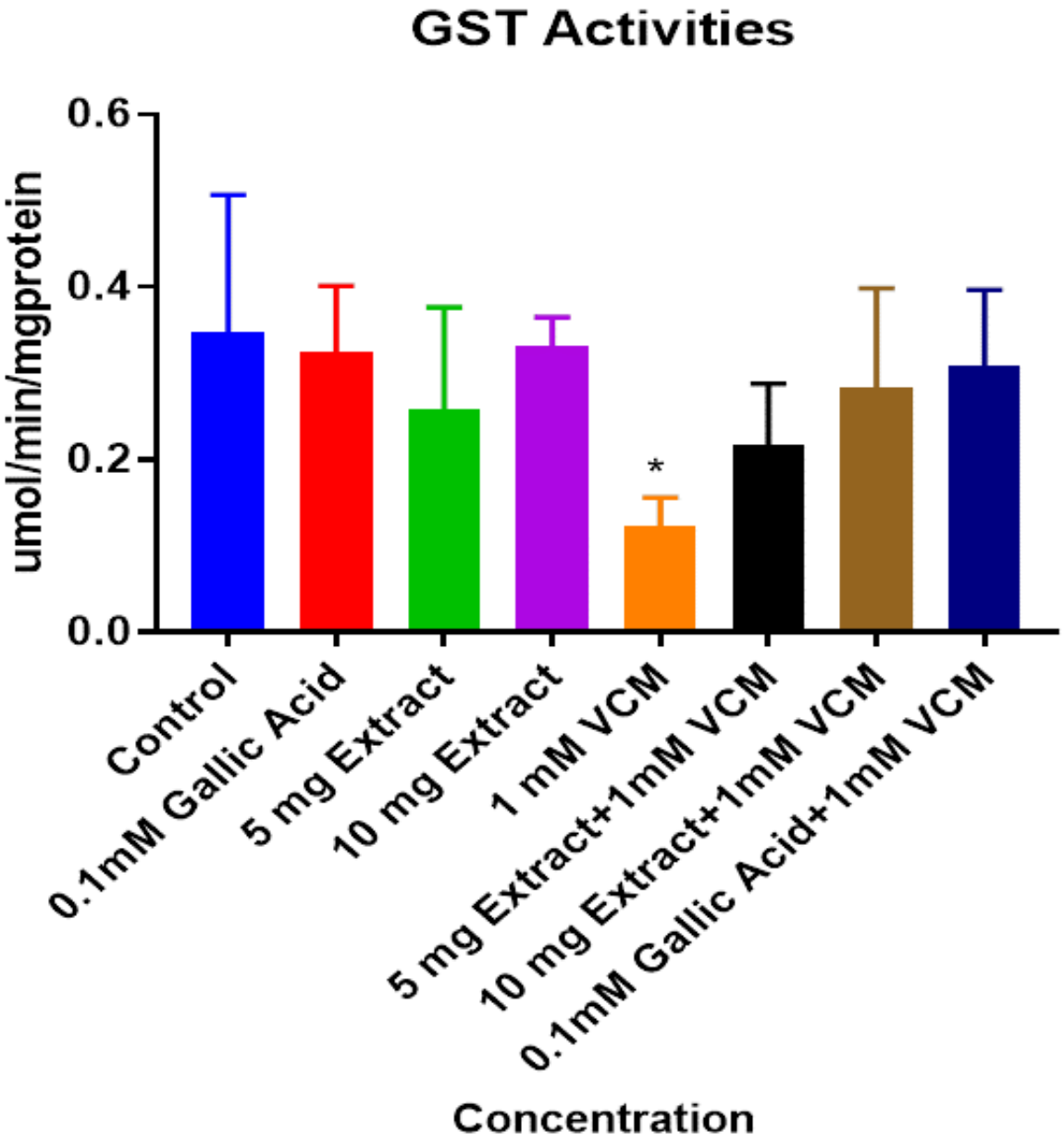


Figure 6: **GST Activities of Fruit Flies Administered Aqueous Ethanolic *Luffa cylindrica* Sponge Extract. 1 mM VCM, significantly declined ($p<0.05$) GST activities compared to any other group. There was a non-significant elevation ($p>0.05$) of GST activities of 5 & 10 mg extract + 1mM VCM, and 0.1mM gallic acid + 1mM VCM fed fruit flies compared to 1 mM VCM-fed fruit flies.**

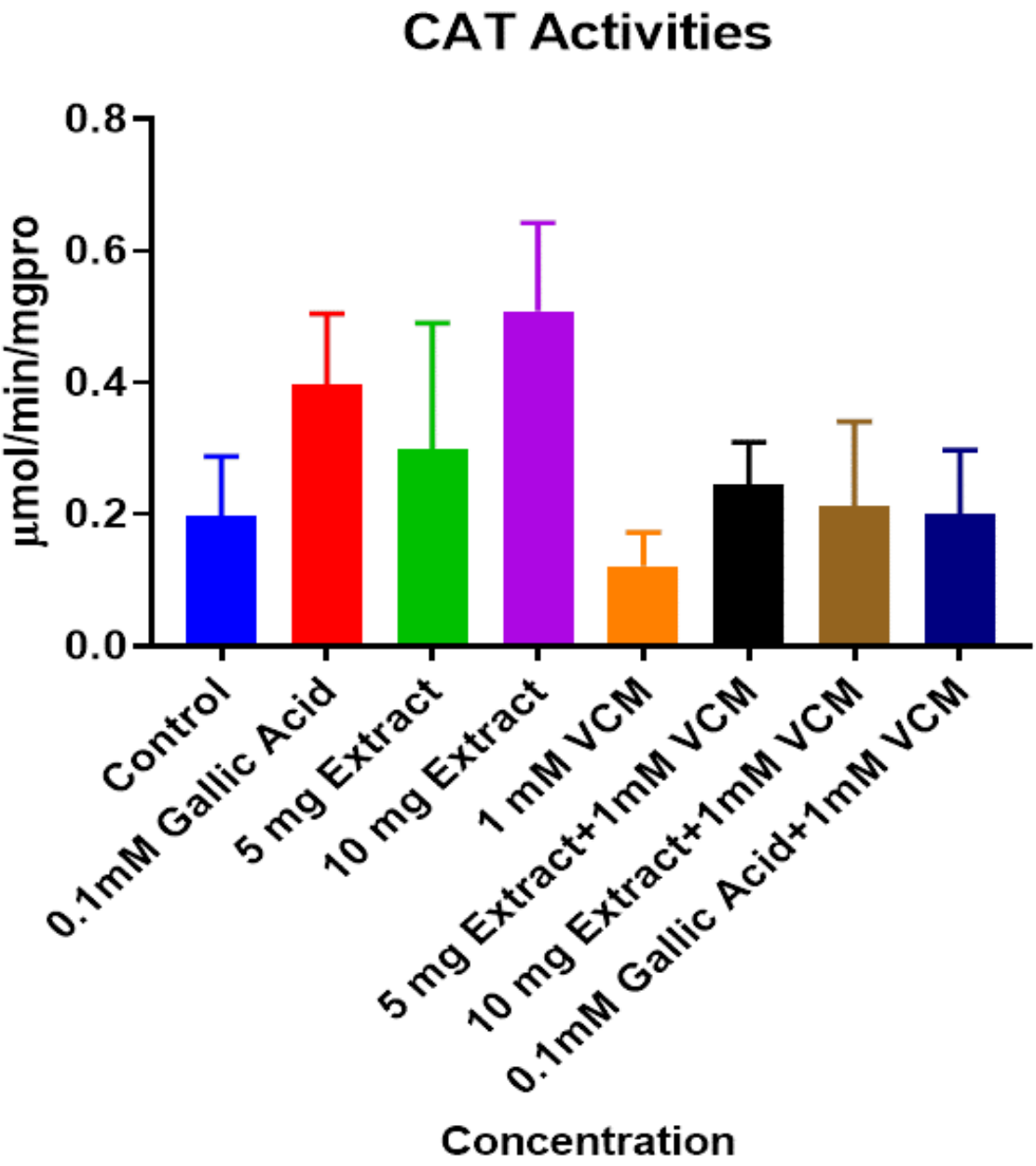
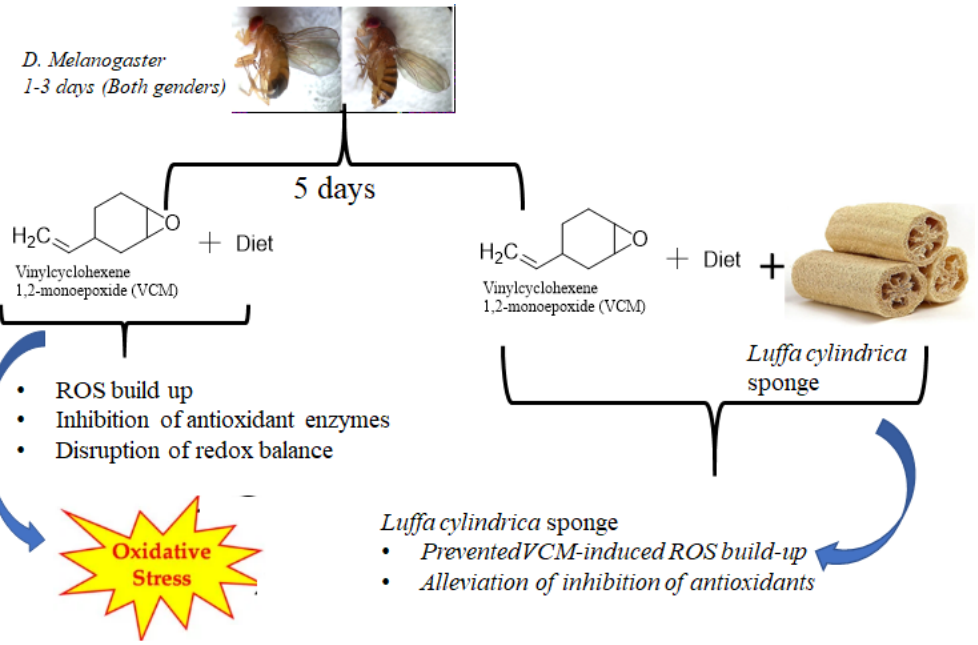


Figure 7: **CAT activities of Fruit Flies Administered Aqueous Ethanolic *Luffa cylindrica* Sponge Extract. Figure 7 revealed a non-significant elevation (p>0.05) of CAT activities in 0.1mM gallic acid, 5 mg and 10 mg extract compared to control. 1 mM VCM, non-significantly declined (p>0.05) CAT activities compared to any other group. 0.1mM gallic acid + 1mM VCM, 5mg extract+ 1mM VCM and 10 mg extract + 1mM VCM non-significantly elevated (p>0.05) CAT activities compared to 1mM VCM-fed fruit flies**



Scheme 1: Mechanism of VCM-induced oxidative stress and the ameliorative role of *Luffa cylindrica* Sponge

DISCUSSION

Free radicals have been responsible for the generation of oxidative stress which is implicated in many chronic and degenerative diseases such as cancer, diabetes mellitus, Alzheimer, Parkinson's diseases and cardiovascular diseases (Ojerinde *et al*., 2022). Medicinal plants, reservoirs of phytochemicals, have received extensive study due to their capacity to act as antioxidants. These antioxidants from natural products prevent free radicals from causing damage to the human DNA, protein and cells by neutralizing the harmful effects of the radicals via electron donation (Ojerinde *et al*., 2022). This property has made medicinal plants a major source of natural phytochemicals such as flavonoids, tannins, phenols, terpenoids and alkaloids that could be used to scavenge free radicals generated in human system (Ojerinde *et al*., 2022). The antioxidant property of medicinal plants has received extensive study using DPPH free radical scavenging method due to its sensitivity (Kumar *et al*., 2008).

The extract of *Luffa cylindrica* showed significant DPPH free radical scavenging activity when compared with gallic acid used as a standard (Figure 1). This finding corroborated the previous study by Du *et al*. (2006) that *Luffa cylindrica* sponge possesses significant antioxidant property. Bulbul *et al*. (2011) also reported DPPH free radical scavenging activity of *Luffa cylindrica* leaves extracts which shows that the plant possesses antioxidant property. The antioxidant property of *Luffa cylindrica* cannot be unconnected with the occurrence of phytochemicals such as flavonoids, tannins, phenols and others in the plant. An increase in extensive research in antioxidant potentials of medicinal plants has continued to gain interest over the years for their applications in the management of oxidative stress-related diseases. This study substantiated the ethnomedical applications of *Luffa cylindrica* for the treatment of chronic diseases.

Oxidative stress has been linked with the neuroinflammation which has resulted in neurodegenerative diseases such as Alzhemier and parkinson's diseases (Velagapudi *et al*., 2014). This study investigated some selected neurotoxic markers in *Drosophila melanogaster* such as the negative geotaxis assay and acetylcholinesterase activity (AChE). Negative

geotaxis is employed to investigate the behavioural changes in flies in response to exposure to toxic substances, aging and neurodegenerative disorders (Balogun *et al*., 2021). Negative geotaxis behavioural of flies has been linked to a drastic change in AChE activity which affects the normal motor activity of the flies (Abolaji *et al*., 2014). Inhibition of the AChE activity results in the accumulation of the neurotransmitter, acetylcholine (ACh) in the synaptic cleft which results in the generation of neurodegenerative diseases (Colangelo *et al*., 2019). Results from our study have shown that the flies co-administered with VCM and 10 mg/10g diet of *Luffa cylindrica* sponge extract restored the AChE activity while the extract also mitigated VCM-induced behavioural shortfall in the flies by reinstating the flies climbing activity.

Normal physiological function and cellular metabolism in an organism require a balanced redox state which is very essential for proper functionality (Balogun *et al*., 2021). The total thiols play an important role in biological system, most especially in the cellular redox state reactions where they act as antioxidant agents. Glutathione, one of the protein thiols in the biological system, is employed by the glutathione peroxidase to reduce the harmful effects of free radicals, thereby protecting the cells from the oxidative damage (Bagu *et al*., 2020). The findings in this study show that *Luffa cylindrica* sponge extract was able to improve the total thiol content in the treated VCM-induced *Drosophila melanogaster* and also displayed ameliorative potentials via the increase in total thiol content. This indicates the *in vivo* antioxidant property of the *Luffa cylindrica* sponge.

Glutathione S-transferase is one of the enzymes involved in the detoxification of toxic substances in the cell (Kim *et al*., 2017). GSTs act as an antioxidant agent transferring the glutathione to the oxidized substances from the products of oxidative stress, thereby protecting the cells from the harmful effects of free radicals (Kim *et al*., 2017). The findings in this study show that *Luffa cylindrica* sponge increased the activities of GST which is indicative of its antioxidant property. In oxidative stress, GST levels rise in response to the imbalance between the oxidants and antioxidants in the cells, thereby, defending the cells against the damaging effects of the oxidative stress (Balogun *et al*., 2021).

Catalase is an enzyme that catalyses the decomposition of H_2O_2 (Hydrogen peroxide) to H_2O and O_2 by using either an iron or manganese co-factor, hence protecting the cell against oxidative stress induced by H_2O_2 or consequently formed hydroxyl radical (Harman, 1993). In this study, the toxicant (VCM) reduced the CAT activities compared to all the other treatment groups. Reduction in CAT activity results in the generation of a more dangerous reactive oxygen species, hydroxyl radical by Fenton reaction which is highly cytotoxic (Balogun *et al*., 2021). Interestingly, *Luffa cylindrica* sponge extract meritoriously reinstated CAT activity when co-administered with VCM in *Drosophila melanogaster*. Therefore, the extract of *Luffa cylindrica* sponge was able to ameliorate the oxidative stress induced in the flies via VCM.

CONCLUSION

Several data and studies have attested to the contribution of oxidative stress in the pathogenesis of chronic diseases. Results from this study have shown that oxidative stress was induced by the administration of VCM in *D. melanogaster,* via the modification of oxidative stress-antioxidant homeostasis, AChE inhibition and redox imbalance. Conversely, to a great extent, *Luffa cylindrica* sponge extract prevented VCM-induced toxicity by restoring redox and antioxidant balance, as well as AChE activity in flies co-treated with VCM. These outcomes have shown that *Luffa cylindrica* sponge extract has ameliorative effects on VCM-induced toxicity compared with the standard and also upheld the ethnomedical uses of *Luffa cylindrica* for the management of oxidative stress-related diseases.

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