EVALUATION OF GENOTOXIC AND BIOCHEMICAL EFFECTS OF SOFT DRINK (BIGI COLA AND ORANGE) USING *Allium cepa* AND *Drosophila melanogaster* BIOASSAYS

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A DISSERTATION SUBMITTED TO THE DEPARTMENT OF BIOCHEMISTRY, COLLEGE OF BASIC AND APPLIED SCIENCES, MOUNTAIN TOP UNIVERSITY, IBAFO, OGUN STATE, NIGERIA, IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF BACHELOR OF SCIENCE (B.Sc.) DEGREE IN BIOCHEMISTRY

SEPTEMBER, 2022

DECLARATION

I, Petra Vera Esther, hereby declare that this project titled "**EVALUATION OF GENOTOXIC AND BIOCHEMICAL EFFECTS OF SOFT DRINK (BIGI COLA AND ORANGE) USING *Allium cepa* AND *Drosophila melanogaster* BIOASSAYS".**

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Petra, V. E **Date**

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CERTIFICATION PAGE

This is to certify that the content of this project work entitled "**EVALUATION OF GENOTOXIC AND BIOCHEMICAL EFFECTS OF SOFT DRINK (BIGI COLA AND ORANGE) USING *Allium cepa* AND *Drosophila melanogaster* BIOASSAYS"** was carried out and submitted by **PETRA VERA ESTHER** in partial fulfilment of the requirements for the Degree of **BACHELOR OF SCIENCE IN BIOCHEMISTRY** and is approved for its contribution to knowledge and literary presentation.

…….. ……..

Prof. G. O. Ajayi (Supervisor) **Date**

…….. …….....

(HOD Biochemistry) **Date**

DEDICATION

This project is dedicated to Almighty God, my parent, Mr and Mrs Petra and my family

ACKNOWLEDGEMENT

My utmost gratitude goes to the Almighty GOD, who in his infinite mercies inspired the conception of this project write-up and also made it possible to be a great success. My special thanks also go to **Prof. G. O. Ajayi** who supervised my project and also put me through my project write-up.

I want to use this opportunity to thank every member of my family, Dr. Sodiya, Dr. Ogunsuyi, Mrs Afolabi, my colleagues for their love, support, and encouragement towards the successful completion of my project.

I sincerely wish to express gratitude to the entire staff of Biochemistry Department of Mountain Top University for their wonderful lectures and knowledge they imparted into me.

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Petra, V.E

ABSTRACT

The genotoxicity of carbonated soft drinks were investigated using the *Allium cepa and Drosophila* assay, this present practical has been conducted to study the genotoxic effect of three concentrations of Bigi drinks "Cola and Orange" with concentrations (6.25, 12.5 and 25) respectively with time exposure of 72hours and root growth inhibition test is performed on root tips of *Allium cepa* while pipe borne water served as negative control and was also exposed for 72hours, lead nitrate $[PbNO_3]_2$ served as positive control and was exposed for 72hours.

However, the toxic effect of soft drinks on *Drosophila melanogaste*r was conducted with three concentrations of Bigi drinks "Cola and Orange" {100, 50 &25} with time exposure of 10 days in each vials having triplicate of each group and negative control, negative control which is the distilled water that was exposed for 10 days. Unfortunately, promising results were not obtained from the *Allium cepa* assay.

This conducted research on assessment of the genotoxic effects of soft drinks by bioassay methods will make it possible to extend the understanding of the processes and mechanisms of this toxicity and form more rational concept of consumption.

Keywords: Genotoxicity, Root growth inhibition, soft drinks "Cola & Orange, *Drosophila melanogaster.*

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CHAPTER ONE

INTRODUCTION

1.1 BACKGROUND OF THE STUDY

Soft drinks are non-alcoholic water based flavoured drinks that are optionally sweetened, acidulated, carbonated and may contain fruit juice and salts, their flavour may derive from vegetable extracts or other aromatic substances (Chandraker, *et al*., 2014). The term *soft drink* was originated to distinguish the flavoured drinks from hard liquor, or distilled spirits.

In certain soft drinks caffeine is also present. This include certain soft drinks (primarily Cola drinks) and energy drinks (Monster, Red Bull, Predator, Fearless and Amber) designed as stimulants and to perpetuates activity at times when the user might be asleep. The consumption of caffeinated drinks is often intended entirely or partly for the physical and mental effects of caffeine. Caffeine in soft drinks causes change in the DNA repair system (Edward *et al*.., 2022).

In this study, some Bigi products (a subsidiary of Rites Foods) would be employed which are; Bigi (Cola and Orange). A clear explanation, definition and composition of soft drinks used would be further discussed in chapters to come.

The term *"Genotoxicity"* simply refers to the damage to DNA in the form of gene mutations, large-scale chromosomal damage, recombinant &numerical chromosome (Georg *et al*., 2007).

Allium cepa (Onion) and *Drosophila* (common fruit fly) assays were employed as plant and animal models to evaluate the toxicity of soft drinks in consonance with chemical analysis (Fiskesjö *et al.*, 1993)

1.1.1 Scientific Classification of *Drosophila melanogaster*:

Kingdom: Animalia

Phylum: Arthropoda

Class; Insecta

Order: Diptera

Family: Tephritidae

Genus: Drosophila

Species: *D. melanogaster* (Newman*et al*..,1834)

The *Allium cepa* test is an easy, fast and genetic assay to detect the potential genotoxicity of polluted water and other chemicals on mitosis and chromosome structure (Mercykutty *et al*., 1980)

Scientific Classification of *Allium cepa*:

Kingdom: Plantae

Subkingdom: Tracheobionta

Super Division: Spermatophyta

Division: Liliopodia

Subclass: Liliales

Order: Liliaceae

Family: Alliaceae

Genus: Allium

Species: *Allium Cepa* L. (Burnie *et al.*, 1999)

1.2 STATEMENT OF PROBLEM

The Bigi drinks that would be used for this practical is a new product in market, likely problem would be seen during the use of the drinks (Cola & Orange) for the plant and animal model.

1.3 Aim and Objectives of the Study

The study aims at evaluating the genotoxicity and biochemical effects of soft drinks in *Allium Cepa* and *Drosophila Melanogaster*, by achieving specific objectives which include:

i. To evaluate the genotoxic effect of soft drinks on *Allium Cepa*

ii. To investigate the effect of the soft drinks on biochemical parameters in *Drosophila Melanogaster*

1.4 SCOPE OF THE STUDY

The study aims to look into and determine the genotoxic and biochemical effects of the soft drinks on *Allium Cepa* and *Drosophila Melanogaster*

1.5 SIGNIFICANCE OF THE STUDY

The findings from this study may reveal more information on the toxicological effects on both bioassays.

CHAPTER TWO

LITERATURE REVIEW

2.0 LITERATURE REVIEW

The first commercially available soft drinks came in the 17th century as a combination of water and lemon juice flavored with honey. The Compagnie de Limonadiers was founded in Paris in 1676 and was awarded a monopoly for the selling of its products. Vendors walked around with tanks on their backs, dispensing cups of lemonade. Carbonated beverages and waters evolved from 17th-century European attempts to emulate the popular and naturally effervescent waters of famous springs, with a primary focus on their purported therapeutic qualities. The effervescent nature of the fluids was recognized early on as being crucial. Jan Baptista van Helmont, a Flemish scientist, used the term "gas" in reference to the carbon dioxide content. Gabriel Venel, a French physician, alluded to aerated water, misunderstanding the gas with ordinary air. The gaseous ingredient fixed air was called by British physicist Joseph Black (Britannica, 2014).

English priest and scientist for his research on gas obtained from a brewery's fermenting vats, Joseph Priestley is known as "the father of the soft drinks business." In 1772, he exhibited a tiny carbonating device to the College of Physicians in London, implying that by using a pump, water might be more heavily impregnated with fixed air. In 1773, French chemist Antoine-Laurent Lavoisier made the same suggestion. The first manufacture of carbonated water is credited to Thomas Henry, a pharmacist in Manchester, England, who created it in 12-gallon barrels using an apparatus based on Priestley's design. Swiss jeweler Jacob Schweppes read Priestley and Lavoisier's works and decided to create a similar gadget. By 1794, he was selling his highly carbonated fake mineral waters to his acquaintances in Geneva; later, he started a business in London (Britannica, 2014).

Bottled waters were originally used medicinally, as revealed by a letter written in 1794 by English entrepreneur Matthew Boulton to philosopher Erasmus Darwin:

J. Schweppes manufactured three kinds of mineral waters. No. 1 is for everyday drinking with dinner. No. 2 is for nephritic patients, while No. 3 contains the most alkali and is only used in the most severe instances.

Around 1820, advancements in manufacturing technologies enabled significantly higher output, and bottled water became popular. Mineral salts and flavors were added—ginger around the time of soft-drink bottling in the 1820s, lemon in the 1830s, and tonic in 1858. Coca-Cola, the first cola drink, was introduced in 1886 by John Pemberton, a pharmacist in Atlanta, Georgia. (Britannica, 2021).

Onion, (*Allium cepa L.*), on the other hand, is one of the most consumed and grown vegetable crops in the world. Onion bulb, with its characteristic flavor, is the third most essential horticultural spice with a substantial commercial value (*Abdel-Salam et al., 2014*). Apart from its culinary virtues, *A. cepa* is also used traditionally for its medicinal virtues in a plethora of indigenous cultures. Several publications have been produced in an endeavor to validate such traditional claims. Nonetheless, there is still a dearth of up-to-date, detailed compilation, and critical analysis of the traditional and ethno pharmacological propensities of A. cepa (Adeshina *et al.., 2011*).

Drosophila melanogaster is known to be one of the most common model organisms used for different biomedical science research purposes. Advantages to using the Drosophila model is its low cost, fast generation time, easy to care for, and is an excellent tool for genetic manipulation (Tolwinsk *et al.., 2017*).

2.1 SOFT DRINKS

Soft drinks are a type of nonalcoholic beverage that is usually but not always carbonated and contains a natural or artificial sweetener, edible acids, natural or artificial flavors, and sometimes juice. Natural flavors come from fruits, nuts, berries, roots, herbs, and other plant sources. Soft drinks do not include coffee, tea, milk, chocolate, and undiluted fruit and vegetable juices. The term "soft drink" was coined to differentiate flavoring drinks from hard liquor or distilled spirits. In an effort to modify the hard-drinking habits of early Americans, soft beverages were promoted as a substitute. Indeed, modern customers' health concerns prompted the creation of new categories of soft drinks that emphasized low calorie count, low salt level, no caffeine, and "all natural" components (Britannica, 2014). There are numerous specialty soft drinks available. In Europe and Latin America, mineral waters are extremely popular. People in Fiji and other Pacific islands use kava, which is prepared from the roots of a bushy shrub called Piper methysticum. People in Cuba drink carbonated cane juice, which is flavored with unrefined syrup. Soft drinks containing soybean flour have been promoted in

tropical locations where diets usually lack sufficient protein. Carob (locust bean) extract is utilized in Egypt. Maté is used as the base for a soft drink in Brazil. In North Africa, the whey obtained from the production of buffalo cheese is carbonated and drunk as a soft drink. Some Eastern Europeans consume a beverage made from fermented stale bread. A popular drink contains honey and orange juice in Israel. (Britannica, 2014).

2.1.1 FORMS OF SOFT DRINKS

Soft drinks as at of today can be seen in two main forms, which are;

i. Powdered Soft Drinks: These are created by combining the flavoring substance with dry acids, gums, artificial color, and so on. If the sweetener is already present, the user merely needs to add the appropriate amount of plain or carbonated water.
ii. Iced or readily made Soft Drinks: The original iced soft drink was a cup of ice coated in flavored syrup. To prepare the completed beverage, sophisticated dispensing devices now blend calibrated amounts of syrup with carbonated or plain water. The machine cools the beverage to between 5 and 2 °C (22 and 28 °F) to create soft ice or slush (Britannica, 2014).

Nowadays, soft drinks are sold in glass or plastic bottles, tin-free steel, aluminum, or plastic cans, treated cardboard cartons, foil pouches, or huge stainless steel cans.

2.1.2 COMPONENTS OF SOFT DRINKS

All ingredients used in soft drinks must be of high purity and food grade to obtain a quality beverage. These include the water, carbon dioxide, sugar, acids, juices, and flavors.

i. Water: Although water is normally collected from a safe municipal supply, it is usually processed further to assure the finished product's homogeneity; the level of contaminants in the municipal supply may fluctuate from time to time. Water-treatment equipment in some bottling operations may be as simple as a sand filter to remove minute solid matter and an activated carbon purifier to remove color, chlorine, and other tastes or odors. However, in most plants, water is treated using a process known as hyper chlorination and coagulation. The water is then passed through a sand filter and activated carbon after being exposed for two hours to a high concentration of chlorine and a flocculants, which eliminates organisms such as algae and bacteria (Britannica, 2014).

ii. Carbon dioxide and Carbonation: Carbon dioxide gas adds sparkle and zest to the beverage while also preventing spoiling. It is delivered to the soft drink manufacturer in either solid (dry ice) or liquid form, both of which are kept in massive steel containers under pressure of around 1,200 pounds per square inch (84 kilograms per square cm). When liquid carbon dioxide is kept refrigerated, lightweight steel containers are employed. Internal pressure is around 325 pounds per square inch in that situation. Carbonation (either of the water or the finished beverage mixture) is accomplished by cooling the liquid and cascading it in thin layers over a succession of plates in an enclosure holding under pressure carbon dioxide gas. The amount of gas that the water will absorb increases as the pressure and temperature rise.

iii. Flavoring Syrup: Flavoring syrup is typically a concentrated solution of a sweetener (sugar or artificial), an acidulant for tartness, flavoring, and, if necessary, a preservative. The flavoring syrup is prepared in two stages. First, a "simple syrup" is made by combining water and sugar. If the sugar quality is low, this simple sugar solution can be treated with charcoal and filtered. The remaining components are then added in a certain order to create a "finished syrup." (Britannica, 2014)

iv. **SOFT DRINKS IN NIGERIA** (Britannica, 2014)

There are several types of soft drinks in Nigeria, but the majorly consumed ones are;

i. Coca-Cola
ii. Fanta
iii. Fayrouz
iv. Sprite
v. Pepsi
vi. 7up
vii. Mirinda
viii. Smoov
ix. Schweppes
x. Lacasera
xi. Lipton
xii. Royal crown
xiii. Mountain dew
xiv. Bigi (Lime, Apple, Cola, Orange e.tc) which are the main focus of this research work.

Figure 1: Most consumed Nigerian Soft Drinks (Britannica, 2014).

v. **2.1.4 BIGI VARIANTS AND THEIR COMPOSITION** (Britannica, 2014)

'Bigi' a subsidiary of Rite Foods Limited which embarked on its beverage journey, July 2016 with six (6) Bigi flavours which are; Bigi Cola, Orange, Apple, Bitter Lemon, Soda Water and Lemon & Lime. In Lagos, Nigeria, bigi cola, orange, and apple flavors are the most popular. The test solvents employed in this project work are those with the most popular flavours, and their primary constituents are:

- Bigi Cola: Water, Sugar, Carbon Dioxide, Colour Sulphite, Ammonia, Caramel E150d, Acid Phosphoric Acid E338, Vitamin C, Sodium Benzoate E211, Flavouring: Caffeine, Natural Flavouring.

- Bigi Orange: Water, Sugar, Carbon Dioxide, Acid Citric Acid E330, Vitamin C, Potassium Sorbate E202, Sodium Benzoate E211, Stabilisers, Gum Arabic E414 and

Glycerol Esters of Wood Rosins E445, Flavouring, Colours Sunset Yellow FCF E110, Quinoline Yellow E104 and Azorubine E122

i. Figure 2: Rites own Bigi soft drink variants (Britannica, 2014)

2.2 BIOCHEMICAL ASSAYS

2.2.1 Lipid peroxidation (MDA):

The principle is based on the reaction between thiobarbatituric acid {TBA} and alondialdehyde {MDA}, an end product of lipid peroxidation. On heating in acidic PH, a pink coloured product is formed, which absorbs maximally at 532nm. The absorbance was read on a spectrophotometer. The result is expressed as the amount of free MDA produced.

2.2.2 Superoxide Dismutase (SOD):

This principle is based on the ability of superoxide dismutase to inhibit the auto oxidation of adrenalin at PH 10.2, superoxide anion generated by the xanthine oxidase reaction causes the oxidation of adrenalin to adrenochrome. One unit of enzymes was defined as the amount of the enzyme required for 50% inhibition of oxidation of adrenalin to adrenochrome in one minute.

2.2.3 Catalase (CAT):

The disappearance of peroxide was followed spectrophotometrically at 240nm. Unit definition: one unit of catalase will decompose 1.0 micromole of hydrogen peroxide per minute at PH 7.0 at 250^0C, while the hydrogen peroxide concentration fall from 10.3 mM. The rate of disappearance of hydrogen peroxide is followed by observing the rate of decrease the absorbance at 240nm.

2.2.4 Cholesterol (CHOL):

It based on the following reactions:

Cholesterol +water in the presence of cholesterol esterase to give cholesterols and fatty acid.

Cholesterol + oxygen in the presence of cholesterol esterase to 4-cholesten -3-one + water

Hydrogen peroxide +phenol + 4-aminophenazone in the presence of peroxide to give quinonimine + $4H_20$.

2.2.5 Triglyceride (TRIG):

The colorimetric reaction described by Tietz [1995] was used for the concentration of triglyceride in the serum. The triglyceride was determined after enzymatic hydrolysis with lipases. The indicator is a Quinone lime formed from hydrogen peroxide, 4-aminophenazone and 4-chlorophenol under the catalytic influence of peroxidase.

2.2.6 High density lipoprotein (HDL):

HDL Cholesterol was determined by CHOD-PAD method described by Frieduarld *et al* in 1972

2.2.7 Low density lipoprotein (LDL) and very low density lipoprotein (VLDL):

The fractions are precipitated quantitatively by addition of phosphotungstic acid in presence of magnesium ions. After centrifugation, the cholesterol concentration in HDL fractions which remains in the supernatant is determined by the following changes in absorbance

2.3 Genotoxicity and Mutagenotoxicity of Soft drinks in *Allium cepa ASSAY*

As said earlier, the term ‘genotoxicity’ is defined as a destructive effect affecting the integrity of the genetic material of the cells. On the other hand, ‘mutagenotoxicity’ or ‘mutagenicity’ is the process of inducing permanent alterations in a genetic material’s structure or amount (World Health Organization, 2020). A conducted research on assessment of hemotoxic and mutagenic effects of soft drinks by bioassay methods will make it possible to extend the understanding of the processes and mechanisms of this toxicity and form more rational concept of consumption.

(Leme *et al..,* 2009) carried out an extensive review on the Allium cepa test and its use in environmental contamination, where they reported that vascular plants are recognized as excellent genetic models for detecting environmental mutagens and are frequently used in monitoring studies. Allium cepa is among the plant species used to evaluate DNA damages (genotoxicity), chromosomal alterations (mutagenotoxicity) and disturbances in the cycle.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Collection and preparation of plant material:

The onions *(Allium cepa)* was purchased from Ile-epo oja, a market located at Iyana-Ipaja, Lagos State in May 2022.

The onions was spread for two weeks in the sun, for the scale to be dried so it would be able to peel easily, Seventy-two (72) onion bulbs were used in which sample of the experiment was done to get a grips of the project, the onions was spread out in the sun daily before use.

3.2 Materials

Allium cepa (onions), Disposable cups, Tray, Bowl, Distilled Water, INHCL, Acetocarmine, Board, Microscope slides, Cover slips, Tissue papers, Nail vanish, Microscope, Counter, Autoclave, Water bath, Weigh balance, PH meter, Centrifuge, Petri dish, Paper tape, Needles, Beakers, Measuring cylinders, Test tube and Test tube rack, Razor Blade, Bigi drinks (Cola, Apple, Orange), *Drosophila* (fruit fly), Hot plate, Disposable cups, Stirring rod, Foams, Bama bottles (medium sizes), Plain bottles, Agar powder Corn meal, Spatula, Nipagin, Ethanol, Water, Funnel, Microliter pipette(100-1000), Ice block, Centrifuge plastics/ plain bottles(2.94-3.0 g), Brush, Filter paper, Ruler, Forceps .Phosphate Buffer Saline, Sodium chloride, Potassium chloride, Sodium phosphate, Potassium phosphate monobasic, Randox HDL, Cholesterol,

3.2.1 Chemicals and Reagents

- 1N HCL
- Ethanol
- Glacial Acetic Acid
- 70% Ethanol.

3.3 *Allium cepa* ASSAY

The following steps and methods make up the preparation of the allium cepa assay:

a. A large number of commercially available onions were obtained,

b. The outer scales of the bulb that is brownish were removed,

c. The peeled bulbs were washed in clean water,

d. The bulbs were placed in different doses/concentration of test liquids and then put in the dark for 72hours. The test liquid was changed every 24hrs and 12 bulbs/doses were started with.

e. After 48hours, the root tips of 2 of the growing bulb were cut off and fixed in methanol/ethanol; glacial acetic acid [3:1v/v] for 24hours

For Root Growth Inhibition, the length of the root of the remaining 10 bulbs were measured at 72hours and the average for tested concentration would be calculated, using this calculation the $E.c_{50}$ or $Ic_{50.}$

3.4 Plant Study Design

3.4.1 Experimental Plants

Seventy-two (72) healthy onion bulbs were used for this study and kept in a room temperature, it was kept in a drawer/locker to avoid sunlight so it would not have effect on the planted onions, Department of Biochemistry, College of Basic and Applied Sciences, Mountain Top University, Nigeria, the concentration of the drinks were changed every 24hours in order to have fresh drink so it would not ferment, before, commencement of the experiment.

3.4.2 Experimental design

The Seventy-two (72) bulb onions were randomly distributed into four (4) groups (I-III) of three Bigi drinks in triplicates:

Group I: Negative Control; water and onions

Group II: Bigi Cola; 6.25, 12.5, 25%

Group III: Bigi Orange; 6.25, 12.5, 25%

Group IV: Positive control; Lead nitrate and water

3.4.3 Planting of Onions (*Allium cepa*)

The scale of the onions was peeled till it was a fresh scale/body, the upper part of the primordial ring was scrapped off cutting off any long root growth, and the inner part was cut off gently so it would not be damaged. The drinks were poured into the disposable cup according to their concentration, the primordial with control ring was placed inside the cup with the base on top of the drink. It was labelled respectively according to its concentration. The method is represented by the figure below;

Removal of the outer papery layer of onions

↓

Onion bulbs placed at various concentrations of soft drinks along with control

3.4.4 Root Harvest

The root tips was harvested after 48hours of exposure, the tips was cut off from the base and cut into 1N HCL, dipped inside water and blotted on filter paper. The root tips was dipped into Ethanol Glacial Acetic acid, using a bijoux bottle. The root tips were prepared for examination under microscope, the slides were coded, viewed, using oil immersion under the light microscope, at 1000 × (**OLYMPUS**, China) magnification and scored blind for normal and aberrant cells in the different stages of the cell cycle. The most representative ones for each structure aberration slides were photographed by "**SONY OPTICAL STEADY SHOOT"**

3.4.5 Root Growth Inhibition Test

The effect of the concentration (Drinks) on the morphology of growing roots of *Allium cepa* was examined. The root growth inhibition assay was performed as a 72hours semi static exposure test (Bakare *et al*., 2009), that is the onions was exposed for 72hours to different concentrations (6.25, 12.5, and 25%) of the Bigi drinks. At the end of the exposure, the length of the root bundle was measured for the remaining four bulbs at each concentration for each Bigi drinks, water was used for the control.

3.5.0 Collection and preparation of Animal Material

The fruit fly was collected from Biology Laboratory, Department of Biological Sciences, College of Basic and Applied Sciences, Mountain Top University, Prayer City, Ogun State, Nigeria in May 2022.

The fruit fly was cultured for three-five days for the use of the practical work to attain the adult stage, diet preparation was prepared for the fruit flies.

3.5.1 Chemicals and Reagents Used

Nipagin, Ethanol, Phosphate Buffer Saline (Sodium chloride, Potassium chloride, Sodium phosphate, Potassium phosphate monobasic), Adrenalin, Hydrogen peroxide.

3.6 Animal Study Design

3.6.1 Experimental Animals

Six-hundred (600) fruit flies were used for this study and kept well in a ventilated location in the Biology laboratory**,** Department of Biological sciences, College of Basic and Applied Sciences, Mountain Top University, Nigeria. There were 20 fruit flies per plain bottles cover with foam for air also for the fruit flies not to escape, for the exposure of the fruit flies.

3.6.2 Experimental design

The Six-hundred (600) fruit flies were randomly distributed into three (3) groups (I-III) of two Bigi drinks in triplicates:

Group I: Negative Control; Distilled Water

Group II: Bigi Cola; 100, 50, 25%

Group III: Bigi Orange; 100, 50, 25%

3.6.3 Preparation of Diet

1000ml of water was boiled in a beaker, 150 ml of distilled water was used to dissolve the cornmeal, and little quantity of the boiling water was used to dissolve the yeast. 7.9g of Agar was added to the boiling water and stirred for 10 minutes, 52g of dissolved corn meal was added to the stirred agar also stirred for another 10 minutes. After 10 minutes, add 5g of dissolved yeast was added to the agar and yeast, it was left on the hot plate for 25 minutes. 5ml of ethanol was poured into 0.5g nipagin using a measuring cylinder, it was left to dissolve for 5 minutes, add the dissolved ethanol to the cooked diet and stir the diet for 2 minutes, the diet was transferred into the vials (Bama bottles). It was let to cool till the next day.

3.6.4 Transfer of fruit flies

Transferring of the 3-5 days old fruit flies into the vials of the freshly prepared diet, was done using a funnel, by transferring the fruit flies with the old diet into new diet.

3.6.5 Exposure of Fruit flies

The fruit flies was exposed to the drinks (Cola, Apple, Orange), the freshly prepared diet was scooped with a spatula into the plain bottles weighing 9.8g each, label all the plain bottle of each concentration of drinks respectively (100, 50 &25%). Using the microliter pipette 20 microliter of drinks was used for each of the concentration (50 &25%) press into the diet and dilute respectively;

For 50% concentration;

100microliter of drink + 100 microliter of Distilled water = 50%

For 25% concentration;

50 microliter of drink + 50 microliter of Distilled water = 25%

While, the 100% is undiluted which is 100 microliter.

As the concentrations were been added into the diet, it was stirred immediately and clean properly. The fruit flies were transferred from the new diet back into the induced diet and cover with foams, it was exposed for 10 days and observed daily for the number of death and the living ones.

3.6.6 Homogenization

After 10 days of the administration, the flies were anesthetized in iced, weighed, and homogenized in 0.1 M phosphate buffer (pH 7.4) , with dilution factor 5ml of PBS. The homogenization was performed using a mortar and pestle at room temperature, it was kept in plain bottles, for biochemical assays.

3.7 Biochemical Assay

3.7.1 Lipid peroxidation (MDA)

Reagents Composition

Thiobarturic acid (TBA)

Alondialdehyde

PROCEDURE

Tissue supernatant (0.4ml) was mixed with 1.6ml of phosphate buffer and 0.5ml of 30% TCA was added followed by 0.5ml of 75% TBA and placed in a water bath for 45 minutes at 80^0C, cooled in ice and centrifuged for 10 minutes at room temperature. The absorbance of the clear supernatant was read against blank.

	Blank	Standard	Sample
Supernatant	-	-	0.4ml
Mixed buffer	-	-	1.6ml
TCA	-	-	0.5ml
TBA	-	-	0.5ml
Distilled water	10 µl	-	-

The absorbance of the clear supernatant was read against the blank (distilled water) at 532nm using spectrophotometer.

CALCULATION:

MDA (Unit /mg protein) = DOD X V X 1/ Molar absorbency index for MDA x v x mg protein

Molar absorbency index for MDA = 1.56 x 10^5M-1CM^1

DOD = absorbance at 532nm, V = total volume of reaction mixture, v = volume of sample

3.7.2 Superoxide Dismutase (SOD)

Reagents Composition

Pips buffer (0.05M, pH 10.2)

Adrenalin

Epinephrine

PROCEDURE

An aliquot of 0.2ml of the diluted tissue supernatant was added to 2.5ml of 0.05M of carbonate buffer (pH 10.2) to equilibrate in the spectrophotometer and the reaction was started by the addition of 0.3ml of freshly prepared 0.3mM epinephrine to the mixture which was quickly mixed by inversion. The reference cuvette contained 2.5ml of carbonate buffer, 0.3ml of epinephrine and 0.2ml of water. The increase in absorbance at 480nm was monitored every 30 seconds for 150 seconds.

	Blank	Standard	Sample
Supernatant	-	-	0.2ml
Carbonate buffer`	2.5ml	-	2.5
Distilled water	0.2ml	-	-
Epinephrine	0.3ml	-	0.3ml

The increase in absorbance at 480nm was monitored every 30 seconds for 150 seconds.

CALCULATION:

Increase in absorbance per minute = A3-A0/1.5

Where A0 = Absorbance after 30 sec; A3 = Absorbance after 150 sec.

1 unit of SOD activity is given as the amount of SOD necessary to cause 50% inhibition of epinephrine

3.7.3 Catalase (CAT)

Reagents

Pipes buffer (pH 7.0, 250^0C)

Hydrogen peroxide 1.0 µmole

PROCEDURE

The enzyme source was diluted in 0.5M phosphate buffer (pH 7.0) to obtain a rate of 0.03-0.07 ΔA/min, the assay mixture was thereafter constituted by adding 1.0ml hydrogen peroxide to 1.9ml of distilled water. The mixture was incubated in spectrophotometer for 5 minutes to achieve temperature equilibrium, this was followed by the addition of 0.1ml of the enzyme source (appropriately diluted). Decrease in absorbance was monitored at 240nm for 2 mins immediately after the addition of the appropriately diluted enzyme source.

	Blank	Standard	Sample
Reagent	-	-	1ml
Distilled water	1ml	-	1.9ml
Sample	-	-	0.1ml

The decrease in absorbance was monitored at 240nm for 0seconds, 45 seconds and 2 minutes immediately after the addition of the appropriately diluted enzyme source.

CALCULATION:

Change in absorbance (change A240/min) was calculated from the initial 45 seconds

Catalase activity was obtained from the following expression:

Catalase activity (units/mg) = Change A240/min x 1000/43.6 x mg protein /ml reaction mixture

3.7.4 Cholesterol (CHOL)

Cholesterol was analysed with whole fruit flies, using Randox Kit from Randox laboratories limited UK. Protocols were followed according to the manufacturer manure procedures.

Reagents

1. **Reagents composition**

   Content

PRODUCURE:

A known volume (0.02ml) of appropriately diluted testicular supernatant was pipette into a test tube and 2.0ml of the working reagent as added. Also, 2.0ml of the working reagent was added to 0.02ml of distilled water in another test tube to constitute the blank. For the standard, 2.0ml of working reagent was added to 0.02ml of the standard reagent in another test tube. The reaction constituents were thoroughly mixed and incubated at 37 degree Celsius for 5 mins. Absorbance was read against the blank spectrophotometrically at 546nm.

Blank	**Standard**	**Sample**	
Reagent	10µl	10µl	1ml
Distilled water	-	-	-
Standard	-	10µl	-
Sample	-	-	10µl
Cholesterol	1000µl	1000µl	1000µl
	1010µl	1010µl	1010µl

The solution was incubated for 5 minutes at 37^0C. The absorbance of the sample (A sample) and standard (A standard) was read at 505nm against the reagent blank.

CALCULATION:

Concentration of cholesterol (mmol/l) = Absorbance of sample x concentration of standard / Absorbance of standard

Concentration of standard = 5.10mmol/l

3.7.5 Triglyceride (TRIG)

Reagent composition

4-chlorophenol

Magnesium ion

4-aminophenaz

PROCEDURE:

Sample was prepared by pipetting 0.01ml and adding 1ml of the enzyme reagent (pipes buffer containing 4-chlorophenol, magnesium ion, 4-aminophenazone and various enzymes) in a test tube. Standard and blank were constituted by adding 1ml of the enzyme reagent to 0.01ml standard and distilled water respectively. Samples were mixed, incubated at 37 degree celsius for minutes and absorbance read at 500nm.

	Blank	**Standard**	**Sample**
Sample	--	-	0.1ml
Blank	0.1ml	0.1ml	0.1ml
(Rlb		1000 µl	1000 µl

The samples were mixed, incubated at 37^0C for minutes and absorbance read at 500nm.

CALCULATION:

Concentration of triglyceride (mg/dl) = Absorbance of sample x concentration of standard / Absorbance of standard

3.7.6 High density lipoprotein (HDL)

Reagents Composition

Phosphotungstic Acid/Magnesium Chloride

Cholesterol Standard

Randox HDL Rl

PROCEDURE

A known sample (0.2ml) of serum was added to 0.5ml of phosphotungstic acid/ magnesium chloride solution, mixed and left for 10 mins at 250C, after which it was centrifuged for 10mins in order to remove non-HDL lipoproteins. After that, samples were prepared by pipetting 0.05ml of serum (supernatant) into test tubes. Standards and blank were prepared by pipetting 0.05ml each of the standard and distilled water into appropriately labelled tubes. This was followed by the addition of 1ml phosphotungstic acid (CHOL reagent) into each well. Samples were mixed, incubated at 370C for 5mins and absorbance read at 500nm within 60 mins.

	Blank	**Standard**	**Sample**
Cholesterol Standard	20 µl	-	-
Randox HDL RL	500 µl	-	500 µl
Sample	-	-	20µl

The samples were mixed, centrifuge at 4000rpm, incubated at 37^0C and absorbance read at 500nm within 60 minutes.

CALCULATION:

Concentration of HDL cholesterol (mg/dl) = Absorbance of sample x concentration of standard/ Absorbance of standard

3.7.7 Low-density lipoprotein (LDL):

The low density lipoprotein was gotten from friedweld formula, where the Triglyceride is divided by a total number of 5.

For low-density lipoprotein, we have;

LDL-cholesterol (mg/dl) = TG/5

Where TC = Total cholesterol, TG = Triglyceride, HDLC = HDL-cholesterol total cholesterol, TG = triglyceride, HDLC = HDL-cholesterol

3.7.8 Very low-density lipoprotein:

The VLDL is gotten by subtracting the total cholesterol from the answer gotten from Triglyceride divided by a total number of 5.

For very low-density lipoprotein, we have;

VLDL = Total cholesterol - Trig-HDL/5

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Biochemical Assay

4.1.1 Effect of Bigi brands (cola and orange) soft drink on MDA of treated *D. melanogaster*

Biochemical effects of the MDA (LIPID PEROXIDATION) on the activity of Drosophila Melanogaster on soft drinks at different given concentration are significantly different when experimented ($p = 0.012 < 0.05$, $F = 4.295$, $df = 6$). The most effective activity took place on 100 Concentration (Bigi Cola) (Mean = 170.150, 53.400), followed by 100 Concentration (Orange), (mean = 115.953, 4.54), next to it is 50 concentration (Bigi Orange) (Mean =115.803, 15.805) while 25 Concentration (Cola) show least activity (mean = 60.693, 2.065) Therefore null hypothesis is rejected

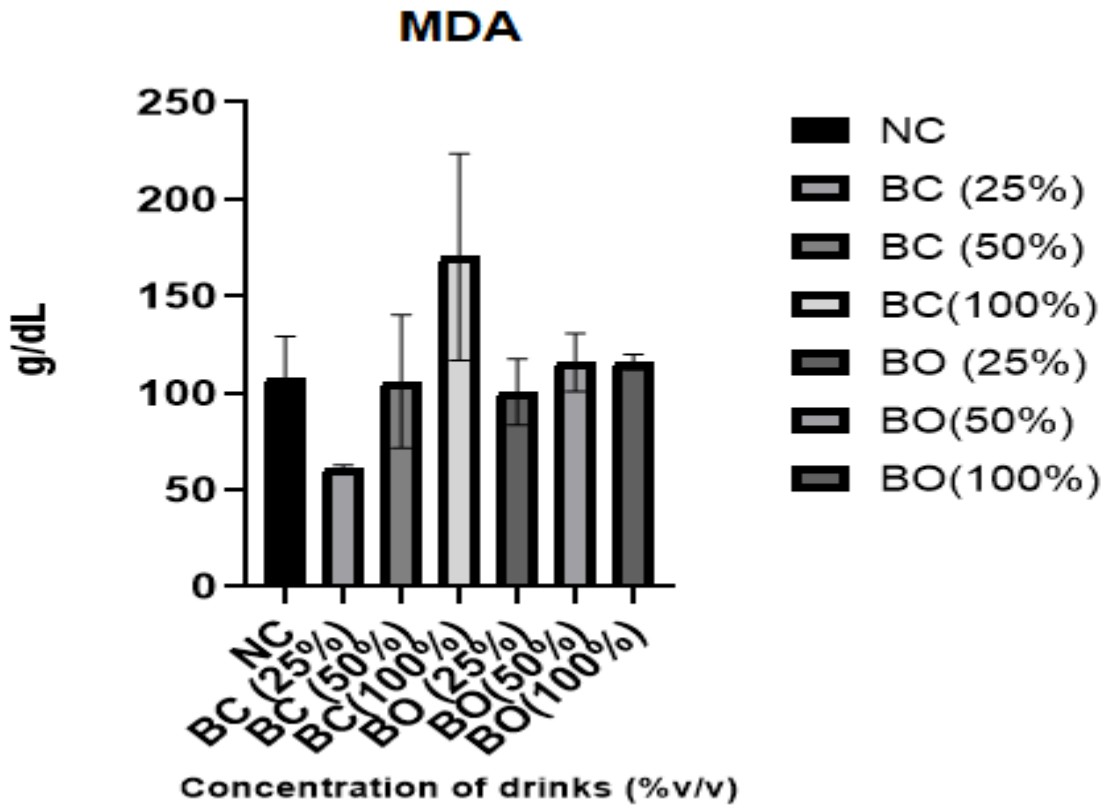


Figure 4: Effect of Bigi brands (cola and orange) soft drink on MDA of treated *D. melanogaster*

Mean ± SD, where; p value = 0.012(< 0.05).

NC – Negative Control

BC – Bigi Cola soft drink

BO – Bigi Orange soft drink.

4.1.2 Effect of Bigi brands (cola and orange) soft drink on SOD of treated *D. melanogaster*

The effects of SOD (Super Oxide Dismutase) activity on the soft drinks (Bigi cola and Bigi orange) at different given concentration are significantly different when examined ($p = 0.054 < 0.05$, $F = 2.681$, $df = 6$). The most potent activity took place on Negative Control (NC)

(Mean = 99670.00, 130.00), followed by 25 concentration (Orange), (mean = 99626.673, 102.643), next to it is 50 concentration (Orange) (mean = 99500.016, .021) while 100 concentration (Cola) show least activity (mean = 99113.337, 7370.22), Therefore null hypothesis is rejected

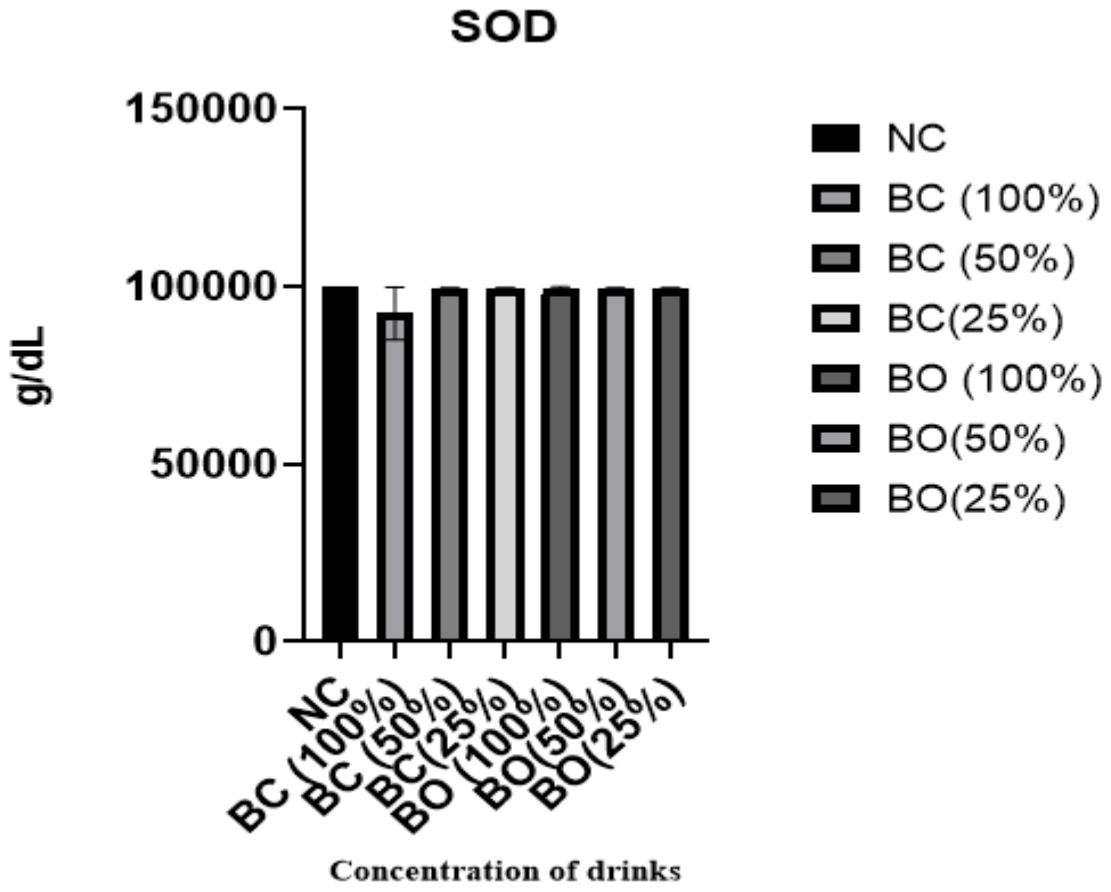


Figure 5: Effect of Bigi brands (cola and orange) soft drink on SOD of treated *D. melanogaster*

Mean ± SD, where; p value = 0.054(< 0.05).

NC – Negative Control

BC – Bigi Cola soft drink

BO – Bigi Orange soft drink.

4.1.3: Effect of Bigi brands (cola and orange) soft drink on CAT of treated *D. melanogaster*

Biochemical effects of the CATALASE on the activity of Drosophila Melanogaster on soft drinks at different given concentration are significantly different when investigated ($p = 0.032 < 0.05$, $F = 3.266$, $df = 6$). The most potent activity took place on 100 concentration Bigi Orange (Mean = 254.65, 43.51), followed by Negative control, (mean = 246.7167, 68.185), next to it is 50 concentration (Bigi Cola) (Mean = 203.240, 77.805) while 25 concentration (Bigi Cola) show least activity (mean = 121.2467, 4.895) Therefore null hypothesis is rejected.

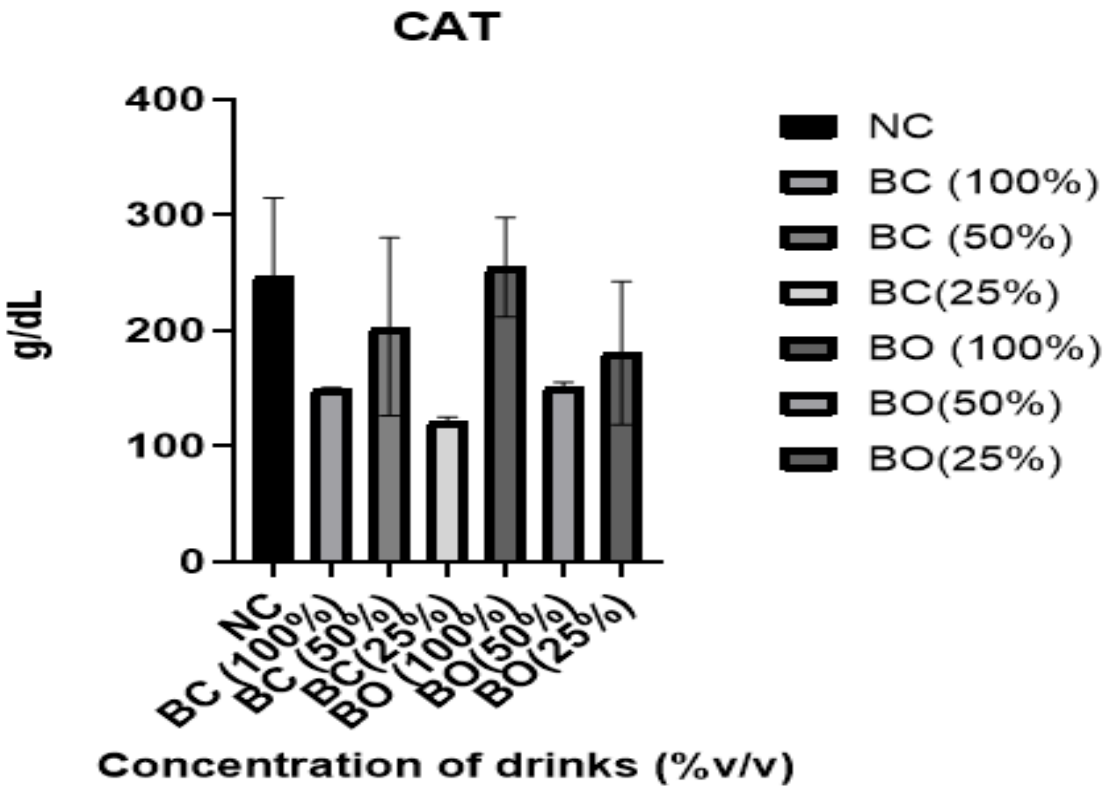


Figure 6: Effect of Bigi brands (cola and orange) soft drink on CAT of treated *D. melanogaster*

Mean $\pm$ SD, where; p value = 0.032(< 0.05).

NC – Negative Control

BC – Bigi Cola soft drink

BO – Bigi Orange soft drink.

4.1.4: Effect of Bigi brands (cola and orange) soft drink on CHOL of treated *D. melanogaster*

Biochemical effects of the CHOLESTEROL on the activity of *Drosophila Melanogaster* on soft drinks at different given concentration are significantly different when experimented ($p = 0.053 < 0.05$, $F = 2.515$, $df = 6$). The most effective activity took place on 50 concentration Bigi Orange (Mean = 73.33, 6.66), followed by 25 concentration Bigi Cola, (mean = 57.999, 4. 666), next to it is 50 concentration (Bigi Cola) (Mean =50.666, 4.000) while 25 concentration (Bigi Orange) show least activity (mean = 41.00, 3.000) Therefore null hypothesis is rejected.

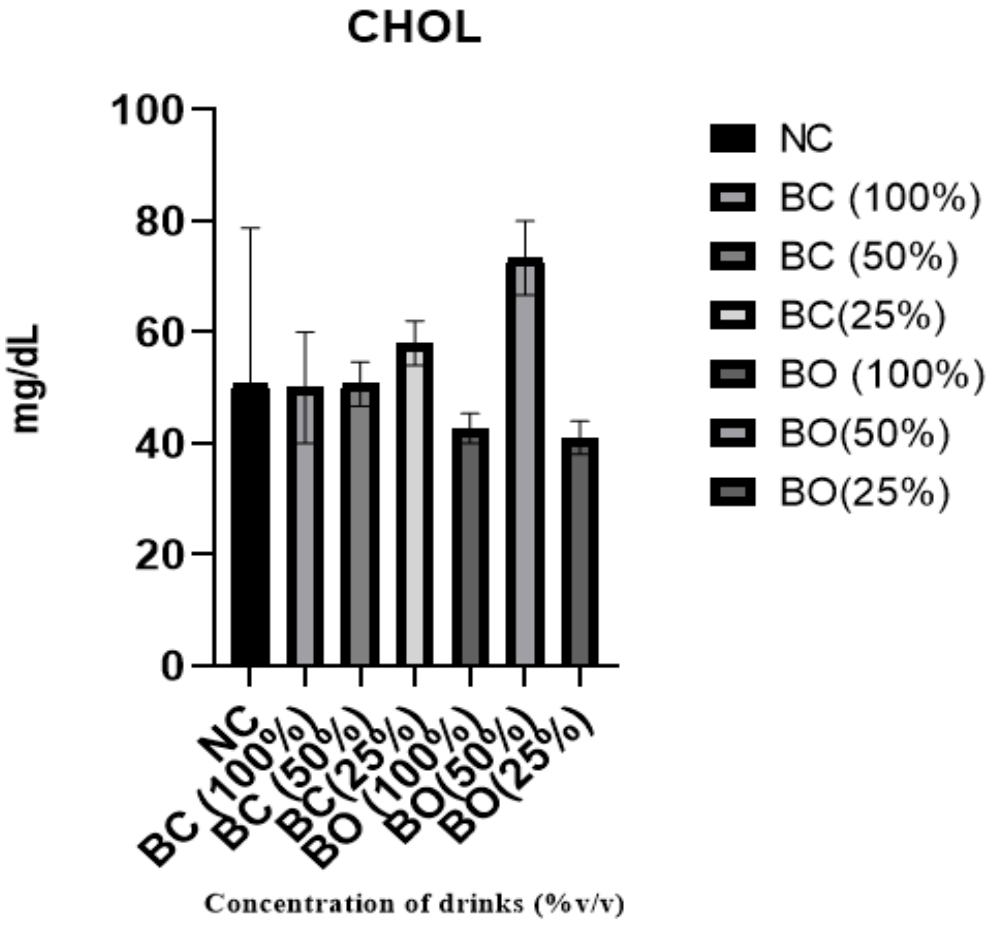


Figure 7: Effect of Bigi brands (cola and orange) soft drink on CHOL of treated *D. melanogaster*

Mean ± SD, where; p value = 0.053(< 0.05).

NC – Negative Control

BC – Bigi Cola soft drink

BO – Bigi Orange soft drink.

4.1.5 Effect of Bigi brands (cola and orange) soft drink on TRIG of treated *D. melanogaster*

Biochemical effects of the **TRIGLYCERIDE** on the activity of *Drosophila Melanogaster* on soft drinks (Bigi cola and Bigi orange) at different given concentration are significantly different when investigated ($p = 0.000 < 0.05$, $F = 11.749$, $df = 6$). The most potent activity took place on Negative Control (NC) (Mean = 573.3267, 215.02), followed by 100 concentration (Bigi Cola), (mean = 366.66, 20.00), next to it is 50 concentration (Bigi Cola) (Mean = 356.66, 3.335) while 100 concentration (Orange) show least activity (mean = 123.66, 3.67), Therefore null hypothesis is rejected.

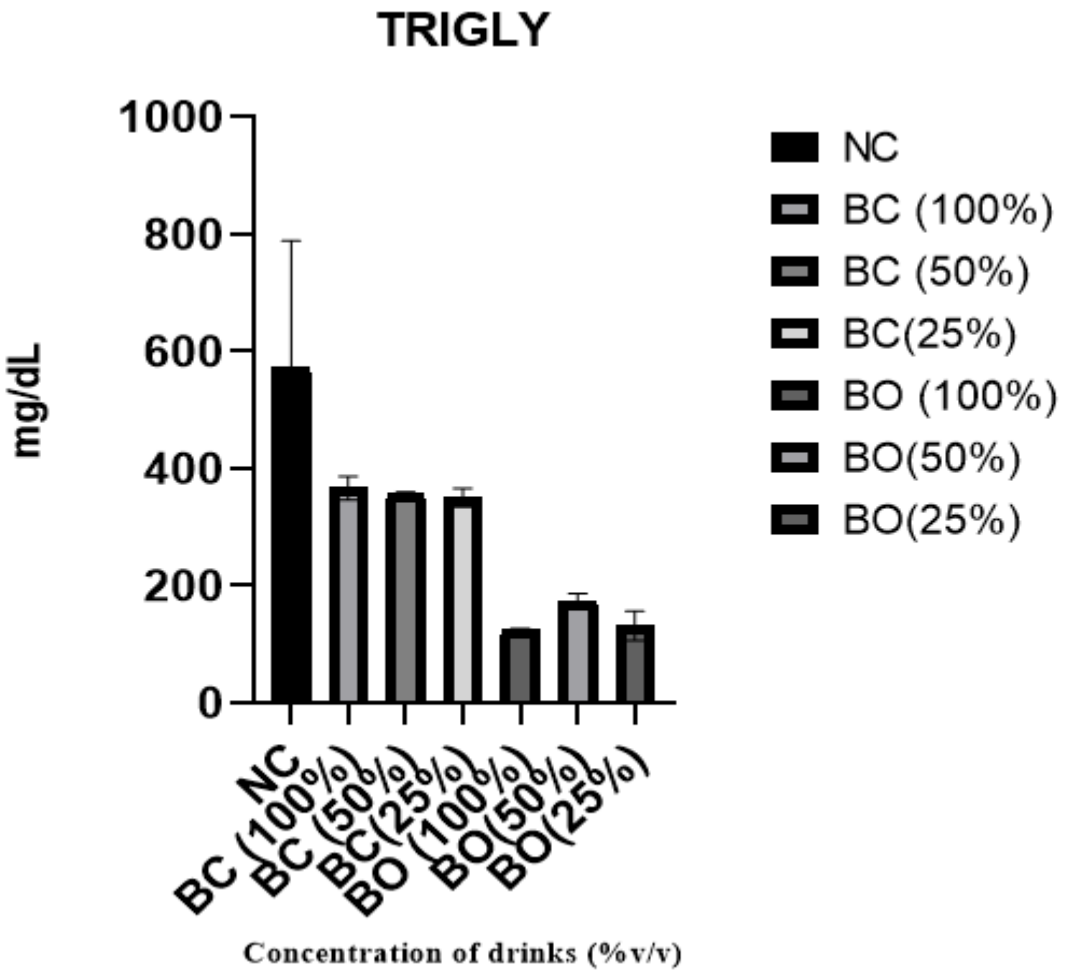


Figure 8: Effect of Bigi brands (cola and orange) soft drink on TRIG of treated *D. melanogaster*

Mean ± SD, where; p value = 0 (< 0.05).

NC – Negative Control

BC – Bigi Cola soft drink

BO – Bigi Orange soft drink.

4.1.6 Effect of Bigi brands (cola and orange) soft drink on HDLC of treated *D. melanogaster*

Biochemical effects of the HIGH DENSITY LIPOPROTEIN (HDLC) on the activity of *Drosophila Melanogaster* on soft drinks at different given concentration are significantly different when experimented ($p = 0.008 < 0.05$, $F = 2.181$, $df = 6$). The most active activity took place on 25 Concentration (Cola) (Mean = 44.57, 89.664), followed by 100 Concentration (Orange), (mean =-53.33, 101.094), next to it is 25 Concentration (Orange) (Mean =-58.109, 8.756) while Negative Control (NC) show least activity (mean = -184.67, 127.363) Therefore null hypothesis is rejected.

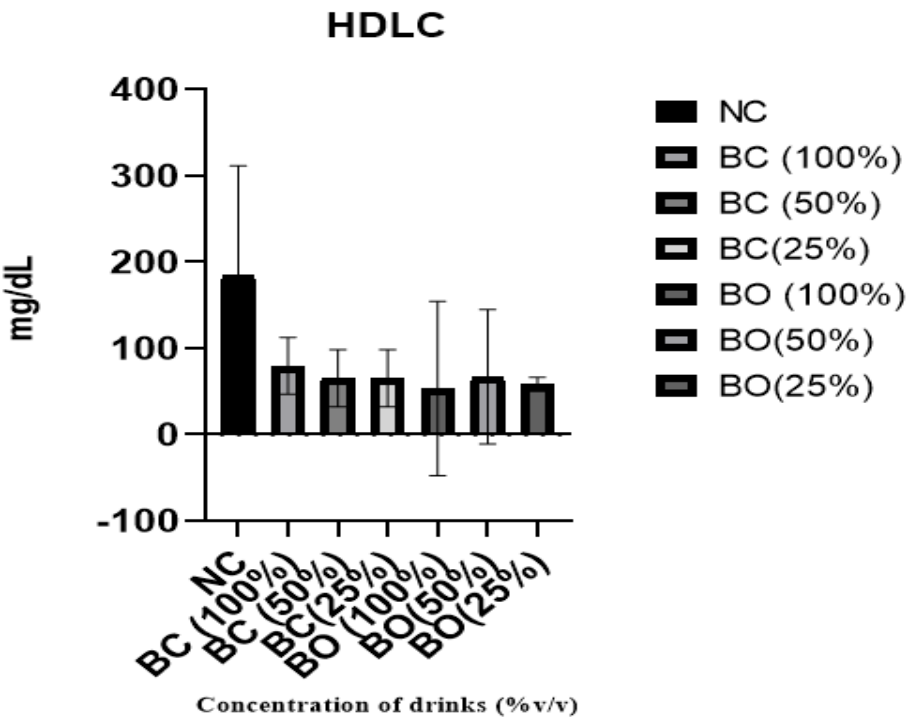


Figure 9: Effect of Bigi brands (cola and orange) soft drink on HDLC of treated *D. melanogaster*

Mean ± SD, where; p value = 0.008 (< 0.05).

NC – Negative Control

BC – Bigi Cola soft drink

BO – Bigi Orange soft drink.

4.1.7 **Effect of Bigi brands (cola and orange) soft drink on LDLC of treated *D. melanogaster***

Biochemical effects of the **LOW DENSITY LIPOPROTEIN (LDLC)** on the activity of *Drosophila Melanogaster* on soft drinks at different given concentration are significantly different when experimented ($p = 0.000 < 0.05$, $F = 2.515$, $df = 6$). The most effective activity took place on Negative Control (NC) (Mean = 114.665, 43.003), followed by 100 Concentration (Cola), (mean = 73.33, 4.00), next to it is 50 concentration (Bigi Cola) (Mean =71.332, .66700) while 100 concentration (Bigi Orange) show least activity (mean = 24.732, 0.733) Therefore null hypothesis is rejected.

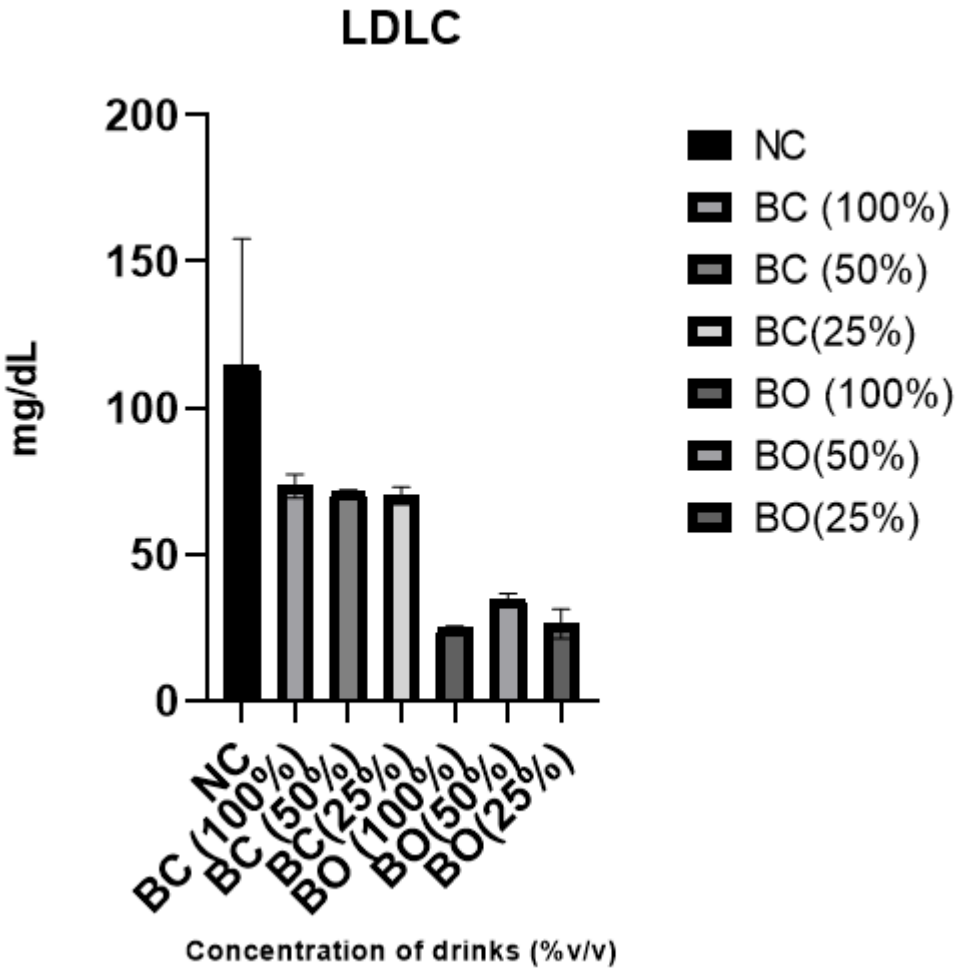


Figure 10: Effect of Bigi brands (cola and orange) soft drink on LDLC of treated *D. melanogaster*

Mean ± SD, where; p value = 0 (< 0.05).

NC – Negative Control

BC – Bigi Cola soft drink

BO – Bigi Orange soft drink.

4.1.8 Effect of Bigi brands (cola and orange) soft drink on VLDL of treated *D. melanogaster*

Biochemical effects of the VERY LOW DENSITY LIPOPROTEIN (VLDL)**,** activity of *Drosophila Melanogaster* on soft drinks at different given concentration are not significantly different when tested ($p = 0.228 > 0.05$, $F = 1.570$, $df = 6$). It means that various concentration of the soft drinks have no effects of activities of *Drosophila Melanogaster* at 95% significant level. Therefore null hypothesis is accepted.

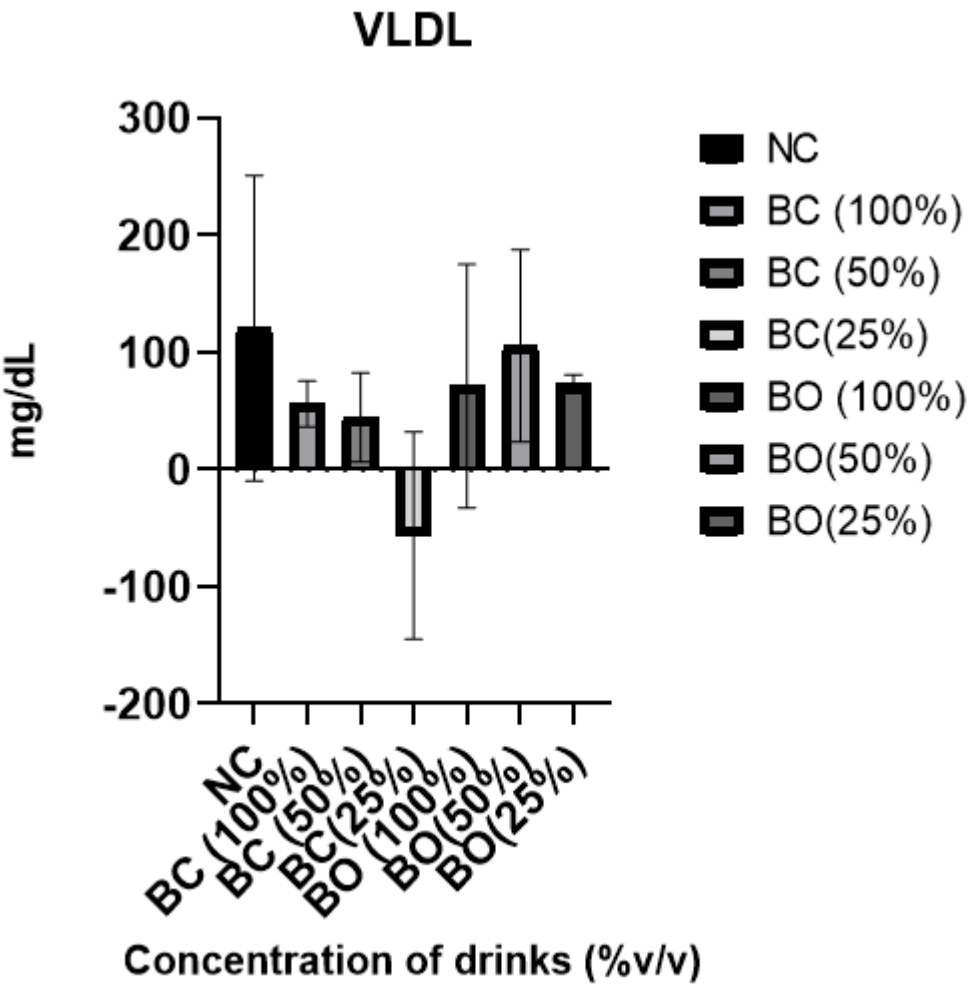


Figure 11: Effect of Bigi brands (cola and orange) soft drink on VLDL of treated D. melanogaster

Mean ± SD, where; p value = 0.228 (< 0.05).

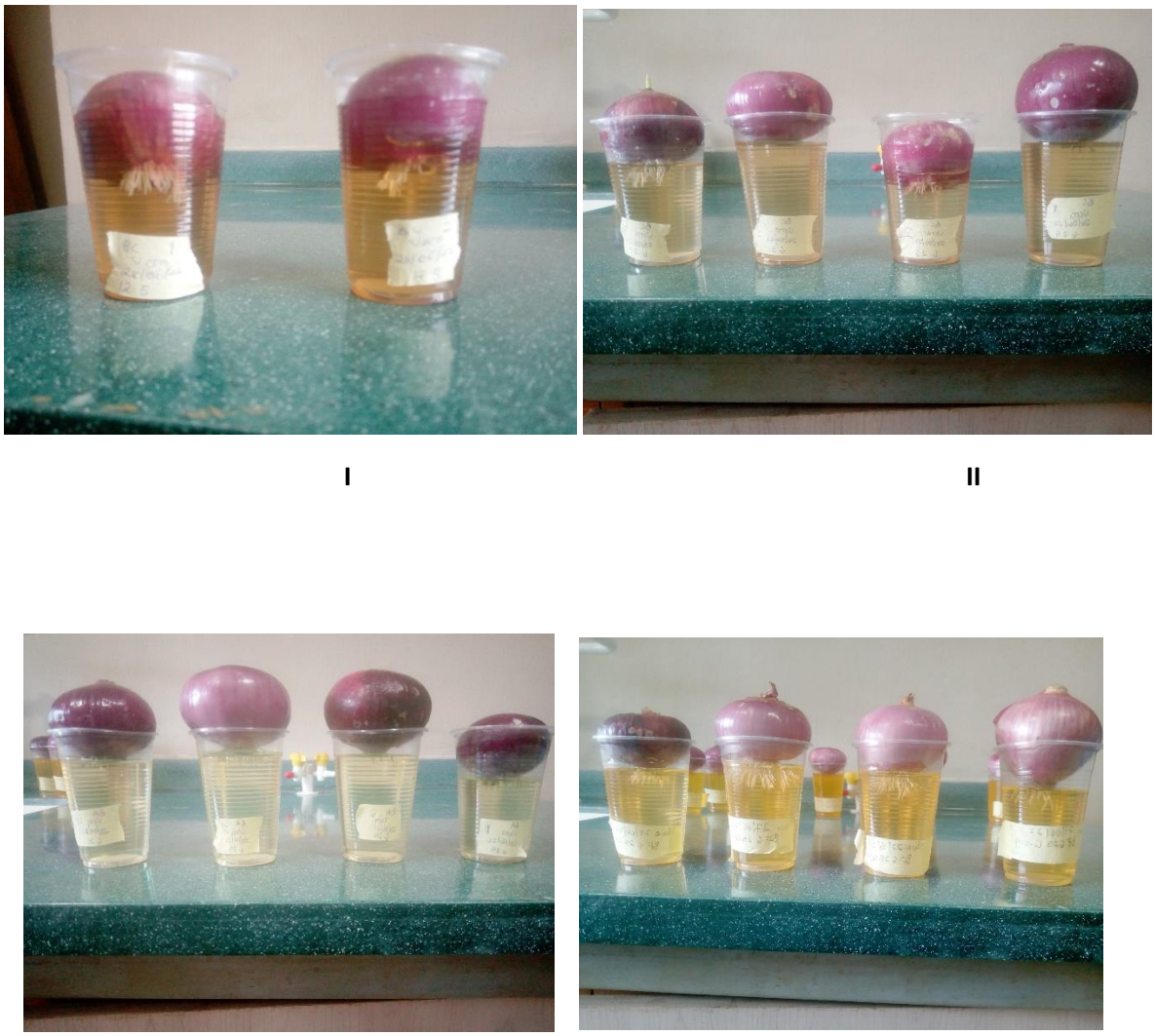
NC – Negative Control

BC – Bigi Cola soft drink

BO – Bigi Orange soft drink.

4.2Toxic Effect of Bigi brands (cola and orange) soft drink on Root Growth in treated *A. Cepa*

There was good growth in the Negative control borehole water, the roots of bulb grown in the water was visible. The Bigi cola concentrations at 6.25% &12.5% the root tips were short, scanty, pointed root tips but visible, at 25% it was little or stunted growth. The Bigi orange for the root tips at 6.25% there was visible growth, at 12.5% there were scanty growth, at 25% there were little or no growth. Although subsequent observation on the bigi orange it still maintained its colour of its root tips.



I II

III IV

Figure 12: Toxic Effect of Bigi brands (cola and orange) soft drink on Root Growth in treated *A. Cepa*

(I)Short, scanty, pointed root tips (II) little or stunted growth (III) Visible growth (IV) little or no growth.

4.3 Effect of Bigi brands (cola and orange) soft drink on Root Growth of treated *A.cepa*

The root growth inhibition indicates the length of the root tips, also the negative control is the standard used for comparison with concentrations the Bigi cola 25%concentrat]]ion ($P<0.05$).has more root growth than 6.25% & 12.5%, when the negative control was compared with the samples/concentrations. However, Bigi orange cola 25 concentration ($P<0.05$).has more root

growth than 6.25% & 12.5%, when the negative control was compared with the samples/concentrations. However, the survival results discuss more on the rate at which the fruit flies lived, after that was later homogenized for biochemical parameters to take place.

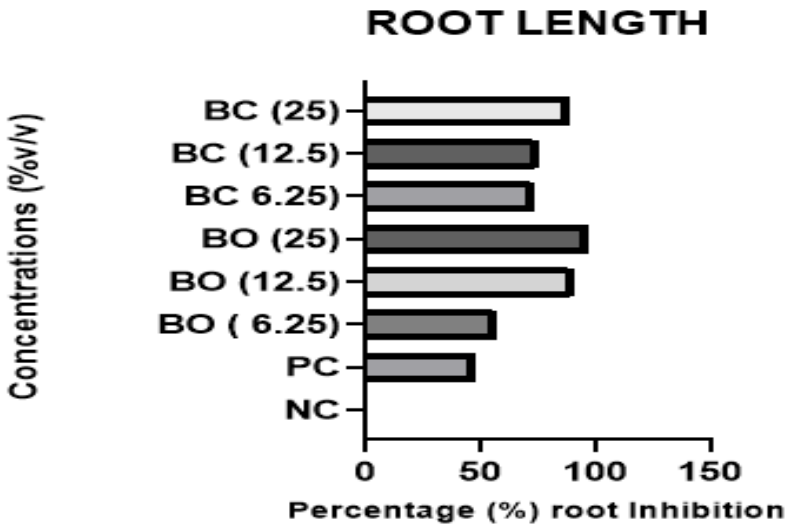


Figure 13: Effect of Bigi brands (cola and orange) soft drink on Root Growth of treated *A.cepa*

4.4 DISCUSSION

The effects of the concentration of the soft drinks on biochemical parameters in the induced *D. melanogaster* were investigated in this study. The ingredients/components of the drinks might not be healthy for human beings so a mimicry of the project was performed on a model which is widely known "As *Drosophila melanogaster (D. melanogaster)*, a type of fruit fly, because of its physiological effect that occurs in humans/mammals, this fly has become the main invertebrate model used to study developmental genetics which has been used for over a century.

The fruit fly was exposed for 10 days, homogenization took place and biochemical parameters were conducted, the effective activities of some parameters TRIG, HDL, LDL, MDA, and CAT were of significant values ($P<0.05$). Moreover for the **MDA,** at 100 µ*l* bigi cola, there was a significant increase when compared with the control, at 50 µ*l* there is no significant difference between the cola and negative control, while 25 µ*l* bigi cola decreased in lipid peroxidation in comparison between the bigi cola and control. Also, 100µ*l*, 50 µ*l,* 25 µ*l*, of bigi orange had no significant difference in comparison between the bigi orange and control. It is said that, an increase in the MDA level in the cerebrospinal fluid can cause oxidative damage to the brain (Mahadik,. *et al,* 2001). This type of damage can be prevented with antioxidants and cyto-protective enzymes.

However for the **SOD**, at 100 µ*l* bigi cola there was a decrease in the activity, for 50 µ*l* & 25 µ*l* bigi cola did not produce this kind of effect. Also, 100µ*l*, 50µ*l*, &25µ*l*, there was no significance in comparison between the bigi orange and the control. (*Fabrizio. et al*, 2003) postulated that, in Saccharomyces cerevisiae and Drosophila models, increase of SOD protects against oxidative damage and extend life span. Feeding SOD mimetic drugs, such as Euk-8 and MitoQ, increase lifespan in Drosophila melanogaster.

Moreover, for the **CAT** 100µ*l*, 50µ*l* & 25 µ*l* there is a decrease in the activity in comparison between the bigi cola and the control. Also, 100 µ*l* there was no significant difference in comparison between the orange and control, at 50 µ*l* & 25 µ*l* there was a decrease in CAT in comparison between the orange and control. An increase in **CAT** should not be considered as a bad, but a decrease in it can destroy the cell membranes, cause pain, makes the hair turn grey, and it causes peroxidation in the lipids which leads to bad cholesterol ratios, diabetes and heart attack. (Goodsell., 2004).

However, for the **CHOL** at 100 µ*l* & 50 µ*l* there was no significant difference in comparison between bigi cola and negative control, 25 µ*l* had a significant increase in the activity when compared with the bigi cola and control. Also, at 100 µ*l* there was a decrease in the activity, 50 µ*l* there was a significant increase in comparison between the bigi orange and control, 25 µ*l* there was a decrease in the activity. According to, (Mahley. *et al*, 2016) an increase in cholesterol can develop fatty deposits in the blood vessels. Eventually, these deposits grow, making it difficult for enough blood to flow through the arteries. Sometimes, those deposits can break suddenly and form a clot that causes a heart attack or stroke.

Moreover, the **TRIG** at 100 µ*l* 50 µ*l*, 25 µ*l*, there is a significant decrease in the activity between the bigi cola and control. Also, 100 µ*l* 50 µ*l*, 25 µ*l*, there is a significant decrease in the activity between the bigi orange and negative control. Hardening of the arteries or thickening of the artery walls (arteriosclerosis) — which increases the risk of stroke, heart attack and heart disease are the effects of the increase of Triglycerides in the body. Extremely high triglycerides can also cause acute inflammation of the pancreas (pancreatitis). (Karen,2008)

The **HDL** at 100 µ*l*,&50 µ*l*, there is a significant decrease in the activity between the cola and negative control, 25 µ*l* there was a significant increase in comparison between the bigi cola and control. Also, at 100 µ*l* 50 µ*l*, 25 µ*l*, there is a significant decrease in the activity between the orange and control. Increase in HDL levels could slow the process of clearing LDL cholesterol from the arteries. (Razin, *et al*, 2006)

Moreover, the **LDL** at 100 µ*l*, 50 µ*l*, 25 µ*l*, there is a significant decrease in the activity between the bigi cola and control. Also, 100 µ*l*, 50 µ*l*, 25 µ*l*, there is a significant decrease in the activity between the bigi orange and control. When LDL cholesterol builds up in these blood vessels, it forms clumps called plaques that slow or block blood flow. Eventually a chunk of plaque can break free and form a clot, which could lead to a heart attack or stroke. (Gordon, et al, 2001).

Lastly, the **VLDL** at 100 µ*l*, 50 µ*l*, 25 µ*l*, there is a significant decrease in the activity between the bigi cola and control. Also, 100 µ*l*, 50 µ*l*, 25 µ*l*, there is a significant decrease in the activity between the bigi orange and control. VLDL carries triglycerides in the bloodstream. Once the body extracts the triglycerides, cholesterol-rich LDL remains. Increase in VLDL will only yield to increase in LDL after extraction. (Gibbons G.F, *et al*, 2004)

CHAPTER FIVE

5.0 CONCLUSION

Based on this study, it was observed that 100 BC concentration elevated MDA (Lipid peroxidation), reduced Catalase, Triglyceride, High density lipoprotein activity levels. Moreover, care must be taken on the consumption of this product because it has the ability to induce oxidative stress & distort the lipid profile in the D. melanogaster. The results for both drinks on 6.25% & 12.5 % had shorter root tips in comparison with the 25%, this might be as a result of the of the caffeine content in soft drinks that causes change in the root tips of the onions, although the lesser the dilution the concentration with water the longer the root tips.

5.1 RECOMMENDATION

Further investigation should be done on the effect of various extracts with soft drinks (ethanol) on the biochemical assays.

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APPENDIX

1. **Preparation of Phosphate Buffer Saline**

800ml of distilled water was prepared in a suitable measuring cylinder, 8g of sodium chloride, 0.2g of potassium chloride, 1.44g of sodium phosphate dibasic, 0.245g of potassium phosphate monobasic, was poured into conical flasks separately, the chemicals were dissolved in little quantity of water, the dissolved chemicals was poured into a beaker and stirred gently. The solution was poured into the 800ml of distilled water, the PH was checked to attain 7.4.

2. **Preparation of Ethanol Glacial Acetic Acid (fixative)**

5ml of Acetic acid was dissolved into 15ml of Ethanol. It was warm.

3. **Preparation of 70% Ethanol**

70ml of Ethanol and 30ml of Distilled water was poured into a measuring cylinder separately, the 70 ml ethanol was diluted into the distilled water.

4. **Preparation of 1µ HCL**

8.6 ml of Hydrochloric acid was dissolved in 91.4ml of water. In a 1000ml measuring cylinder, the warmness of the 1µ HCL indicated that the hydrochloric acid was concentrated, and it's pungent/choking smell.

5. **Dilution of drinks**

For the drinks respectively Bigi (Cola & Orange), for each concentration the calculate measurement was used;

6.25% of Cola and Orange

$400/100 \times 6.25$

= 25ml of drink,

375ml of water.

12.5% of Cola and Orange

500/100 × 12.5

= 437.5ml of water,

6.25ml of drink.

25% of Cola and Orange

400/100 × 25

= 100ml of drink,

300ml of water

6. MALONDIALDEHYDE (MDA) PARAMETERS

MDA (LIPID PEROXIDATION)	Activity
Negative control (NC)	
1	86.18
2	129.16
3	107.67
Bigi cola	
100 concentration 1	116.75
100 concentration 2	223.55
100 concentration 3	170.15
50 Concentration 1	140.38
50 Concentration 2	71.59
50 Concentration 3	105.98
25 concentration 1	62.76
25 concentration 2	58.63
25 concentration 3	60.69
Bigi Orange	
100 concentration 1	110.75
100 concentration 2	119.16
100 concentration 3	117.95
50 Concentration 1	131.61
50 Concentration 2	100.00
50 Concentration 3	115.80
25 concentration 1	117.85
25 concentration 2	83.27
25 concentration 3	100.56

7. SOD PARAMETERS

SOD (Super Oxide Dismutase)	Activity
Negative control (NC)	
1	99540
2	99800
3	99670
Bigi cola	
100 concentration 1	99740
100 concentration 2	85000
100 concentration 3	92470
50 Concentration 1	99740
50 Concentration 2	99340
50 Concentration 3	99200
25 concentration 1	99740
25 concentration 2	98534
25 concentration 3	99137
Bigi Orange	
100 concentration 1	98340
100 concentration 2	99800
100 concentration 3	99200
50 Concentration 1	99500
50 Concentration 2	99500
50 Concentration 3	99500
25 concentration 1	99740
25 concentration 2	99540
25 concentration 3	99600

8. CATALASE (CAT) PARAMETERS

CATALASE	Activity
Negative control (NC)	
1	178.56
2	314.93
3	246.66
Bigi cola	
100 concentration 1	148.56
100 concentration 2	150.99
100 concentration 3	148.96
50 Concentration 1	125.43
50 Concentration 2	281.04
50 Concentration 3	203.25
25 concentration 1	126.14
25 concentration 2	116.35
25 concentration 3	121.25
Bigi Orange	
100 concentration 1	211.15
100 concentration 2	298.16
100 concentration 3	254.65
50 Concentration 1	155.96
50 Concentration 2	146.78
50 Concentration 3	151.37
25 concentration 1	249.50
25 concentration 2	164.89
25 concentration 3	127.43

9. CHOLESTEROL PARAMETERS

CHOLESTEROL	Activity
Negative control (NC)	
1	22.6666
2	78.6666
3	50.6666
Bigi cola	
100 concentration 1	60.0000
100 concentration 2	40.0000
100 concentration 3	50.0000
50 Concentration 1	46.6666
50 Concentration 2	54.6666
50 Concentration 3	50.6666
25 concentration 1	53.3333
25 concentration 2	62.6666
25 concentration 3	57.9999
Bigi Orange	
100 concentration 1	45.3333
100 concentration 2	40.0000
100 concentration 3	42.6666
50 Concentration 1	66.6666
50 Concentration 2	80.0000
50 Concentration 3	73.3333
25 concentration 1	44.0000
25 concentration 2	38.0000
25 concentration 3	41.0000

10. TRIGLYCERIDE (TRIG) PARAMETERS

TRIGLYCERIDE	Concentration
Negative control (NC)	
1	336.66
2	626.66
3	756.66
Bigi cola	
100 concentration 1	346.66
100 concentration 2	386.66
100 concentration 3	366.66
50 Concentration 1	360.00
50 Concentration 2	353.33
50 Concentration 3	356.66
25 concentration 1	333.33
25 concentration 2	366.66
25 concentration 3	350.00
Bigi Orange	
100 concentration 1	120.00
100 concentration 2	127.33
100 concentration 3	123.66
50 Concentration 1	160.00
50 Concentration 2	186.66
50 Concentration 3	173.33
25 concentration 1	160.66
25 concentration 2	120.00
25 concentration 3	113.33

11. HIGH DENSITY LIPOPROTEIN (HDL) PARAMETERS

HIGH DENSITY LIPOPROTEIN (HDL)	Activity
Negative control (NC)	
1	-57.3134
2	-312.0398
3	-184.6766
Bigi cola	
100 concentration 1	-46.1691
100 concentration 2	-113.0348
100 concentration 3	-79.6019
50 Concentration 1	-31.8407
50 Concentration 2	-98.7064
50 Concentration 3	-65.2735
25 concentration 1	-54.1293
25 concentration 2	120.9950
25 concentration 3	66.8657
Bigi Orange	
100 concentration 1	-154.4278
100 concentration 2	47.7611
100 concentration 3	-53.3333
50 Concentration 1	11.1442
50 Concentration 2	-144.8756
50 Concentration 3	-66.8657
25 concentration 1	-66.8656
25 concentration 2	-49.3532
25 concentration 3	-58.1094

12. LOW DENSITY LIPOPROTEIN (LDL) PARAMETERS

LOW DENSITY LIPOPROTEIN (LDL)	Activity
Negative control (NC)	
1	67.332
2	125.332
3	151.332
Bigi cola	
100 concentration 1	69.332
100 concentration 2	77.332
100 concentration 3	73.332
50 Concentration 1	72.000
50 Concentration 2	70.666
50 Concentration 3	71.332
25 concentration 1	66.666
25 concentration 2	73.332
25 concentration 3	70.000
Bigi Orange	
100 concentration 1	24.000
100 concentration 2	25.466
100 concentration 3	24.732
50 Concentration 1	32.000
50 Concentration 2	37.332
50 Concentration 3	34.666
25 concentration 1	32.132
25 concentration 2	24.000
25 concentration 3	22.666

13. VERY LOW DENSITY LIPOPROTEIN (VLDL) PARAMETERS

VERY LOW DENSITY LIPOPROTEIN (VLDL)	Activity
Negative control (NC)	
1	12.6480
2	265.3744
3	84.0112
Bigi cola	
100 concentration 1	36.8371
100 concentration 2	75.7028
100 concentration 3	55.2699
50 Concentration 1	6.5073
50 Concentration 2	82.7064
50 Concentration 3	44.6081
25 concentration 1	40.7966
25 concentration 2	-131.6604
25 concentration 3	-78.8658
Bigi Orange	
100 concentration 1	175.7611
100 concentration 2	-33.2271
100 concentration 3	71.2679
50 Concentration 1	23.5224
50 Concentration 2	187.5436
50 Concentration 3	105.5330
25 concentration 1	78.7336
25 concentration 2	63.3532
25 concentration 3	76.4434

14. Effect of Bigi brands of soft drink on MDA of treated *D. melanogaster*

Chemical/Enzyme Activities		
Products/ Concentrations	Mean	Standard Deviation (STD)
Negative control	107.6700	21.49000
100 concentration (Bigi Cola)	170.1500	53.40000
50 concentration (Bigi Cola)	105.9833	34.39500
25 concentration Bigi Cola	60.6933	2.06500
100 concentration Bigi Orange	115.9533	4.54665
50 concentration Bigi Orange	115.8033	15.80500
25 concentration Bigi Orange	100.5600	17.29000

	Sum of squares	df
Between Groups	18667.006	6
Within Groups	10140.149	14
Total	28807.155	20

Mean square	F	P
3111.168	4.295	0.012
724.296		

15. Effect of Bigi brands of soft drink on SOD of treated *D. melanogaster*

Chemical/Enzyme Activities		
Products/ Concentrations	Mean	Standard Deviation (STD)
Negative control	99670	130
100 concentration (Bigi Cola)	92403.33	7370.23
50 concentration	99426.677	280.24

(Bigi Cola)		
25 concentration Bigi Cola	99137.001	603.00
100 concentration Bigi Orange	99113.34	733.85
50 concentration Bigi Orange	99500.02	102.02
25 concentration Bigi Orange	99626.67	102.64

	Sum of squares	df
Between Groups	127178739.6394	6
Within Groups	110656710.6547	14
Total	237835450.2941	20

Mean square	F	P
21196456.60656	2.681	0.054
7904050.761052		

16. Effect of Bigi brands of soft drink on CAT of treated *D. melanogaster*

Chemical/Enzyme Activities		
Products/ Concentrations	Mean	Standard Deviation (STD)
Negative control	246.7167	68.18502
100 concentration (Bigi Cola)	149.5033	1.30293
50 concentration (Bigi Cola)	203.2400	77.80500
25 concentration	121.2467	4.89500

Bigi Cola		
100 concentration Bigi Orange	254.6533	43.50500
50 concentration Bigi Orange	151.3700	4.59000
25 concentration Bigi Orange	180.6067	62.53424

	Sum of squares	df
Between Groups	46338.806	6
Within Groups	33105.516	14
Total	79444.322	20

Mean square	F	P
7723.134	3.266	.032
2364.680		

17. Effect of Bigi brands of soft drink on CHOL of treated *D. melanogaster*

Chemical/Enzyme Activities		
Products/ Concentrations	Mean	Standard Deviation (STD)
Negative control	50.6666	28.00000
100 concentration (Bigi Cola)	50.0000	10.00000
50 concentration (Bigi Cola)	50.6666	4.00000
25 concentration Bigi Cola	57.9999	4.66665

100 concentration Bigi Orange	42.6666	2.66665
50 concentration Bigi Orange	73.3333	6.66670
25 concentration Bigi Orange	41.0000	3.00000

	Sum of squares	df
Between Groups	2117.997	6
Within Groups	1964.667	14
Total	4082.664	20

Mean square	F	P
352.999	2.515	.053
140.333		

18. Effect of Bigi brands of soft drink on TRIG of treated *D. melanogaster*

Chemical/Enzyme Activities		
Products/ Concentrations	Mean	Standard Deviation (STD)
Negative control	573.33	215.01938
100 concentration (Bigi Cola)	366.66	20.000
50 concentration (Bigi Cola)	356.66	3.335
25 concentration Bigi Cola	349.997	16.665
100 concentration Bigi Orange	123.66	3.665
50 concentration	173.33	13.330

Bigi Orange		
25 concentration Bigi Orange	131.33	25.6185

	Sum of squares	df
Between Groups	481084.20	6
Within Groups	95539.216	14
Total	576623.416	20

Mean square	F	P
80180.700	11.749	0
6824.230		

19. Effect of Bigi brands of soft drink on HDLC of treated *D. melanogaster*

Chemical/Enzyme Activities		
Products/ Concentrations	Mean	Standard Deviation (STD)
Negative control	-184.6766	127.36320
100 concentration (Bigi Cola)	-79.6019	33.43285
50 concentration (Bigi Cola)	-65.2735	33.43285
25 concentration Bigi Cola	44.5771	89.66446
100 concentration Bigi Orange	-53.3333	101.09445
50 concentration Bigi Orange	-66.8657	78.00990
25 concentration	-58.1094	8.75620

Bigi Orange		

	Sum of squares	df
Between Groups	80160.595	6
Within Groups	85757.829	14
Total	165918.423	20

Mean square	F	P
13360.099	2.181	0.008
6125.559		

20. Effect of Bigi brands of soft drink on LDLC of treated *D. melanogaster*

Chemical/Enzyme Activities		
Products/ Concentrations	Mean	Standard Deviation (STD)
Negative control	114.6653	43.00388
100 concentration (Bigi Cola)	73.3320	4.00000
50 concentration (Bigi Cola)	71.3327	.66700
25 concentration Bigi Cola	69.9993	3.33300
100 concentration Bigi Orange	24.7327	.73300
50 concentration Bigi Orange	34.6660	2.66600
25 concentration Bigi Orange	26.2660	5.12371

	Sum of squares	df

Mean square	F	P

Between Groups	19243.368	6	3207.228	11.749	0
Within Groups	3821.569	14	272.969		
Total	23064.937	20			

21. Effect of Bigi brands of soft drink on VLDL of treated *D. melanogaster*

Chemical/Enzyme Activities		
Products/ Concentrations	Mean	Standard Deviation (STD)
Negative control	120.6779	130.29195
100 concentration (Bigi Cola)	55.9366	19.44143
50 concentration (Bigi Cola)	44.6073	38.09955
25 concentration Bigi Cola	-56.5765	88.36268
100 concentration Bigi Orange	71.2673	104.49410
50 concentration Bigi Orange	105.5330	82.01060
25 concentration Bigi Orange	72.8434	8.29814

	Sum of squares	df	Mean square	F	P
Between Groups	59657.516	6	9942.919	1.570	0.228
Within Groups	88654.228	14	6332.445		

Total	148311.743	20