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Assessment of the *In Vivo* Catalase Enzyme Activity and Anti-Obesity Effects of the Stem Bark Extract of *Erythrinaabyssinica* on *Drosophila Melanogaster* Model of Diet Induced Obesity.

Asiimwe Oscar Hilary, Edward Wampande and Hellen Kinyi

Department of Biochemistry, Kampala Internatonal University, Western Campus, Uganda.

ABSTRACT

Though physio-nutritional therapy has shown appreciable results in obesity management, the results are limited due to failure to adhere to a strict routine. In developing countries, alternative and complementary medicine has been promoted for the management of metabolic conditions but information on its efficacy and mechanism of action is scarce. *Erythrinaabyssinica* has anti-inflammatory, cytotoxic and anti-microbial properties; however there is scanty information on its *in vivo* antioxidant activity and anti-obesity effects. The aim of this study was to quantify the antioxidant activity of the stem bark extract of *Erythrinaabyssinica,* evaluate its *in vivo* catalase activity, and to determine its anti-obesity effects in obese male *Drosophila melanogaster*. Male *Drosophila melanogaster* (W^{1118}) were used in this study. Obesity was induced by feeding male *Drosophila melanogaster* flies on 10% w/v coconut food for two weeks. A mixed solvent extract (20% ethanol and 80% water) of *Erythrinaabyssinica* stem bark was prepared into concentrations of 25%, 50% and 75% v/v using standard protocols. Flies were divided into seven groups each containing 30 flies each. Group 1-3 were fed on the coconut food (HFD) mixed with 3 graded concentrations of fenofibrate. Groups 4-6 were fed on the coconut food mixed with graded concentrations of the extract, while group 7 was the control and was fed on coconut food only. The flies were fed on the respective diets for 30 days. *In vitro* antioxidant activity of *Erythrinaabyssinica* stem bark was determined using the reducing power activity protocol. Catalase enzyme activity was measured in obese *Drosophila melanogaster* treated with fenofibrate and the extract. Anti-obesity effect was determined by measuring the triglyceride levels. The study showed that the reducing power activity of the extract was concentration dependent**.** Catalase levels increased in a dose dependent fashion in obese flies fed on the extract. Triglyceride levels were decreased in a concentration dependent fashion. The study showed that the stem bark extract of *Erythrinaabyssinica* modulates and increases *in vivo* catalase enzyme activity in the obese *Drosophila melanogaster*. It also showed that the extract has concentration dependent anti-obesity activity in obese *Drosophila melanogaster*.

Keywords: *Erythrinaabyssinica,* catalase, *Drosophila melanogaster,* Obesity, fenofibrate

INTRODUCTION

Obesity is an abnormal accumulation of triglycerides in adipose tissue [1]. This disorder is strongly associated with local and systemic chronic low grade inflammation that increase the risk of vascular events due to metabolic abnormalities, adipocyte tissue dysregulation and dysfunction [2].Central to this condition is higher calorie intake and low calorie expenditure, which could be due to increased uptake of processed foods rich in saturated fat and sugars, physical inactivity, genetic factors, excessive alcohol use, Cushing's syndrome and smoking [3]. This excessive positive energy balance in obese states results mainly from dysregulated normal responses to both the body's energy and diet cues, thus increasing the amount of stored energy in adipose tissue inform of mainly triglycerides [3]. Clinically one is considered obese if the Body Mass Index (BMI) is ≥ 30.0 Kg/m^2 [4]. Fat accumulation in non-adipose tissue is responsible for

lipotoxicity under obese states [5]. High fat diet induced cellular inflammatory cascades bring about excessive immune responses which disrupt cellular pathways and receptors, thus the multifactorial adverse effects of obesity such as insulin resistance, foam cell formation, increased cytokine production and hypoxia which manifest clinically as type II diabetes, dyslipidemia, osteoarthritis, some cancers, Coronary artery disease, stroke, hypertension, liver disease, psychological challenges and polycystic ovary syndrome [3].

Excessive adipocyte hyperplasia and ectopic lipid accumulation under obese conditions increases Reactive oxygen species production, mitochondrial and endoplasmic reticulum stresses [6]. This in turn disrupts cellular pathways for energy homeostasis such as Adenosine Monophosphate Protein Kinase pathway, those that regulate pro-inflammatory cytokines such as Mitogen Activated Protein Kinase pathway, those that regulate gene expression such as the Calcium-Calmodulin dependent protein kinase II pathway and the Phosphoinositol-3-protein kinase/Akt pathway [1]. However exogenous antioxidant replenishment has of recent been found to boost the endogenous antioxidant defense system which in the end reverses and/or curtails obesity markers and its complications [7]. Previous studies indicate that rutinandquercetin increase catalase enzyme activity in obese *Drosophila* [8]. Exogenous antioxidants include both synthetic and natural antioxidants such as plant antioxidants [9]. The mechanism of action of this antioxidant system is by either interference or breaking of reactive oxygen species chain initiation and propagation [10]. This action brings about anti-lipid droplet accumulation in the body and is attributed to antioxidants such as phenols and alkaloids [11].

Current interventions in the prevention and management of obesity include, high calorie diet restriction, regular exercise and use of drugs such as Orlistat and fenofibrate alone or in combination [2].The use of Orlistat is associated with side effects such as fecal incontinence, bloating, flatulence, dyspepsia and diarrhea [11]. Fenofibrate is a third generation fibric acid derivative with a very high lipid lowering potency which is attributed to an additional benzene ring unlike other fibrates. This potentiates its very high specificity as a molecule [12].

Plants have of recent increasingly proven to be a very effective alternative to conventional drug therapy in the management of obesity and its complications [13]. The therapeutic effects of these plants have been attributed to phytochemicals or phytonutrients like flavonoids, terpenoids, alkaloids, sterols, saponins, tannins, lignans, stilbenes and phenolic acids all of which have been proven to have anti-inflammatory and antioxidant properties [14].

Medicinal uses of *Erythrinaabyssinica* in the greater tropical and sub-tropical regions of the world include; treatment of cough, bronchitis, asthma and insomnia [15]. More so, it is also used as an anti-ulcer, anti-malarial, in the treatment of stomach ache, sexually transmitted diseases, amoebiasis, diabetes, liver inflammation and measles [16].

In Western Uganda, the crushed flowers are used in the treatment of dysentery (Esheshe), as an abortifacient (okutorozyaenda) [17]. The leaf decoction is used as an emetic (okucunduura) and as treatment for diarrhoea. The roots are used in the treatment of malaria (omushwiija) and epilepsy (entsimbo). The stem bark is used to manage the adverse effects of poison from snake bites (okurumuura), treatment of sexually transmitted diseases such as gonorrhea (Enziku) and syphillis (Ebinyoro), measles (Obusheru). Fresh sap from the stem bark is used as an antihelminth (okutambaenjoka), chlamydia and conjunctivitis. More so, the powdered and roasted stem bark is applied externally on burns, scalds and swellings (Okwomooraebironda).

The medicinal properties of *Erythrinaabyssinica* have been attributed to alkaloids and phenolic compounds such as flavonoids, chalcones,

pterocarpans and benzofurans [18]. Plants rich in antioxidants have been shown to be beneficial in the management of obesity [7].A previous *in vitro* pancreatic lipase assayonsigmoidin A from the stem bark of *Erythrinaabyssinica* revealed its strong inhibitory effect on pancreatic lipase, an important target in obesity management [19].

Problem statement

Worldwide obesity has nearly tripled since 1975 and most of the world's population live in countries where overweight and obesity kill more people than underweight [4]. High calorie diets are the leading cause of healthy years lost world wide and by 2018,the world obese percentage had increased to 13.1% from 8.7% in 2000 [20]. In sub-Saharan Africa, approximately 9-13% of deaths are attributable to cardiovascular diseases (CVDs) as a complication of obesity [21]. NCD account for 27% of Uganda's total mortality with coronary heart disease (CHD) alone accounting for a 3.26% of total mortality [4]. More so, 26.4% of Uganda's population is hypertensive and the percentage for Western Uganda is 26.3% [22].

Conventional anti-obesity therapies are associated with many side effects, for example the use of rimonabant is associated with psychiatric disorders [12]. Other methods in the management of obesity like regular exercises, dietary restriction and dietary changes have shown appreciable results [3]. However, these results are susceptible to relapses due to the requirement of strict timeline adherence and discipline a feat that many people find hard to follow [23].

Thus as an alternative, exogenous antioxidants from plants have of recent been suggested as an alternative in the management of excessive adipose tissue accumulation [11]. Examples of plants used in the management of obesity include *Nigella sativa*, *Zingibarofficinale*, *Allium victorialis*, Pomegranate leaf, *Panaxjaponicus*, *Kocbiascoparia*, black Chinese tea and Oolong tea [7]. Studies have also shown that species of the genus *Erythrina* have anti-inflammatory, cytotoxic and anti-microbial properties. These have been attributed to the presence of phytochemicals like polyphenols that have been shown to lower stored lipid levels and protect from complications of obesity [24]. There is thus a need to screen local plant species for their anti-obesity potential in an effort to manage obesity.

Aim of the study

This study thus aims to evaluate the effect of *Erythrinaabyssinica* stem bark extract on catalase enzyme activity and obesity in *Drosophila melanogaster* model of diet induced obesity

Objectives of the study include the following:

1. To quantify the antioxidant activity of the stem bark extract of *Erythrinaabyssinica* using the reducing power method
2. To evaluate the effect of *Erythrinaabyssinica* stem bark extract on catalase enzyme activity of obese *Drosophila melanogaster*.
3. To determine the effect of *Erythrinaabyssinica* stem bark extract on triglyceride levels of obese *Drosophila melanogaster*.

Research questions:

1. How much antioxidant activity is present in the stem bark extract of *Erythrinaabyssinica*?
2. Does the stem bark extract of *Erythrinaabyssinica* have an effect on catalase enzyme activity of obese *Drosophila melanogaster*?
3. Does the stem bark extract of *Erythrinaabyssinica* have an effect on triglyceride levels of obese *Drosophila melanogaster*?

Justification of the study

Drosophila melanogaster animal disease model is increasingly being used because of the prohibitively high cost of screening and maintenance of mammalian models [25]. Most of the major metabolic enzymes in mammals are conserved in *Drosophila* and the genes that regulate the uptake, transport, storage and mobilization of lipids are also well conserved [26].

Lipids in *Drosophila melanogaster* are stored as triglycerides in the fat body similar to lipid triglyceride storage in

adipose tissue of mammals [27]. Additionally, the molecular mechanism that controls the mobilization, transport and storage of neutral lipids in cellular lipid droplets (lipophorins) resembles that in mammalian pathways of lipoproteins [28]. More so, *Drosophila melanogaster* accumulates lipids in both the fat and non-fat tissue in a dose dependent manner in a shorter period unlike mice which take months to become obese [29].Previous studies have used *Drosophila melanogaster* to model obesity [30].

Significance of the study

Establishing the effect of *Erythrinaabyssinica* stem bark extract on obese *Drosophila melanogaster* will enable the greater scientific community to understand its potential in the management of obesity. Since the plant is already widely used to manage several conditions in the region and is already accepted by the community, scientific validation of its activity will provide a basis for its adoption by the local community as a cheaper alternative in the management of obesity as well as its conservation. More so, the validation of its activity could help its commercialization thus improving the economic status of the local community.

Scope of the study

The study focused on the effect of the stem bark extract of *Erythrinaabyssinica* on obese *Drosophila melanogaster.* Reducing power was used to measure the *in vitro* antioxidant activity of the extract. The effect of the extract on catalase enzyme activity and TG levels in obese male flies was assessed.

MATERIALS AND METHODS

Study setting

All laboratory experiments were carried out at the Institute of Biomedical Research (IBR) laboratory of Kampala International University western campus. It had an operational fly laboratory with all the required material and reagents for this study.

MATERIALS

Reagents

Methanol, distilled water, methanol, sulphuric acid, alpha naphthol, absolute alcohol, copper sulphate, sodium sulphate, anhydrous sodium carbonate, cupric acetate, lactic acid, iron III chloride, lead acetate, magnesium ribbon, hydrochloric acid, chloroform, acetic anhydride, ammonium hydroxide, formaldehyde, potassium ferricyanide, ferric chloride, phosphate buffer, trichloro acetic acid, ascorbic acid, mercuric chloride, potassium iodide, benzene, triton X-100, hydrogen peroxide, PBS, tween 20 and fenofibrate

Reagent 1 (4-aminophenzone, p-chlorophenol, lipoprotein lipase, glycerol kinase peroxidase, glycerol-3-oxidase, ATP, good buffer pH 6.3) and standard triglycerides aqueous for triglycerides determination.

Reagent 1 buffer (TRIS buffer pH 7.4, phenol), reagent 2 enzymes (glucose oxidase, peroxidase, 4-aminophenazone) and standard aqueous glucose for glucose determination.

Reagent 2 buffer (pipes pH 6.9, phenol), reagent 2 enzymes (peroxidase, cholesterol esterase, cholesterol oxidase, 4-aminophenazone) and standard aqueous cholesterol for total cholesterol determination.

Equipment

Equipment used included; fly incubator, weighing balance, spectrophotometer (model 722), quartz cuvettes (0.3ml - 0.7ml), centrifuge (centaur 2 Germany), water bath (Memmert Germany model), laboratory oven (BTI Bio-Tech model Germany), oven, hot plate, weighing scale, blender, mechanical shaker, timer, test tube racks, refrigerator, sauce pan, Whatman No.1 filter paper, aluminum foil, micro pipette, pipette tips, mortar and pestle, gloves, micropipettes (0.5 - 10µl and 100 - 1000µl), Eppendorf tubes and a deep freezer (solstar).

Plant collection and extraction

Erythrinaabyssinica stem bark was sourced from an *Erythrinaabyssinica* tree in Rwemirokora, Ishaka municipality, Bushenyi district and taxonomic

identification of the collected stem bark and leaf samples (collection number AOH001) was done by a taxonomist at Mbarara University of Science and Technology (MUST). The stem bark was first pulverized to make it soft using a mortar and pestle, it was then air dried at room temperature until no more changes in weight. The dried stem bark was ground again using a mortar and pestle into a fine powder and then sieved. In the extraction process, 100g of the fine powder stem bark material was agitated in 100ml of 20% ethanol extraction solvent using a mechanical shaker (Stuart Scientific Orbital Shaker, UK) for 48 hours [31]. The extract was then filtered using a Buchner funnel and filter paper (Whattman No.1) overnight and the resultant extract dried in a hot air oven at 35^{0}C until a thick paste was formed. This was considered 100% extract concentrate which was weighed and stored at 4^{0}C in the refrigerator in amber jars. Serial dilution concentrations such as 25%, 50% and 75% extracts were prepared as follows. A stock solution was prepared by adding 15g of harvested extract in 15ml of distilled water after which 2.5ml, 5.0ml and 7.5ml of the stock solution were dispensed in separate conical flasks and 7.5ml, 5.0ml and 2.5 ml of distilled water added respectively to make up the 25%, 50% and 75% v/v extract solutions of 10ml as shown in **Table 1**.

Table 1: Preparation of stock solution and serial dilutions

Serial dilutions (v/v)	**Stock solution/ml**	**Extract/g** 15	**Distilled water/ml** 15	**Total ml** 15
25%	2.5	.....	7.5	10
50%	5.0	.....	5.0	10
75%	7.5	.....	2.5	10

Fly rearing, collection and treatment

The study was done using *w*^{1118} Yellow white strain of *Drosophila Melanogaster* which were provided by the *Drosophila* fraternity of Kampala International University Western campus, Uganda and originally from National Species Stock Center (Bowling Green, OH, USA).The flies were kept and fed on their respective diets at constant temperature (23 ± 1^{0}C) in the Institute of Biomedical Research Laboratory at Kampala International University. Food was prepared as standard corn meal and placed in plastic vials as regular food (RF) using the standard *Drosophila* food preparation recipe. The coconut food (CF) was made by adding coconut oil to the regular food as a source for increased saturated fat in the diet. The recipe was specialized for the current model with minor modifications according to the method by [32] with coconut food diet consisting of 10% weight per volume of food grade coconut oil. For the *Erythrinaabyssinica* stem bark extract treatment, the extract at 25%, 50% and 75% v/v was mixed with the coconut food (CF) and placed in plastic vials as feed for the intervention group of flies.

Study population

Flies were bred for one month as2100 male flies (N) were being selected in due course at 0-3 days and transferred to separate vials of standard corn meal medium in room air. After aging for 3 more days, these flies were fed on a HFD (coconut food) for one week.

Sampling techniques

Purposeful sampling of 623 phenotypically obese male flies (n) was carried out(Etikan, Musa and Alkassim, 2016). They were subjected to treatments as postulated in the experimental design [33].

Sample size and its determination

A 'resource equation' method formula was used [34]. Thus;

E = Total number of animals – Total number of groups

(30 x 3 x 7) - 7

(where 30 = sample triplicates of 10 flies, 3 = intervals, 7 = groups)

630 – 7

623 animals

Where E was the degree of freedom of analysis of variance (ANOVA), N was population size and n was the sample size.

Therefore since E was much greater than 20, the probability of getting significant results was more possible since E >10 < 20 for adequate statistical significance results

Experimental design

A total of 2100 male flies (N) were bred in standard corn meal medium. Three day old flies were transferred and fed on food supplemented with coconut oil for two weeks to induce obesity (increased fat storage). *Drosophil*a standard corn meal medium contained, cornmeal 7% w/v, dextrose 7.5% w/v, yeast 1.5% w/v, nipagin 2.33% v/v, agar base 1.05% w/v, propionic acid 0.37% w/v in a liter of food. High fat diet (HFD) or coconut food (CF) was prepared by adding 10% w/v food-grade coconut oil to the standard corn meal medium [32; 35]. Phenotypically obese flies were divided into 7 groups. Groups 1-3 were fed on coconut food (CF) + *Erythrinaabyssinica* stem bark extract at concentrations of 25%, 50%, and 75% respectively. Groups 4-6 were fed on coconut food (CF) + standard drug fenofibrate at concentrations of 25mM, 50mM and 75mM respectively. For the control experiments, flies were placed in plastic vials with coconut food only. Observations of the acute effects (within 30minutes to 2 hours) and chronic effects (after 48 hours) of the extract and the standard drug were noted [36]. Ten fly samples from each experimental group were picked on day 10. They were immobilized using cold shock, weighed and then rinsed using cold phosphate-buffered saline (PBS) solution to remove all traces of food. Whole fly samples were then homogenized in 100µl of cold 0.05% phosphate-buffered saline tween (PBST) solution and the homogenate centrifuged at 13,000 rpm for 3 minutes [37]. The supernatant was immediately stored at 4^0C for later assay of catalase enzyme activity and total triglyceride levels. This procedure was repeated thrice for all the experimental groups.

Survival rate analysis.

In order to determine the duration of exposure to be used in the experiment, an initial cohort of *Erythrinaabyssinica* stem bark feeding experiment was carried out to know the number of dead flies during the whole lifespan. A population of 120 obese male flies was raised in 4 vials at a density of 30 flies per vial for the control and each extract concentration. Obese *Drosophila Melanogaster* (male) were divided into four groups namely; one control (with coconut food alone) and coconut food + *Erythrinaabyssinica* stem bark extract (25%, 50% and 75%). There were three replicates for each of the concentrations of *Erythrinaabyssinica* and the control group shown above. The diet mixed with *Erythrinaabyssinica* stem bark extract was changed twice a week. Survival rate was determined across the extract concentrations by recording the number of live and dead flies daily. At the end of 15 days, data was analyzed and plotted as percentage of live flies. Based on this data, one day of picking samples was selected (day 10) on which the survival rates were comparable with that of the control. A second cohort of *Erythrinaabyssinica* feeding experiment was conducted to collect flies for mass, catalase activity and triglyceride level determination.

Determination of reducing power activity of *Erythrinaabyssinica* stem bark extract

The reducing power of *Erythrinaabyssinica* stem bark extract was determined according to the method described by [38]. The absorbance values of ascorbic acid at different concentrations were obtained and a standard curve was plotted according to the protocol below. 1ml of ascorbic acid at 5%, 15%, 30%, 50%, 75% and 90% w/v and the stem bark extracts at 25%, 50% and 75% v/v were pipetted into clean and

dry test tubes. 2.5ml phosphate buffer (0.2M, pH 6.6) and 2.5ml potassium ferricyanide (1% w/v) were added and the mixture was kept in a water bath at 50°C for 20 minutes. The mixture was then cooled and 2.5ml trichloroacetic acid was added and centrifuged at 3000rpm for 10 minutes, the upper layer of the solution (2.5ml) was mixed with 2.5ml distilled water and 0.5ml freshly prepared ferric chloride solution (0.1% w/v) after which, the absorbance values were determined spectrophotometrically at 700nm.

Potassium ferricyanide + Ferric chloride $\xrightarrow{\text{Antioxidan}}$ Potassium ferrocyanide + Ferrous chloride

Catalase enzyme activity determination

Catalase enzyme activity of the fly samples was determined by following a protocol developed by [39] whereby, a calibration curve was generated in the form y=mx + c using standard catalase concentrations for which the corresponding foam heights were determined with the defined unit of catalase activity following the procedure below. 100µl of catalase solution was pipetted in 13mm diameter x 100mm height test tubes, 100µl of 1% Triton X-100 and 100µl of undiluted hydrogen peroxide (30%) were added to the solutions, mixed thoroughly and incubated at room temperature. Following the completion of the reaction, the height of O_2-forming foam that remained constant for 15minutes in the test tube was finally measured using a ruler after which the corresponding catalase activity was ascertained and expressed as mg/ml of protein. A standard curve was constructed from which an equation was generated in the form y =mx + c i.e. Absorbance (y) = 0.0432 concentration (x) + 0.013.; $R^2 = 0.9973$. The above experimental protocol together with the standard curve generated were used to determine catalase activity for both the control and intervention group fly samples.

Determination of total triglycerides

The supernatant from the samples collected and stored at 4°C was used for triglyceride quantification using Cypress Diagnostics triglyceride kit. A commercial coupled colorimetric assay (CCA) protocol was used to measure the total triglyceride levels of the whole fly samples indirectly in the form of a quinoneimine dye whose absorbance was taken at 540nm [40].

Validity and reliability of instruments

A well calibrated visible spectrophotometer (model: 722) was used to obtain absorbance readings for the various parameters. Standard quartz cuvettes with a narrow tube (0.5ml) specifically for minute sample volumes were used. Also standard protocol and reagents for various parameter determination were followed and used strictly following the manufacturer's instructions.

Data analysis plan

Graph pad prism version 6 software was used for statistical analysis of the biochemical assay tests. The results were reported as mean ± standard error of mean (S.E.M) using figures and tables. A one way ANOVA, followed by the post hoc Tukey's test, where *p*value of $P< 0.05$ was considered to represent statistical significant difference in the metabolic and antioxidant assay results.

Ethical considerations

Ethical considerations were followed. This research model was selected on the basis of following the replacement protocol for ethics in animal research. The reduction and refinement procedures were adhered to also [41]. There was no use of infectious agents, caution was taken to use minimum number of flies for this research, there were no transgenic flies used in this research to raise biosafety issues. Flies were not subjected to any form of pain and distress by strictly taking care of the fly welfare such as exposure of flies to 12hour day and night cycles, invasive procedures were not involved in this research and the flies were properly disposed of after use in a fly morgue containing a disinfectant (Jik). Approval for this research from the research ethics committee was sought and upon approval, it given the number, Nr.UG-REC-023/201916.

RESULTS

Antioxidant activity of the stem bark extract of *Erythrinaabyssinica*

Reducing power activity

The reducing power activity of the extract significantly increased in a dose dependent manner. The antioxidant activity at 50% and 75% was significantly different from that at 25% at a ($P < 0.05$) as shown in **Figure 1.**

Key: Statistical comparison between groups is indicated by superscripts (letters) above bars. Similarity in superscripts indicates statistically similar effects/activity amongst experimental groups while a difference in superscripts indicates statistically de-similar effects/activity amongst experimental groups.

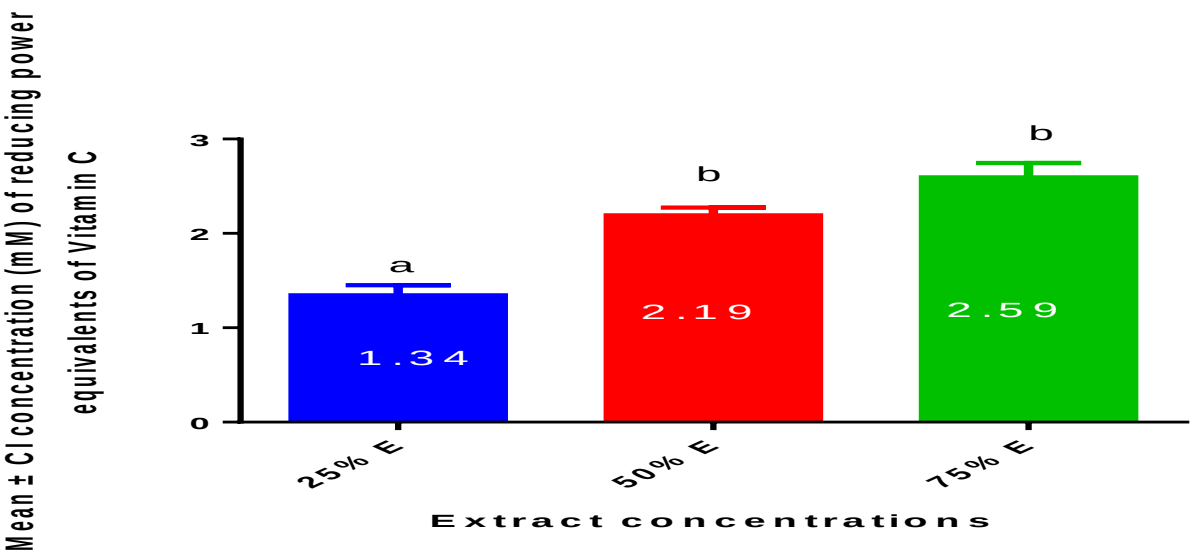

Figure 1: Reducing power activity at varying extract concentrations.[a and b] represent statistically significant differences in reducing power activity between all extract concentrations.

Effect of *Erythrinaabyssinica* stem bark extract on catalase enzyme activity of Obese *Drosophila melanogaster*

Changes in catalase levels after 10 days

Generally, the extract significantly increased catalase activity with increasing concentrations ($P > 0.05$) after 10 days .This was indicated by a marked statistically significant difference in catalase activity between 50% and 75%extract whereby 75% activity> 50% activity.

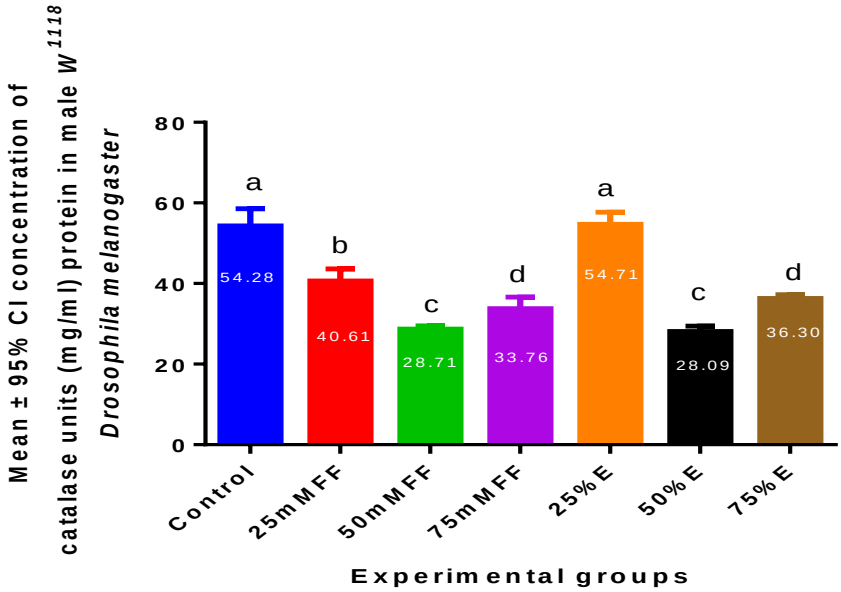

Figure 2: Changes in catalase activity in obese *Drosophila melanogaster* following exposure to the extract after 10 days.[a,b,c and d] represent group statistically significant differences in catalase enzyme activity of flies fed on different concentrations of fenofibrate and the extract from that of flies fed on coconut food alone (control) and then, between all treatment experimental groups.

Effects of *Erythrinaabyssinica* stem bark extract on triglyceride levels of obese *Drosophila melanogaster*.

Triglyceride levels after 10 days

After 10 days, TG levels had significantly decreased in all extract experimental groupsand this decrease was concentration dependent as shown in **Figure 3**. TG levels in obese *Drosophila melanogaster* decreased with an increase in extract concentration.

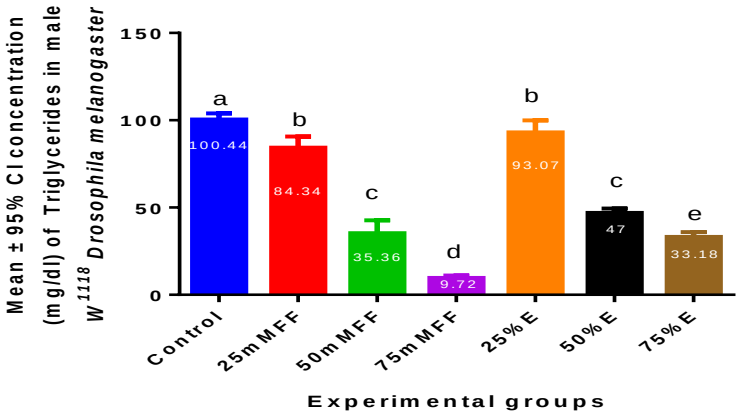

Figure 3: Changes in TG levels in obese *Drosophila melanogaster* following exposure to the extract after 10 days.[a, b, c, d and e] represent group statistically significant differences in triglyceride levels of flies fed on different concentrations of fenofibrate and the extract from that of flies fed on coconut food alone (control) and then, between treatment experimental groups after 10 days.

DISCUSSION

The study aimed at describing the anti-obesity activity of *Erythrinaabyssinica* stem back extract. Specifically its effect on catalase enzyme activity and triglyceride level accumulation in obese *Drosophila melanogaster*. Data from this study showed that *Erythrinaabyssinica* stem bark extract modulates and increases catalase enzyme activity in obese *Drosophila melanogaster*. The extract also reduces triglyceride level accumulation in obese *Drosophila melanogaster*.

Determination of *in vitro* reducing power activity of natural products helps in the rapid screening of their antioxidant and probable *in vivo* effects [42; 43]. The reducing power activity of antioxidants in the stem bark extract of *Erythrinaabyssinica* showed a concentration dependent activity (**Figure 1)**. As the concentration increased, the reducing power activity of the stem bark extract of *Erythrinaabyssinica* also increased and this trend was similar to that of previous studies on other plant extracts [42; 44; 45]. Since reducing power activity of natural products determines their electron donation ability and thus the potential to minimize oxidative damage under pathological conditions [38]. The stem bark extract of *Erythrinaabyssinica* had higher electron donation ability at higher concentration.

Increased endogenous catalase enzyme activity in the initial stages of oxidative stress caused by allergens and/or various disease pathologies serves as a natural defense mechanism against cellular stressors [46]. However, this natural defense mechanism can only be sustained under chronic pathological disease conditions such as obesity by exogenous antioxidant replenishments [47]. Catalase levels were increased in a dose dependent fashion in obese flies fed on the extract after 10 days (**Figure 2**). This mechanism of action by the *Erythrinaabyssinica* stem bark extract on obese flies was similar to that in previous studies that used plant extracts in the management of dysregulated storage lipid homeostasis [48; 49]. Though the catalase levels were much higher at low concentration of the extract (25%) than at higher concentration after 10 days, this could have been an indication of the extract's mechanism of action to exert protective effects under stress through catalase enzyme up-regulation at lower potentials of reactive oxygen species reducing activity, a mechanism of action that was similar to other anti-obesity natural remedies [50]. The moderate increase of catalase levels with increasing concentration could have been an indication of both the enzymatic and increased reactive oxygen species reducing mechanism in stress arrest and anti-obesity effects of the stem bark extract at a higher concentration.

Triglyceride storage in fat cells serves as a defensive mechanism against oxidative stress due to a positive energy balance. The triglyceride lowering mechanism of plant derived antioxidants such as polyphenols is through lipolysis stimulation, suppression of adipose tissue growth, inhibition of pre-adipocyte differentiation and apoptosis induction to reduce adipose tissue mass [7].This improves on the fat turn-over rate under obese conditions and thus improving the general wellbeing [51]. Triglyceride levels were safely and significantly reduced after 10 days by the extract at 75% (**Figure 3**). This showed the dose dependent potency of the antioxidants in the stem bark extract of *Erythrinaabyssinica*, the results of which were similar to those of previous studies on potent triglyceride lowering plant extracts [52]. However, by the 15^{th} day, fly samples in the control and low concentration of extract at 25% had died. This could have been due to the life shortening effects due to high fat diet as previous studies indicated [53].These results could validate the dose dependent efficacy of *Erythrinaabyssinica* stem bark extract in improving longevity, lipid profile and conferring protection in the management of obesity and its associated effects such as increased mortality. The effects of *Erythrinaabyssinica* stem bark extract at high concentration in this study were similar to those of plant

antioxidants in high fat diet fed and stressed *Drosophila melanogaster* of previous studies [54].Also, since one of the function of lipid droplets is to safely sequester toxic lipids such as an over abundant fatty acids supply from high fat diet feeding [55,56,57,58,59], the stem bark extract at higher concentration could have improved on this function of lipid droplets in obese *Drosophila melanogaster* and thus improving fly triglyceride profile and longevity.

Several bioactive compounds in plant extracts have been hypothesized to act on complex biological pathways involved at different stages of adipocyte life cycle. The effect of these compounds in combination has been found to far exceed the favorable effects of each individual compound, thus their multi-factorial beneficial additive or synergistic effect on adipose cell development regulation under obese states [52,60,61]. In this study, *Erythrinaabyssinica* stem bark extracts at 75% safely improved *in vivo* antioxidant activity and lowered triglyceride levels in obese *Drosophila melanogaster*. The protective effect of the extract against oxidative stress due to obesity was attributed inhibition of inflammatory cascades, catalase enzyme activity modulation and up regulation, coupled with increased reactive oxygen species reducing activity at higher extract concentration. The triglyceride lowering effects of the extract could have been due to its potential to enhance Brummer lipase enzyme activity on the stored triglyceride homeostasis of obese *Drosophila melanogaster*, thus its anti-inflammatory effect in obese fly tissue, obese cell apoptosis induction, reduction of obese tissue generation, stimulation of lipolysis, calorie restricting enzyme activity increment and reduction in expression of inflammatory response mediators.

CONCLUSION

The study showed that;

1. *Erythrinaabyssinica* stem bark extract has strongest reducing power at 75%
2. *Erythrinaabyssinica* stem bark extract has increased *in vivo* catalase activity at 75%
3. *Erythrinaabyssinica* stem bark extract decreased TG levels significantly at 75%

Novelty: Mass and triglyceride levels were reduced at 75% concentration extract with lesser chronic effects compared to the standard drug.

RECOMMENDATIONS

More studies should be carried out to ascertain the effect of the stem bark extract on Brummer lipase enzyme activity in obese *Drosophila melanogaster.* Also, comparative studies of lipid droplet imaging should be carried out on fly fat body, gut and intestine tissue under obese and extract treatment states. More so, further studies should be carried out to ascertain the combined effect of the standard drug and the extract in obese flies. This was a basic study using a lower invertebrate animal model, further studies on the extract should be carried out using a higher vertebrate animal model. Finally, this was a basic study in which a limited number of markers were assessed, further studies should be carried out with emphasis on, immunological modulators of inflammation, second messengers and molecular modulators of gene expression. This would offer a holistic picture on the mechanism of action of *Erythrinaabyssinica* stem bark extract through which it exerts its triglyceride lowering and protective effects against obesity.

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