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Research article

Anti-obesity effects of *Erythrina abyssinica* stem bark extract in flies exposed to a high fat diet

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ABSTRACT

Background: An *in vitro* assay on Sigmoidin A from *Erythrina abyssinica* stem bark revealed its potency to inhibit pancreatic lipase. However, studies indicate activity of extract bioactive compounds in combination far exceed the favorable effects of each individual compound due to synergy and additive effects. In this study, we provide information on the effect of *E. abyssinica* stem bark extract in *Drosophila melanogaster*. The objective of the study was to determine the safety and effects of *E. abyssinica* stem bark extract on fly survival, body weight, triglycerides, sterol, total protein, and catalase activity of obese male *D. melanogaster*.

Methods: Obesity was induced by exposing *D. melanogaster* white mutant w^{1118} to coconut food for two weeks. Groups 1–3 were fed on coconut food + fenofibrate at 25 mM, 50 mM, and 75 mM. Groups 4–6 were fed on coconut food + *E. abyssinica* stem bark extract at concentrations of 2.5 g/ml, 5.0 g/ml, and 7.5 g/ml. The positive control was exposed to only coconut food while the negative control was on regular food. Fly survival observations were done for 15 days, while acute and chronic effects were done at 30 min and after 48 h respectively following treatment. Body mass, negative geotaxis, reducing power of the extract, triglycerides (TG/TP), sterol, total protein levels, and catalase activity were measured after 10 days of exposure to the experimental diets.

Results: Fly survival changes were observed after 10 days and *E. abyssinica* stem bark extract had the strongest reducing power at 7.5 g/ml extract concentration. *E. abyssinica* stem bark extract reduced body mass, triglyceride levels (TG/TP), sterol levels, and modulated catalase activity at 7.5 g/ml extract concentration. Though the standard drug fenofibrate had the highest fat accumulation reduction potential, the extract at 7.5 g/ml was much safer in reducing fat accumulation in obese male *D. melanogaster* than other concentration used.

Conclusion: Antioxidants in *E. abyssinica* stem bark extract are responsible for the observed anti-obesity activity.

1. Introduction

Obesity is an abnormal accumulation of triglycerides in adipose tissue [1]. Central to this condition is local and systemic chronic low-grade inflammation that increases the risk of vascular events due to metabolic abnormalities, adipocyte tissue dysregulation, and dysfunction [2]. It is associated with increased uptake of processed foods rich in saturated fat and sugars, physical inactivity, genetic factors, excessive alcohol use, Cushing's syndrome, and smoking [3]. Complications of obesity such as type II diabetes, dyslipidemia, osteoarthritis, some cancers, coronary artery disease, stroke, hypertension, liver disease, psychological challenges, and polycystic ovary syndrome have been attributed to adipocyte tissue function dysregulation and dysfunction [4]. The adipocyte hyperplasia present in obesity causes increased reactive oxygen species production which in turn disrupts cellular pathways for energy homeostasis causing multifactorial cellular adverse effects which present clinically as obesity complications [5]. Current interventions in the prevention and management of obesity such as calorie restriction, regular exercise, and use of drugs such as tetrahydrolipstatin and fenofibrate are associated with poor compliance and side effects [2, 6]. Hence there is a need for alternative modes of obesity management.

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Exogenous plant-derived antioxidants have recently been suggested as alternatives in the management of obesity [7]. However, studies on their mode of action are scarce for example, recent studies indicate that plant-derived antioxidants have the potential of boosting *in vivo* antioxidant activity and hence improving redox homeostasis thus reversing and/improving the complications associated with obesity [8, 9]. The tree *Erythrina abyssinica* is usually grown to prevent soil runoff, provide shade, live fence, craft material, wood, and fuel. However, it is also used medicinally in the greater tropical and sub-tropical regions of the world as a treatment for cough, diabetes, bronchitis, asthma, and insomnia [10]. In addition, it is also used as an anti-ulcer, anti-malarial, in the treatment of stomach ache, sexually transmitted diseases, amoebiasis, liver inflammation, and measles [11]. The medicinal properties of *Erythrina abyssinica* have been attributed to alkaloids and phenolic compounds such as flavonoids, chalcones, pterocarpans, and benzofurans [11, 12]. Since this plant is widely accepted and used for the treatment of several conditions and is rich in antioxidants, it was, therefore, the objective of this study to quantify the reducing power activity of the stem bark extract, assess the effect of the stem bark extract on the mass, negative geotaxis, catalase activity, total protein, sterol and triglyceride levels of obese male *Drosophila melanogaster*.

2. Methods

2.1. Plant collection and extraction

E. abyssinica stem bark was sourced from an *E. abyssinica* tree. Taxonomic identification of the collected leaf samples was done by a taxonomist and assigned a number AOH001. The stem bark was split into small pieces, air-dried at room temperature until constant weight, and ground using a mortar and pestle into a fine powder [13]. The powder was cold extracted using 20% ethanol at a ratio of 1:1. The extracts were shaken and left to stand for 48 h at room temperature [14]. They were then filtered using a Buchner funnel and Whatman No.1 filter paper and the resultant extract dried in a hot air oven at 35 °C until a thick paste was formed, which was weighed and stored at 4 °C. Extract concentrations of 2.5 g/ml, 5.0 g/ml, and 7.5 g/ml were then prepared as previously described [15]. Qualitative phytochemical analysis was done on alkaloids and phenolic compounds [11, 12] with emphasis on flavonoids, saponins, tannins, alkaloids, terpenes and anthraquinones as shown in (Supplementary file 1).

2.2. Laboratory animals

D. melanogaster white mutant w^{1118} obtained originally from the National Species Stock Center (Bowling Green, OH, USA) and cultured at the Institute of Biomedical Research. They were kept at a constant temperature (23 ± 1 °C) and fed on a standard cornmeal diet. A total of 2100 male flies (N) were bred on a standard cornmeal medium. Three-day-old flies were transferred and fed on food supplemented with coconut oil for two weeks to induce obesity under 70% humidity, 24–26 °C temperature, and 12:12 h light/dark cycles.

2.3. Experimental design

*Drosophil*a standard cornmeal 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) and coconut food (CF) were prepared by adding 10% w/v food-grade coconut

Table 1. Tukey's multiple comparison test on survival, body mass, negative geotaxis, catalase activity, total protein, sterols, and triglyceride (TG/TP) of *E. abyssinica* stem back extract fed obese male *D. melanogaster*.

Tukey's multiple comparison test groups	N	Mass	Negative geotaxis		Catalase activity	Total protein	Sterols	TG/TP	Survival analysis
		10 days	30 min	48 h	10 days	10 days	10 days	10 days	
		Adjusted *p* values							
Negative control vs. Positive control	60	0.0143	0.9393	0.4418	0.0164	>0.9999	<0.0001	<0.0001	Log-rank (Mantel-Cox) test for curve comparisons; X^2 (7) = 16.80, p = 0.0187
Negative control vs. 25 mM	60	0.8570	0.9933	0.2314	0.0277	0.6608	>0.9999	0.8685	
Negative control vs. 50 mM	60	>0.9999	0.0004	0.7267	<0.0001	<0.0001	0.9511	<0.0001	
Negative control vs. 75 mM	60	0.9963	0.7910		0.0007	<0.0001	0.1125	<0.0001	
Negative control vs. 2.5 g/ml	60	<0.0001	0.0132		>0.9999	<0.0001	0.0004	<0.0001	
Negative control vs. 5.0 g/ml	60	0.0143	0.0007		<0.0001	0.0025	0.1258	<0.0001	
Negative control vs. 7.5 g/ml	60	0.8570	0.0063		0.0027	>0.9999	0.0007	<0.0001	
Positive control vs. 25 mM	60	0.1786	>0.9999	0.0258	>0.9999	0.8604	<0.0001	<0.0001	
Positive control vs. 50 mM	60	0.0277	0.0030	0.1127	0.1125	<0.0001	<0.0001	<0.0001	
Positive control vs. 75 mM	60	0.0038	>0.9999		0.7307	<0.0001	<0.0001	<0.0001	
Positive control vs. 2.5 g/ml	60	0.0073	0.1110		0.0130	<0.0001	<0.0001	<0.0001	
Positive control vs. 5.0 g/ml	60	>0.9999	0.0063		0.0829	0.0052	<0.0001	<0.0001	
Positive control vs. 7.5 g/ml	60	0.0010	0.0560	0.7267	0.9792	>0.9999	<0.0001	<0.0001	
25 mM vs. 50 mM	60	0.9626	0.0015		0.0691	<0.0001	0.9114	<0.0001	
25 mM vs. 75 mM	60	0.4775	0.9933		0.5718	<0.0001	0.0872	<0.0001	
25 mM vs. 2.5 g/ml	60	<0.0001	0.0560		0.0220	<0.0001	0.0006	<0.0001	
25 mM vs. 5.0 g/ml	60	0.1786	0.0030		0.0503	0.0709	0.1605	<0.0001	
25 mM vs. 7.5 g/ml	60	0.1786	0.0274		0.9226	0.7829	0.0006	<0.0001	
50 mM vs. 75 mM	60	0.9626	0.0063		0.8482	<0.0001	0.5511	0.0006	
50 mM vs. 2.5 g/ml	60	<0.0001	0.5754		<0.0001	0.0016	<0.0001	<0.0001	
50 mM vs. 5.0 g/ml	60	0.0277	>0.9999		>0.9999	<0.0001	0.0166	0.0019	
50 mM vs. 7.5 g/ml	60	0.6788	0.7910		0.4570	<0.0001	0.0060	0.0178	
75 mM vs. 2.5 g/ml	60	<0.0001	0.2093		0.0006	<0.0001	<0.0001	<0.0001	
75 mM vs. 5.0 g/ml	60	0.0038	0.0132		0.7640	<0.0001	0.0004	<0.0001	
75 mM vs. 7.5 g/ml	60	0.9963	0.1110		0.9957	<0.0001	0.2136	<0.0001	
2.5 g/ml vs. 5.0 g/ml	60	0.0073	0.7910		<0.0001	<0.0001	0.1190	0.0711	
2.5 g/ml vs. 7.5 g/ml	60	<0.0001	>0.9999		0.0022	<0.0001	<0.0001	0.0078	
5.0 g/ml vs. 7.5 g/ml	60	0.0010	0.9393		0.3668	0.0038	<0.0001	0.9339	

oil to the standard cornmeal medium [16, 17]. For the *E. abyssinica* stem bark extract treatment, the extracts at 2.5 g/ml, 5.0 g/ml, and 7.5 g/ml were mixed with the coconut food (CF) and placed in plastic vials as feed for the intervention group of flies. Phenotypically obese flies were divided into 7 groups. Since physiologically effective concentrations in *Drosophila* experiments vary from 0.01 to 100 mM in the feeding substrate [18], groups 1–3 were fed on coconut food (CF) + standard drug fenofibrate at concentrations of 25 mM, 50 mM, and 75 mM. Groups 4–6 were fed on coconut food (CF) + *E. abyssinica* stem bark extract at concentrations of 2.5 g/ml, 5.0 g/ml, and 7.5 g/ml. For the control experiments, flies were placed in plastic vials with coconut food only (positive control) and normal fly food (negative control). Observations of the acute effects (after 30 min to 2 h) and chronic effects (after 48 h (see [18]) of the extract and the standard drug were noted. The diet supplemented with *E. abyssinica* stem bark extract was changed twice a week.

A population of 240 obese male flies raised in 24 vials at a density of 10 flies per vial were used i.e.,

Sample size = Number of experimental groups × number of replicas × number of flies per vial = 8 × 3 × 10 = 240 flies used for each experiment

Total number of flies used = 240 × 6 experiments = 1440 flies were used

2.3.1. *Survival analysis in* D. melanogaster *under* E. abyssinica *supplementary feeding*

To determine the duration of treatment exposure to be used in the experiment, an initial cohort of *E. abyssinica* stem bark feeding experiment was carried out to know the number of dead flies during their lifespan to establish the day(s) when changes in population numbers stop to appear. Survival rate was determined across the eight groups by recording the number of live and dead flies daily for 15 days. Survival was calculated as below;

Percentage survival = (Number of surviving flies/Total number of flies per vial) = (n/10) × 100

2.3.2. *Mass of flies in* D. melanogaster *exposed to* E. abyssinica

Ten flies from each experimental group were frozen and their body weight was measured on a Sartorius microbalance as previously described [19]. The procedure was repeated three times for each experimental group and this was recorded in grams (g).

2.3.3. *Negative geotaxis assay in* D. melanogaster *exposed to* E. abyssinica

Negative geotaxis was investigated as previously described by [20] with minor modifications. Ten flies from the respective groups were immobilized under light anesthesia with ice. They were placed separately in a vertical glass column (15 cm long and 1.5 cm in diameter). After 10 min of recovery, the flies were gently tapped to the bottom of the column and the number of flies that reached the height of 8 cm in one minute were recorded [21]. The tests were repeated three times for each group at one-minute intervals and the mean number were taken as the number of flies that performed positively in the experiment, expressed as a percentage for each group.

Percentage negative geotaxis = number of flies that cross the 8 cm mark / total number of flies × 100 per group

2.3.4. *Biochemical analysis on* E. abyssinica *effect in* D. melanogaster

After 10 days, more samples of 10 flies were picked from each of the experimental groups, immobilized under light anesthesia with ice, and then rinsed using cold phosphate-buffered saline (PBS) solution to remove all traces of food. Whole fly samples were then homogenized in 100 μl of cold 0.05 % phosphate-buffered saline tween (PBST) solution and the homogenate was centrifuged at 13,000 g for 3 min [22]. The supernatant was immediately stored at 4 °C for later assay of catalase activity, total protein, sterol, and triglyceride levels. This procedure was repeated thrice for all the experimental groups.

2.3.4.1. *Reducing power activity of* E. abyssinica *stem bark extract.*

The reducing power of *E. abyssinica* stem bark extract was determined according to the method described by [23]. The absorbance values of ascorbic acid at different concentrations were obtained and a standard curve was plotted according to the protocol below. 1 ml of ascorbic acid at 5%, 15%, 30%, 50%, 75% and 90% w/v and the stem bark extracts at 2.5 g/ml, 5.0 g/ml and 7.5 g/ml were pipetted into clean and dry test tubes. 2.5 ml phosphate buffer (0.2 M, pH 6.6) and 2.5 ml potassium ferricyanide (1% w/v) were added and the mixture was kept in a water bath at 50 °C for 20 min. The mixture was then cooled and 2.5 ml trichloroacetic acid was added and centrifuged at 3000 g for 10 min, the upper layer of the solution (2.5 ml) was mixed with 2.5 ml distilled water and 0.5 ml freshly prepared ferric chloride solution (0.1% w/v) after which, the absorbance values were determined spectrophotometrically at 700 nm.

2.3.4.2. *Total protein and catalase activity in* D. melanogaster.

Total protein was indirectly measured using the Cypress diagnostics kit and Bradford assay protocol [20].

Catalase activity of the fly samples was determined by following a protocol developed by [24] and customized by [20] 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. 100 μl of catalase solution was pipetted in 13 mm diameter × 100 mm height test tubes, 100 μl of 1% Triton X-100 and 100 μl 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 15 min 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 treatment fly samples.

2.3.4.3. *Total triglycerides and sterol levels in* D. melanogaster.

Triglyceride quantification was done 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 540 nm [25]. A Cypress cholesterol oxidase kit was used to quantify sterol levels using the fluorometric assay protocol to indirectly measure the sterol in the form of resorufin at 590 nm fluorescence [22].

2.4. *Statistical analysis*

Graph pad prism version 6 software (Graph pad software, La Jolla, CA, USA) was used for statistical analysis of the biochemical assay tests. The results were reported as mean ± standard error of the mean (S.E.M) using figures and tables. Survival data was analyzed using Kaplan-Meier survival analysis and a Mantel-Cox was performed on the survival curves. A one-way ANOVA, followed by the post hoc Tukey's test, where a $p < 0.05$ was considered to represent a statistically significant difference in the metabolic and antioxidant assay results. Significance was represented using different superscripts a, b, c, d, and e on the figures.

2.5. *Ethical considerations*

Ethical considerations were followed. This research model was selected based on following the replacement protocol for ethics in animal research. Approval for this research from the Kampala International University research ethics committee was sought and upon approval, it gave the number, Nr.UG-REC-023/201916 as part of my MSc dissertation.

3. Results

3.1. Obese fly survival in all intervention groups was similar to that of the positive control on the 10th day. The highest extract concentration had a higher reducing power and reduced body mass significantly

Fly survival in all intervention groups was similar to that of the positive control on day 10. Though 5.0 g/ml and 7.5 g/ml had a protective effect, this was more pronounced at 7.5 g/ml (Figure 1A) while 7.5 g/ml of the extract significantly had the highest reducing power compared to the lowest concentrations used in the study (Figure 1B). In addition, 7.5 g/ml of the extract significantly (Table 1) reduced fly body mass appreciably when compared to 2.5 g/ml and 5.0 g/ml by day 10 (Figure 1C).

3.2. E. abyssinica at high concentrations improved negative geotaxis in obese D. melanogaster after long term exposure

The neuromuscular activity was assessed using the negative geotaxis experiment in which no significant differences were observed after 30 min (acute effects) in all extract experimental groups (Figure 2A). However, after 48 h (chronic effects), only flies exposed to 7.5 g/ml of the extract recovered, although these effects were significantly comparable ($p > 0.05$) to those in 25 mM fenofibrate (Figure 2B).

3.3. E. abyssinica stem bark extract at high concentration reduced fat accumulation and modulated catalase enzyme activity in obese D. melanogaster

Although triglyceride levels (TG/TP) were reduced by the extract, this reduction was not significantly different across *E. abyssinica* extract concentrations unlike fenofibrate (Figure 3A). *E. abyssinica* stem bark extract significantly reduced sterol levels at higher concentrations than fenofibrate (Figure 3B). In addition, levels of total protein were reduced significantly (Table 1) in the 7.5 g/ml extract while 75 mM fenofibrate had the highest total protein content (Figure 3C). Furthermore, catalase activity was higher in the extract than fenofibrate at the corresponding concentrations (Figure 3D).

4. Discussion

The study showed that *E. abyssinica* stem bark at increased concentration had anti-obesity properties. This was in agreement with previous studies on ethnomedicinal plants due to their shared bioactive profile [26, 27, 28]. In particular, the reducing power was concentration-dependent showing that increased concentration of the plant extract could be used to achieve therapeutical effect in the management of a multifactorial pathological condition [23]. A previous *in vitro* pancreatic lipase assay on sigmoidin A from the stem bark of *E. abyssinica* revealed its strong inhibitory effect on pancreatic lipase, an important target in obesity management [29] demonstrating the importance of the current study in furthering knowledge about the effect of the extract on lipid metabolism. In addition, observations in this study were found to be significant after 10 days showing that effects could be attained after long term exposure as compared to short-term use of the extract.

Physiochemical changes such as reduction in body mass and improved locomotory behavior were observed to be reproducible after long term exposure at higher concentrations of *E. abyssinica* stem bark extract. These findings are in agreement with previous studies supporting the ability of *E. abyssinica* stem bark as a weight-limiting complementary medicinal option for regular use [30]. These observations could be associated with the phytochemical profiles of the extract (in particular flavonoids) since these have strong weight-limiting effects [31, 32]. In obesity, reactive oxygen species disrupt neuromuscular coordination

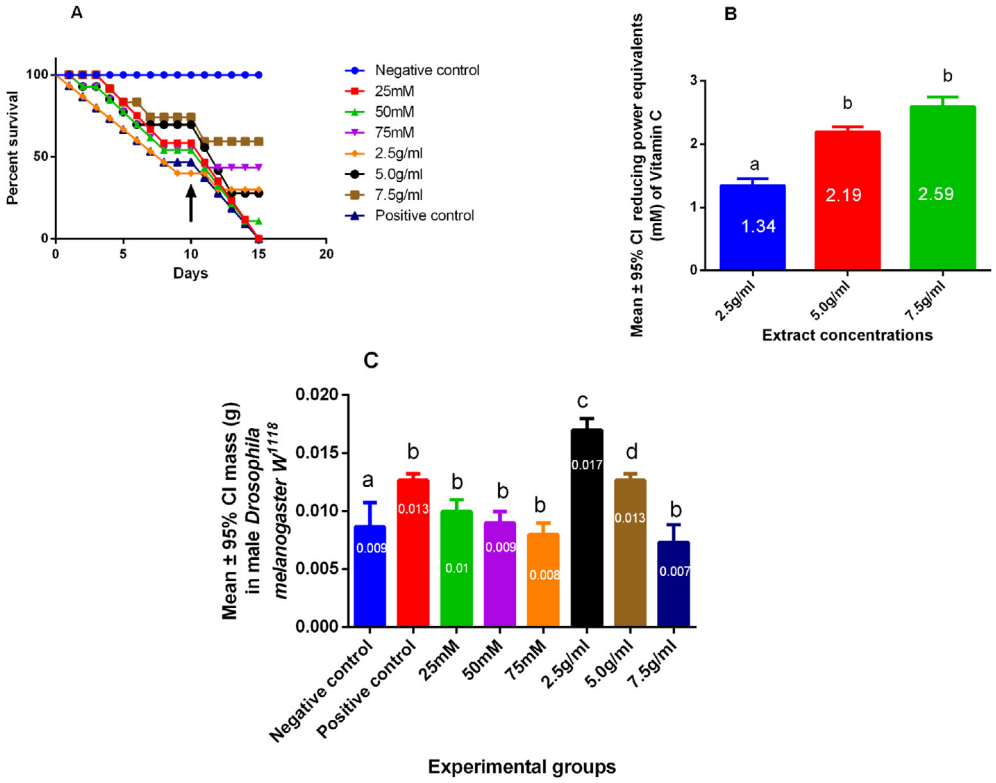

Figure 1. *D. melanogaster* exposed to *E. abyssinica* stem extract showed similar survival rate to the positive control on day 10 **(A)**. Reducing power of the *E. abyssinica* was dependent on the concentration of the extract used **(B)**. Body mass of obese *D. melanogaster* significantly reduced at high concentrations of the extract **(C)**. Similarity of superscripts (a, b, c or d) represent no statistically significant differences ($p > 0.05$) between experimental groups.

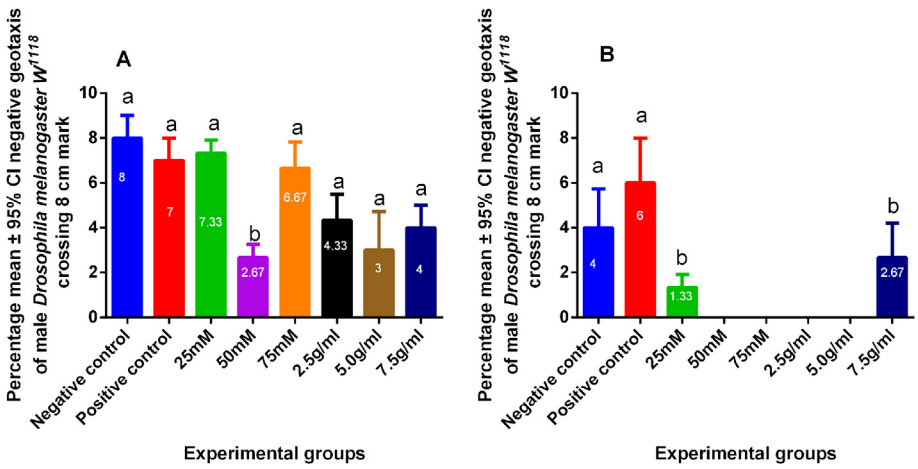

Figure 2. No significant changes in negative geotaxis after 30 min (acute) of exposure to the extract (A). Exposure to the extract after 48 h (chronic) only led to significantly reduced negative geotaxis however, locomotory observations were highest in the 7.5 g/ml *E. abyssinica* stem bark extract (B). Blank space in graph (B) indicate slower fly recovery from light cold anesthesia to reach the 8 cm mark of the vertical column in one minute. Similarity of superscripts (a or b) represent no statistically significant differences ($p > 0.05$) for negative geotaxis between experimental groups.

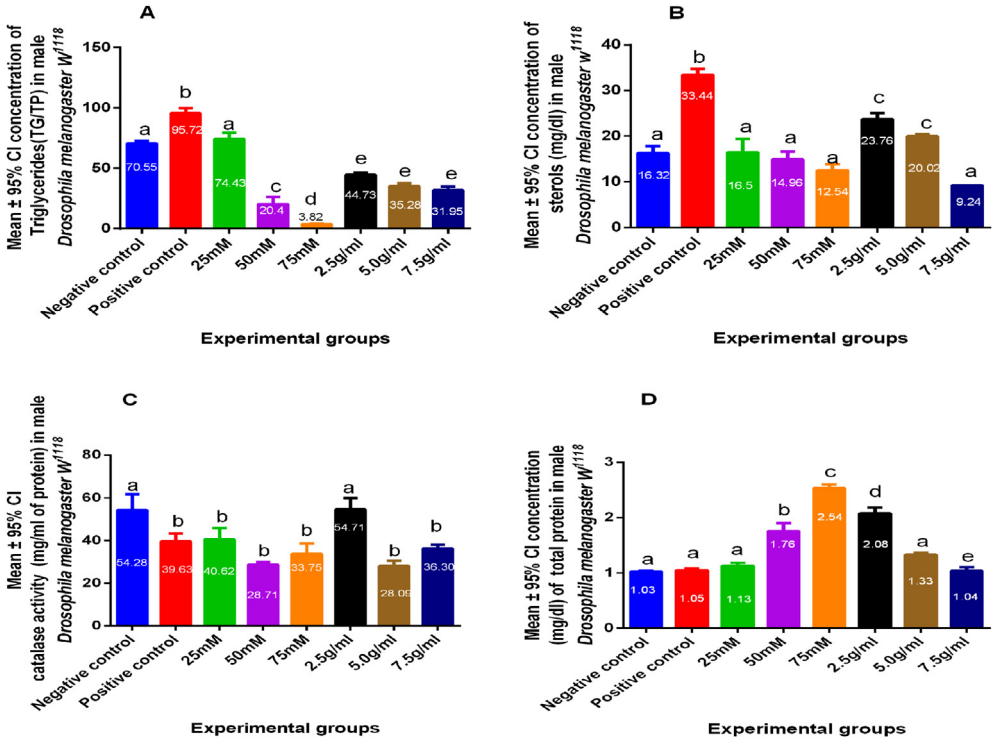

Figure 3. *E. abyssinica* stem bark extract at 7.5 g/ml reduced triglyceride levels (TG/TP) (A), sterol levels (B), total protein (D), and modulated catalase enzyme activity (C) in obese *D. melanogaster*. Similarity of superscripts (a, b, c, d or e) represent no statistically significant differences ($p > 0.05$) between experimental groups.

[33], showing that high concentrations of the extract are associated with beneficial physiochemical and biochemical health effects.

The study also showed that major biochemical properties of *E. abyssinica* stem bark were associated with increased catalase activity. This is important since catalase helps to control oxidative stress [34], by antioxidant replenishments which are reduced in oxidative stress (as a result of reactive oxygen species) during obesity and lipid peroxidation [35, 36]. This offers a novel mechanism through which *E. abyssinica* stem bark disrupts excessive lipid storage in *D. melanogaster* [37, 38]. Furthermore, *E. abyssinica* stem bark reduced triglyceride levels (TG/TP), sterol levels, and total protein providing evidence on the potential mechanism of action. Obesity has been linked to gut microflora alteration and since previous studies showed anti-microbial activity of *E. abyssinica* stem bark extract, *E. abyssinica* stem bark extract could have reduced triglyceride levels in obese flies by restoration of gut microflora balance [3]. This activity of *E. abyssinica* stem bark extract on obese flies was similar to that of other plant extracts in mitigating dietary sterol accumulation as previous studies indicated [35].

5. Conclusion

E. abyssinica stem bark extract exerts protective and obesity lowering effects. This could have been through improved antioxidant-oxidative status at higher concentrations. Reductions in body mass, triglyceride and sterol levels help to promote a healthy metabolic state following long term administration of the extract at high concentrations. Further studies on the effect of the extract on fly lipid histology, cytokines and inflammatory markers could offer more insights on its mechanism of action and place more emphasis on feeding behavioral experiments and molecular markers involved in lipid metabolism.

Declarations

Author contribution statement

Oscar Hilary Asiimwe: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Eddie Wampande, John Rubaihayo, Hellen Wambui Kinyi: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Keneth Iceland Kasozi: Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

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Data availability statement

Data associated with this study has been deposited at https://figshare.com/s/67fec52b94428968c720.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

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