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ORIGINAL RESEARCH

Appetite Suppressing Activity of Rumex Usambarensis Leaf and Stem Aqueous Extract in Wistar Albino Female Rats: an in vivo Experimental Study

Fredrick Atwiine, Albert Mwesigwa, Derick Mwesiga, Polly Mwesigwa, Lawrence Katumba, Patrick Engeu Ogwang

Department of Pharmacy, Mbarara University of Science and Technology, Mbarara, Uganda

Correspondence: Fredrick Atwiine, Mbarara University of Science and Technology, P.O. Box 1410, Mbarara, Uganda, Tel +256759683411, Email fratwiin@must.ac.ug

Background: The burden of obesity and overweight associated morbidity and mortality is increasing in epidemic proportions worldwide. Suppression of appetite is one of the mechanisms that has been shown to reduce weight. Most of the drugs on the market currently for appetite suppression are not readily available or affordable in resource-limited settings. Additionally, previous studies have shown that most of these drugs are associated with significant adverse effects, which demonstrates a need for alternative or complementary options of drugs for appetite suppression. In Uganda, herdsmen commonly chew the raw stems and leaves of Rumex usambarensis, a wild shrub, and this is believed to reduce hunger. This study aimed at determining the effect of Rumex usambarensis aqueous extract on food intake as a measure of appetite in Wistar albino rats.

Methods: This study was carried out in two phases: the fattening phase and the treatment phase. Female albino Wistar rats were fed a high-fat diet for 49 days. The fattened animals were then randomly separated into 4 groups, which received 1 mL of distilled water (negative control), 500 mg/kg body weight of aqueous extract of Rumex usambarensis, 1000 mg/kg body weight of the extract and 20 mg/kg body weight topiramate (positive control), respectively. Food intake was measured every day, and weights were taken every two days for every group.

Results: Rumex usambarensis extract significantly reduced body weight of fattened rats compared to the control group at both doses: for the 500mg/kg dose (Mean difference, MD = 17.2, $p < 0.001$) and for 1000mg/kg dose (MD = 25.9, $p < 0.001$). Additionally, both doses of the aqueous extract showed a significant reduction in food intake: for the 500mg/kg dose (MD = 16.1, $p < 0.001$) and for the 1000mg/kg dose (MD = 37.3, $p < 0.001$). There was a strong correlation between food intake and weight for both doses for the 500mg/kg dose ($r = 0.744$, $p = 0.009$), and the strongest association observed with 1000mg/kg dose ($r = 0.906$, $p < 0.001$).

Conclusion: The aqueous extract of the leaves and stems of Rumex usambarensis has appetite suppressing and weight reduction effects in fattened female Wistar albino rats and could be an efficacious alternative medicine for management of overweight, obesity and other related disorders.

Keywords: Rumex usambarensis, appetite, weight, obesity, dammer

Introduction

Overweight and obesity are defined as abnormal or excessive fat accumulation that may impair health, and their prevalence is increasing in epidemic proportions, with more than 4 million people dying each year as a result of being overweight or obese according to the global burden of disease.[1] These conditions are significant risk factors to various complications and diseases, such as type 2 diabetes mellitus, cardiovascular disorders, osteoarthritis, obstructive sleep apnea, and cancer, among others.[2] Evidence from previous studies indicates that weight loss can significantly reduce the

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risk complications and chronic diseases associated with obesity.[3–5] Diet control, moderate exercise, bariatric surgery and prescription drug treatment are the major interventions for management of obesity and overweight.[6–8]

Appetite regulation plays a major role in the development of obesity.[9] Appetite is the desire to eat food, sometimes due to hunger, and exists in all higher life forms and serves to regulate adequate energy intake to maintain metabolic needs.[10] It is regulated by the close interplay between the digestive tract, adipose tissue and the brain.[11] Dysregulation of appetite leads to overeating disorders which predisposes such individuals to obesity and other associated conditions.[12] Suppression of appetite has shown to be one of the effective ways to reduce weight in obese people.[13] Elucidating the central and peripheral mechanisms regulating appetite has produced anti-obesity drug development programs targeting these pathways, and supplementation with hormonal regulators of appetite reduces appetite which is associated with weight loss.[14] The current drugs used for appetite suppression, such as glucagon-like peptide-1 agonists, bupropion, setmelanotide and amphetamines, have major side effects and long-term toxicity. In resource limited settings, these drugs are not affordable or readily available, hence safer and cheaper alternatives need to be identified.[15]

Rumex genus includes approximately 200 herbaceous species distributed across the world, and some of these species are traditionally used as remedies in different countries and cultures against a variety of disease conditions.[16] *Rumex usambarensis (Dammer)* Dammer [family Polygonaceae] is a wild shrub with stems up to 3 meters or taller.[17] It is commonly found in tropical African countries such as Uganda, Kenya, Tanzania, Sudan, Eritrea, Ethiopia, Somalia, eastern DR Congo and Zambia. It grows commonly in the montane grassland, open mist forest, bushland, exposed rocky slopes and woodlands but also near swamps in lowlands, at elevations from 800 to 2400 meters.[17–19]

The plant is usually harvested from the wild as a local source of food and medicine. Young leaves and stems of the plant are taken raw, and due to their salty flavor, the plant is usually picked from the wild to eat as a snack. The leaves are also pounded and soaked in cold water, and the infusion is used to treat coughs, rheumatism, and stomachache and to reduce gas in the stomach.[20] In Uganda, particularly in western Uganda, herdsmen commonly chew raw stems and leaves as snacks due to their salty taste, as they herd cattle, and it is believed to reduce hunger; a previous study showed that the aqueous extract has an effect on weight and lipid profiles.[19] The aim of this study, therefore, was to determine the effect of *Rumex usambarensis* leaf and stem aqueous extract on appetite, as a possible mechanism by which it causes weight loss.

Material and Methods

Study Design

The study was an in vivo experiment.

Study Site

This study was carried out at Mbarara University of Science and Technology (MUST) located in Mbarara city, Southwestern Uganda. Plant extraction was done at the MUST pharmaceutical analysis laboratory, and In vivo studies were done at the MUST Animal Research Laboratory.

Materials

The feeding plates, pellets, feeding bottles, nose masks, gloves, weighing balances, feeding tubes, beakers and test tubes, redtops, syringes, intragastric cannulas', electric blenders, cotton wool, permanent markers, fans, muslin cloths, and distilled water were used.

Specimen Collection and Identification

The plant was collected from Kiruhura district in southwestern Uganda (0°13'10.0"S 30°48'14.0"E). It was identified by a botanist at MUST, given an Identification number Fredrick Atwiine 001, and the specimen was deposited at the university herbarium.

Induction of Obesity

Thirty healthy female Wistar rats were obtained from the animal facility of Mbarara University of Science and Technology. The animals were allowed to acclimatize in the animal research laboratory for one week while feeding on rat pellets and water at liberty, 12 hours of daylight and 12 hours of darkness. One researcher was blinded and randomly picked six rats without replacing to Group A (control) and fed a normal rat pellet diet (but on day one of the fattening phase, one rat was lost to cannibalism). The other twenty-four (Group B) rats were fed a high-fat diet that consisted of cheese and rat pellets for 49 days. This procedure was performed according to a method modified from that previously described by Buettner et al for the induction of obesity in a rat model.[21] However, another rat from Group B was also lost to cannibalism on day 11, remaining with 23 rats.

Cheese (100 g) was given to the rats in Group B every morning. Both groups had unlimited access to rat pellets and water. After 49 days, the group B animals were weighed, and their average weight was compared with the average weight of the control group.

Preparation of the Aqueous Extract

Fresh leaves and stems of the plants were washed with distilled water, blended and filtered with muslin cloth. The filtrate was concentrated with a fan until a consistent weight was obtained. The percentage yield was calculated as follows:

Percentage yield = (weight of extract powder/weight of the fresh leaves and stems) ×100.

Weight of the fresh leaves and stems = 3151 g;

Weight of the extracted powder = 107 g;

The percentage yield was 3.4%.

Two grams of the dry extract was weighed and dissolved in 20mL of distilled water to make 100mg/mL concentrated extract.

Administration of Extract and Measurement of Food Intake and Weight Changes

On day 50, the Group B rats were sampled into groups by simple randomization to distribute the fattened animals to Groups one, two, three and four. One researcher was blinded and randomly picked one fattened rat without replacement, placing them in each of the four groups. Group one, consisting of 5 rats, was the control group and was given 1mL of distilled water. Groups two and three, each consisting of 6 rats, were given the concentrated extract of *R. usambarensis* at 500 mg and 1000 mg per kilogram body weight, respectively. Group four, which also consisted of 6 rats, was the positive control group and was given topiramate (20 mg/kg body weight), an appetite-suppressing drug.[22] The amount of food given to the rats every morning was weighed. After 24 hours, the amount of food remaining was weighed. The difference in weight was the amount of food consumed by each group per day, and this was done for 20 days.

Data Management and Analysis

All the data collected during the study was recorded and subsequently transferred to Windows Excel 2016. The data was cross-checked for accuracy. Data was then imported SPSS software version 20 for analysis. Descriptive data were generated and are presented as the mean ± standard error of the mean (S.E.) and also presented in tables and graphs. One-way ANOVA was used to detect differences in group means. A student *t* test was used to compare the means if a difference was found in ANOVA and to determine the exact level of significance at $p < 0.05$. Using the Pearson correlation coefficient (r), the association between variables was determined; the closer the Pearson correlation coefficient was to 1, the stronger the association between two variables was.

Results

This study was carried out in two phases: the fattening phase and the treatment phase. In the fattening phase of this study, the rats in group B (high-fat diet) had an average weight (g) ± S.E. of 171.2± 3.6 on day 1, and by day 49, they had 211.1 ± 3.7 with an average weight gain of 23.3% ($p < 0.001$), which was statistically significant. In group A (control group), the rats had an

average weight (g) ± S.E. of 172.6 ± 12.4 on day 1 and on day 49; they had 179.4 ± 13.2 with an average percentage weight gain of 3.9% (p = 0.718), which was not statistically significant (Figure 1).

In the treatment phase of this study, the effect of the aqueous extract of *Rumex usambarensis* on weight and food intake was evaluated as a measure of appetite.

The average weight difference (g) ± S.E. for group 1 (the control group) on day 21 of the treatment phase was 2.2 ± 2.6, that for group 2 was −15.0 ± 0.3, for group 3 was −23.7 ± 1.2, and that for group 4 was −8.3 ± 2.0 (Figure 2). The mean difference (MD) in weight for all treatment groups compared to the control group was significant for group 2 (MD = 17.2, p < 0.001), group 3 (MD = 25.9, p < 0.001) and group 4 (MD = 10.5, p = 0.02).

The average food intake (g) ± S.E. in Group 1 (distilled water) was 71.2 ± 1.7, that in Group 2 (500 mg/kg) was 55.1 ± 1.1, that in Group 3 (1000 mg/kg) was 33.9 ± 2.1, and that in Group 4 (topiramate 20 mg/kg) was 50.1 ± 2.4 (Figure 3). The difference in food intake between the groups was statistically significant (f value = 66.45, p < 0.001). The mean difference in food intake among all treatment groups compared to the control group was also statistically significant for Group 2 (MD = 16.1, p < 0.001), Group 3 (MD = 37.3, p < 0.001) and Group 4 (MD = 21.1, p < 0.001). Food intake was also strongly associated with changes in weight, with the strongest association observed in group 3 (r = 0.906, p < 0.001), followed by group 4 (r = 0.892, p < 0.001) and group 2 (r = 0.744, p = 0.009).

Discussion

During the fattening phase, the weight gain in group B was significantly greater than that in the control group. This is because the cheese that was fed to group B rats was a high-fat diet and which has shown to induce weight gain[21,23] compared to that in the control group, which was fed pellets alone.

In the treatment phase, compared with those in the control group, the mean differences in weight change in the *Rumex usambarensis* aqueous extract and topiramate treatment groups were statistically significant. This finding is in agreement

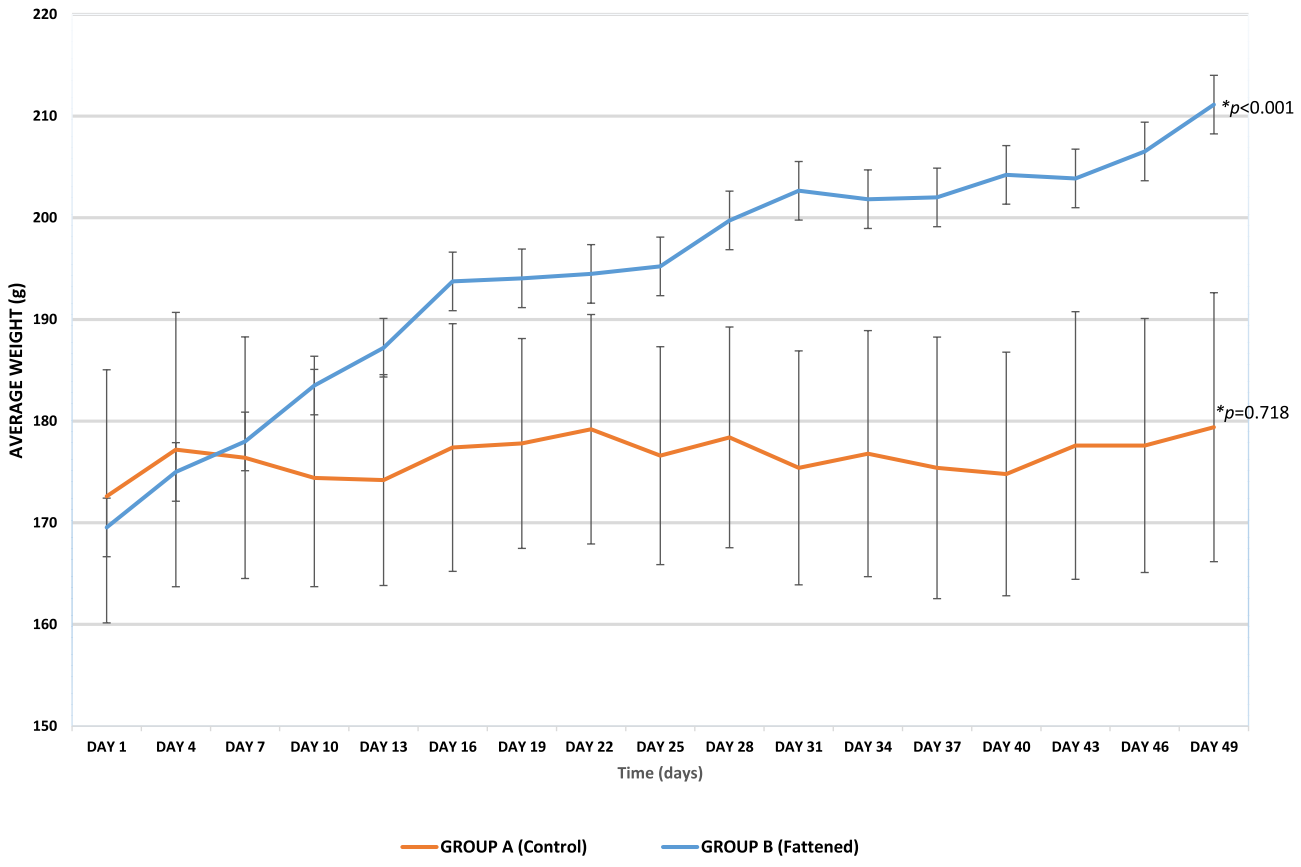


Figure 1 Average weight during the fattening phase against time.

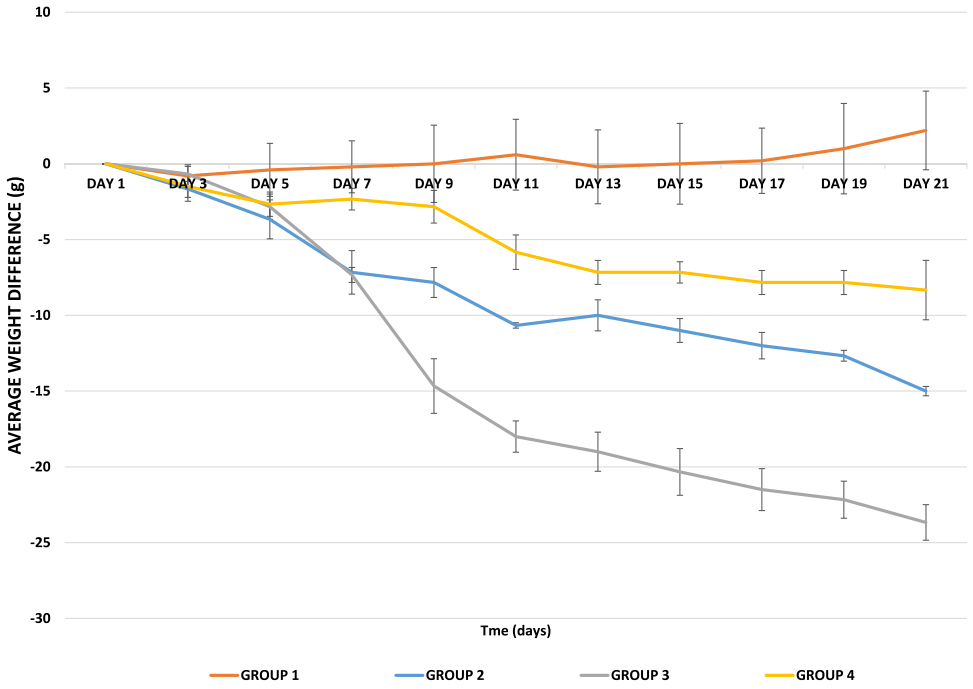


Figure 2 Average weight difference over time in the different treatment groups.

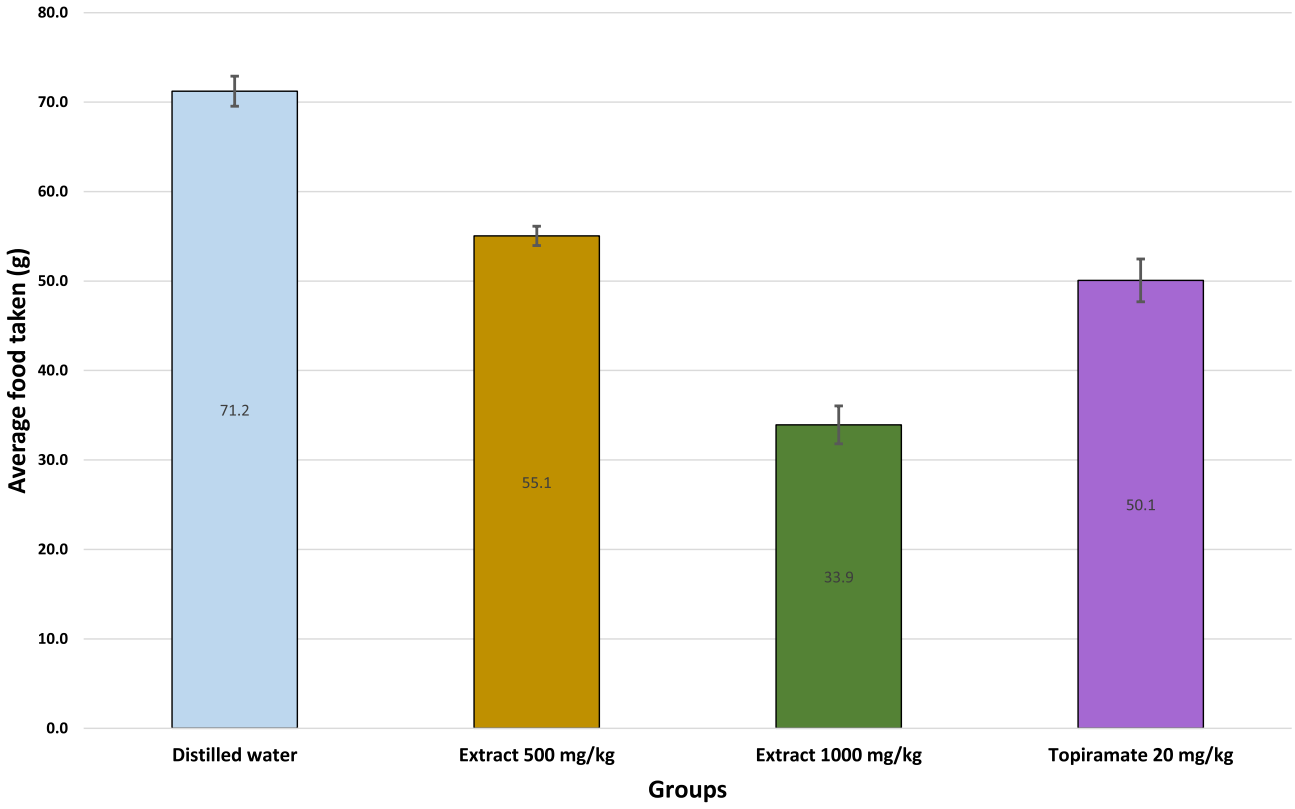


Figure 3 Average food intake of different treatment groups over time.

with that of a study by Hilda et al, which also reported a weight loss effect of the aqueous extract.[19] This could be attributed to the presence of alkaloids and saponins in the aqueous extract of *Rumex usambarensis*, which have shown to have some weight reduction activity.[19,24–26]

Appetite being the desire to eat food, makes it a qualitative outcome and therefore difficult to quantify in a valid and reliable way.[27] Because appetite is directly proportional to food intake, a measure of food intake can indirectly measure appetite.[28] The food intake for all the treatment groups was found to be significantly lower than that of the control group. This could indicate that the aqueous extract has a significant appetite suppressive effect. Both doses of 500 mg/kg and 1000 mg/kg were well tolerated, although the daily dose of 1000 mg/kg *Rumex usambarensis* extract had the greatest appetite suppression effect and was also strongly associated with weight loss, which shows that the effect could be dose dependent. Plants belonging to the Polygonaceae family are known to produce a numerous biologically important secondary metabolites, such as flavonoid glycosides, stilbenoids and phenolic acids.[29] Flavonoids such as flavo-lignan, tannin, and anthocyanins, flavonol, aromadendrin-glycoside, isoflavone, and flavanones are the predominant class in *R. usambarensis* leaves.[17] The stem also has been reported to contain different lipid structures, including long-chain fatty acids such as icosanedioic acid and octadecadienoic acid like as seen in other *Rumex* spp.[17,30] Most of these phyto-chemicals have been reported to have appetite suppressing and weight reduction activity,[31,32] which might explain the activity observed with the *R. usambarensis* extract in this study.

In group four (positive control), which received a daily oral dose of 20 mg/kg Topiramate, the reduction in food intake, and therefore appetite suppression, was statistically significant compared to that in the control group; however, the activity was similar to that in the 500 mg/kg extract group but significantly lower than that in the 1000 mg/kg extract group. The appetite-suppressive activity of topiramate has been documented, and it has been shown to cause a significant reduction in the body weight of obese diabetic patients.[33] Topiramate, however, is associated with numerous side effects[34] and may not be readily available.

Other classes of drugs such as glucagon-like peptide-1 receptor (GLP-1) agonists including beinaglutide, exenatide and liraglutide have shown promise as several animal experiments and clinical trials have demonstrated that these drugs are more effective in treating or preventing obesity with some exhibiting appetite suppressing activity.[6,11,35,36] However, safety concerns have been expressed regarding the significant adverse effects of these drugs on long use, notably on pancreatic and thyroid tissue, as previous animal studies indicate an association of GLP-1 receptor agonists with pancreatitis, pancreatic cancer, and thyroid cancer.[37–39] More so, in resource limited settings, these drugs are very expensive and may not be affordable to the most of the population. Therefore, based on the results in this study, this plant extract may provide a less expensive, more readily available and even a better alternative to some of the conventional appetite suppressing drugs. It is however worthy to note that the safety of this extract has not been established and more studies are recommended on this plant extract, as *Rumex* species have been found to contain high amounts of oxalic acid which is associated kidney problems if consumed in large amounts.[29]

The strong correlation between food intake and weight identified in this study, as shown also in numerous other studies,[40–42] emphasizes the need to control food intake, especially in obese people who have been shown to have a dysregulated appetite due to leptin resistance,[43] and in patients with overeating disorders,[44] which may lead to greater weight gain. Additionally, with the need to control cardiovascular diseases associated with overweight and obesity,[45,46] appetite suppression as a means of weight reduction is key in such patients.[13]

Conclusion

The aqueous extract of the leaves and stems of *Rumex usambarensis* has an appetite suppressing and weight reduction effect in female Wistar albino rats. The high dose of the extract showed the strongest activity in both weight reduction and appetite suppression than topiramate which was the positive control. The active compounds in *Rumex usambarensis*, if purified, could provide an effective alternative for appetite suppression as a way of weight reduction and should be explored in further studies to establish its safety and for possible development into a drug for the management of overweight, obesity, and other related disorders.

Abbreviations

ANOVA, Analysis of variance; MD, Mean difference; MUST, Mbarara University of Science and Technology; NIH, National Institute of Health; S.E, Standard Error; SPSS, Statistical Package for the Social Sciences.

Data Sharing Statement

The datasets used and analyzed during this study are available from the author upon request.

Macy staff and our parents for all the guidance and support during this study.

Ethical Approval and Animal Care

The research proposal was approved by the MUST Department of Pharmacy Research Committee and the Research and Ethics Committee of Mbarara University of Science and Technology. All animals were humanely treated as per the National Institute of Health (NIH) guidelines for the care and use of laboratory animals.[47] This included acquiring, caring for, using and disposing of animals in compliance with current state and local laws and regulations and with professional standards. Reasonable efforts were made to ensure their comfort, health, and humane treatment and minimize pain. Proper animal handling techniques were followed regarding feeding, housing, and general handling throughout the study period.

Consent for Publication

All the authors agreed to the submission of this manuscript for publication.

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Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising, or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be

accountable for all aspects of the work.

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Disclosure

The authors report no conflicts of interest in this work.

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