Journal of Visualized Experiments

Rapid model to evaluate the anti-obesity potential of a combination of Syzygium aromaticum and Cuminun cyminum on C57bl6/j mice fed high-fat diet

--Manuscript Draft--

Article Type:	Methods Article - Author Produced Video
Manuscript Number:	JoVE62087R4
Full Title:	Rapid model to evaluate the anti-obesity potential of a combination of Syzygium aromaticum and Cuminun cyminum on C57bl6/j mice fed high-fat diet
Corresponding Author:	Rosa Pérez Gutiérrez Instituto Politecnico Nacional Secretaria de Investigacion y Posgrado CDMX, CDMX MEXICO
Corresponding Author's Institution:	Instituto Politecnico Nacional Secretaria de Investigacion y Posgrado
Corresponding Author E-Mail:	rmpg01@hotmail.com
Order of Authors:	Rosa Pérez Gutiérrez
	María Arrioja
Additional Information:	
Question	**Response**
Please indicate whether this article will be Standard Access or Open Access.	Standard Access (US$1200)
Please specify the section of the submitted manuscript.	Medicine
Please confirm that you have read and agree to the terms and conditions of the author license agreement that applies below:	I agree to the Author License Agreement
Please provide any comments to the journal here.	None
Please indicate whether this article will be Standard Access or Open Access.	Standard Access ($1400)
Please confirm that you have read and agree to the terms and conditions of the video release that applies below:	I agree to the Video Release

TITLE:

Rapid Model to Evaluate the Anti-Obesity Potential of a Combination of *Syzygium aromaticum* (Clove) and *Cuminun cyminum* (Cumin) on C57BL6/j Mice Fed High-Fat Diet

AUTHORS:

Rosa Martha Pérez Gutiérrez, María Woolrich Arrioja

Laboratorio de Investigación de Productos Naturales. Escuela Superior de Ingeniería Química e Industrias Extractivas. Instituto Politécnico Nacional (IPN) Unidad Profesional Adolfo López Mateos S/N Av. Instituto Politécnico Nacional Ciudad de Mexico, Mexico

rmpg01@hotmail.com
maria.woolrich@hotmail.com

KEYWORD:
Obesity, High-Fat Diet, metabolic syndrome, weight loss, lipid profiles

SUMMARY

This work presents a protocol to explore the anti-obesity effect of two plants used together, for a 5-week duration. There was a combined administration of the extract and high-fat-diet (HFD) in the obese mice. The method can promote the benefits of plants in the treatment of obesity.

ABSTRACT

Several studies have demonstrated that the phytochemical contents of plants are potential anti-obesity agents. In this study we examine the effect of using a combination of dry buttons from *Syzygium aromaticum* and seeds from *Cuminum cyminum* (CC) on C57BL6/J mice induced with obesity via high-fat-diet (HFD). The aim of this study is to demonstrate that the method proposed in the study reduced obesity significantly after several weeks of experimentation. The extract from both plants was extracted using ultrasound to enhance the extraction of phytochemicals. Optimum extraction conditions were obtained with ethanol as follows: 50:50 v/v water with an ultrasound power of 300 W, and ultrasonication time of 30 minutes. The simultaneous administration of the CC extract in HFD for 5 weeks led to the regulation of lipid profiles (cholesterol and triglycerides), reduction of food intake, weight gain, adipose tissue and liver weight. Findings obtained by this obese model indicate that CC extract can prevent obesity. Compared with the traditional 16 weeks method (8 weeks to get fat, and 8 weeks to lose weight), similar results were obtained in the present study obese model in less time of experimentation.

INTRODUCTION

Excess body fat accumulation is a characteristic of obesity. The disequilibrium between energy intake and consumption leads to the storage of excess energy in adipocytes, which is related to metabolic risk factors for hyperglycemia and insulin resistance in type 2 diabetes, hypertension, hypercholesterolemia, and cardiovascular disease[1,2].

Natural products with minimum side effects and low cost have received increased attention since previous studies have reported bioactive phytochemicals and potential anti-obesity agents with mechanisms that reverse or delay metabolic syndrome and associated pathologies[8].

Several medicinal plants have been studied to prevent obesity and related diseases. Among them, *Syzygium aromaticum* has been investigated for its anti-obesity potential in *in vitro* treatment on 3T3-L1 cells and *in vivo* treatment on mice fed with a high-fat diet[11]. In addition, significant anti-overweight effects were observed in a multi-center open trial of *Cuminum cyminum* on extremely obese subjects[12]. In this study, C57BL6/J mice was used to investigate the experimental fast model to evaluate a potential anti-obesity agent using a combination of both edible plants.

PROTOCOL

All animal experiments were approved by the Animal Experiment Committee of Escuela Nacional de Ciencias Biologicas (IPN; Protocol No. 2732).

1. Preparation of the extract of the combination of cloves and cumin

NOTE: The edible plants, *Syzygium aromaticum* (clove) and *Cuminum cyminum* (cumin) were purchased from Central de Abastos CDMX, Mexico.

1.1. Grind 500 g of seeds from the plants and perform ultrasonic-assisted extraction. Sonicate them (320 Watts; 24 kHz frequency, 30 min) with ethanol:water (60:40 v/v) at a temperature range of 30 ± 4 °C.[13]

1.2. Filter the solids using Whatman number 2 filter paper under vacuum and concentrate the extract with a rotary evaporator.

1.3. Measure the UV-vis spectrum of the extract of both plants (CC).

2. Animals

2.1. Use male C57BL6/J mice of six weeks old (48 in total, body weight 24-29 g) for the study.

2.2. House the animals in groups of eight in standard laboratory conditions: temperature (20-22 ± 1 °C), relative humidity (45-54 ± 2%), lighting (08:00–20:00 h) with food and water ad libitum. Acclimate the mice in these conditions for a week before the experiment.

3. Experimental Procedure

3.1. Prepare the high fat diet[14] as in **Table 1**.

3.2. At the beginning of the test, subject each of the groups formed (n = 8) to different treatments: Feed Group 1 on a normal diet; Group 2, on high-fat diet; Group 3, on high-fat diet + 100 mg/kg of CC extract; Group 4, on high-fat diet + 250 mg/kg of CC extract; Group 5, on high-fat diet + 450 mg/kg of CC extract; Group 6, on high-fat diet + phentermine (an antiobesity drug used as positive control)[15].

3.3. Feed all the groups of mice for 5 weeks and record the amount of food consumed daily.

3.3.1. In a test tube with stopper, homogenize the extract or the drug with the dose of water corresponding to the group to which the treatment is being administered. This is done with vortex shaker.

3.3.2. Once homogenized, add all the corresponding treatment into the drinker of each group.

4. Sample collection

4.1. Measure both food intake and body weight once per week. Fast the mice overnight for a 5-week period.

4.2. Sacrifice them by cervical dislocation and necropsy.

4.3. After the mice have been sacrificed, immobilize them in a supine position.

4.4. Using clean surgical forceps and scissors, locate and lift the central skin near the genitals and make a small 0.2 mm incision.

4.5. Insert the flat scissors horizontally into the incision and carefully separate the abdominal skin from the abdominal wall.

4.6. With the forceps, lift the central skin and with the surgical scissors cut it at the level of the rib cage. Then cut the skin above both of the hind limbs to facilitate the collection of adipose tissue.

4.7. Ensure the collection of all adipose tissue from the body by blunt dissection with scissors and forceps. Once collected, place the adipose tissue in aluminum foil. Remember to collect the fat around the reproductive organs. Avoid the accumulation of hair and skin to not alter the results.

4.8. To access the visceral adipose tissue, cut the abdominal muscle with surgical scissors from the genitals to the rib cage, making an incision in the abdominal muscles from the genitals towards the mouse's back.

4.9. Perform a hepatectomy to remove 100% of the liver. To externalize the liver, remove unnecessary organs and cut the hepatic veins and arteries to separate the liver from the rest of the body. As the last step, precisely extract the gallbladder from the liver[16].

4.10. Measure hypercholesterolemia (cholesterol) and hypertriglyceridemia (triglycerides) using commercial assay kits according to the manufacturer's indications.

5. Statistical analysis

NOTE: All experimental results should be representative from three independent assays, expressed as mean ± standard deviation.

5.1. Calculate standard error with a one-way ANOVA analysis of variance followed by Tukey's range test. Consider a $P< 0.05$ as statistically significant.

REPRESENTATIVE RESULTS

Ultrasonic-assisted extraction

The optimum extraction condition for the extraction was ethanol:water (50:50, v/v) with an ultrasound power of 300 W and an ultrasonication time of 30 min. This way, the extraction was faster than conventional extraction methods (**Table 2**). The extract showed an intense absorption peak at 320 nm in UV-visible spectra (**Figure 1**).

Food intake and body weight

The changes in the body weight of the mice given different concentrations of the extract (100, 200 and 450 mg/kg) for 5 weeks are shown in **Figure 2A**. The percentages of weight reduction in groups 3, 4 and 5 were 12.2%, 30%, and 41%, respectively. This shows the lowest body weight gain was observed in all the groups; however, the HFD group had an increase of 2.1x compared to the normal fat diet group. These data indicate that a 450 mg/kg dose of CC is more effective than 30 mg/kg phentermine (39% reduction) in reducing body weight gain.

The mean food intake at the end of the experiment in the HFD + CC (450 mg/mL) was 31% lower than that in the obese group (HFD), with 1.3 g reduction in food intake compared to the obese group (**Figure 2B**). Food intake in the HFD-fed mice was higher by 1.5-fold than in the normal control, thus it significantly ($P<0.05$) decreased in all CC groups in a range of 7.14% to 31%. The diet efficiency rate in HFD group was 16%, which was significantly different ($P< 0.05$) from that of the control group (ND) at 11%. These results demonstrate that CC can reduce HFD-induced body weight gain.

Adipose tissue and liver weight

The weight of epididymal WAT, subcutaneous fat and perirenal WAT adipose tissues was higher in the HFD- group, but not in the HFD-CC groups (**Table 3**). The liver weight was significantly ($P<0.05$) decreased in the CC group (100 to 450 mg/kg) with a range of 15%-28.4% compared to those of the HFD group (**Table 4**).

Liver lipids and serum

The levels of serum triacylglycerol (TG) and total cholesterol (TC) after 5 weeks of treatment are shown in **Figures 3A** and **3B**, respectively. The triglycerides and total cholesterol level were significantly elevated by 1.32-fold and 1.31-fold, respectively, in HFD groups compared to those in the ND groups. The increased levels of plasma TG and TC in the HFD diet were significantly attenuated by the administration of 200 and 450 mg/kg/day CC extract by 18.18% and 22.7% for TG and 16.41% and 20.61% for TC, respectively compared to the control (ND).

FIGURE AND TABLE LEGENDS:

Figure 1. UV-vis spectrum of clove and cumin extract (CC)

Figure 2. Effects of CC on: (A) body weight (g), (B) food intake in High-Fat Diet C57BL6/J mice at 4 weeks of treatment. Data are represented as mean ± SD. For each group, n=8. Different letter showed significant difference ($p<0.05$).

Figure 3. Effects of CC on: (A) Triglycerides, (B) Total cholesterol. All experimental data were Mean ± SD mice (n = 8). Mice were treated with 100, 200 and 450 mg/kg of CC for daily oral administration with simultaneous feeding of HFD for 5 weeks. Different letters indicate significant differences among all the groups ($P < 05$).

Table 1. High fat diet for C57BL6/J mice.

Table 2. Optimal ultrasound conditions for the preparation of the clove and cumin extract

Table 3. Effect of CC in diet-induced obese mice on white adipose tissue weight. Each value is expressed as the mean ± SD (n = 8). Different letter showed significant difference ($P<0.05$) vs white adipose tissue (WAT)

Table 4. Effect of CC in diet-induced obese mice on liver tissue weight. Each value is expressed as the mean ± SD (n = 8). Different letter showed significant difference ($P<0.05$) vs white adipose tissue (WAT).

Discussion

In this study we evaluated, for the first time, the effect of oral administration of a combination of cloves and cumin (CC) extract on lipid profiles and obesity in mice fed with a high-fat diet for 5 weeks. Findings indicated that the HFD groups showed a significantly higher body weight gain compared to the ND group, which showed that the induction of obesity in the obese model was successful. The administered dose of CC (100, 200 and 450 mg/kg/day) produced a reduction in food intake and body weight. Consequently, the reduction in food intake must be due to a decline in the animals' appetite.

The reduction of dyslipidemia is very important to prevent obesity-related disorders. In our study, the total cholesterol levels and triglycerides were lower in the CC group compared to those in

HFD and phentermine groups used as positive control. Findings suggested that CC may inhibit hyperlipidemia and its complications.

Our study showed that the HFD group had increased body fat, including abdominal and liver fat that were significantly reduced by the CC extract supplementation. This shows that the CC extract treatment on the HFD mice reduces lipid absorption due to its anti-adipogenic activity.

In this study, we evaluated a fast model to determine the anti-obesity effect of an extract from a combination of edible plants, *Syzygium aromaticum* (clove) and *Cuminum cyminum* (cumin) on C57BL6/J mice, which rapidly developed obesity with a high-fat diet. The CC treatment significantly prevented the development of obesity and ameliorated hyperlipidemia induced by HFD given to the mice. Altogether, findings in this research provide solid evidence that this fast method of 5 weeks duration developed in our laboratory could be used to assess whether a plant has the capacity to be a potential agent for treating obesity.

ACKNOWLEDGEMENTS:

This research was supported by Instituto Politécnico Nacional México.

DISCLOSURES:

Authors declare no conflict of interest.

REFERENCES

1. Singla, P., Bardoloi, A., Parkash, A. A. Metabolic effects of obesity: A review. *World Journal of Diabetes*. **1** (1), 76–88 (2010).

2. Ojulari, O.V., Lee, S.G., Nam, J.O. Beneficial Effects of Natural Bioactive Compounds from *Hibiscus sabdariffa* L. on Obesity. *Molecules.* **24** (1), 210 (2019).

3. Bahmani, M. et al. Obesity phytotherapy: review of native herbs used in traditional medicine for obesity. *Journal of Evidence-Based Integrative Medicine.* **21** (3), 228-234. (2016).

4. Ríos-Hoyo, A., Gutiérrez-Salmeán, G. New Dietary Supplements for Obesity: What We Currently Know. *Current Obesity Reports*. **5** (2), 262-70 (2016).

5. Hasani-Ranjbar, S., Nayebi, N., Larijani, B., Abdollahi, M. A systematic review of the efficacy and safety of herbal medicines used in the treatment of obesity. *World Journal Gastroenterology*. **15** (25), 3073-3085 (2009).

6. Hardeman, W., Griffin, S., Johnston, M., Kinmonth, A. L., Wareham, N. J. Interventions to prevent weight gain: a systematic review of psychological models and behaviour change methods. *Nternational Journal of Obesity and Related Metabolic Disorders.* **24** (1), 131-143 (2000).

7. Dinda, B., Dinda, M., Roy, A, Dinda, S. Dietary plant flavonoids in prevention of obesity and diabetes. *Advances in Protein Chemistry and Structural Biology*. **120**, 159-235 (2020).

8. Mayer, MA., Hocht, C., Puyo, A., Taira, CA. Recent advances in obesity pharmacotherapy. *Current Clinical Pharmacology.* **4** (1), 53-61 (2009).

9. Rafie, A.Z.M., Syahir, A., Ahmad, W.A.N.W., Mustafa, M.Z., Mariatulqabtiah, A.R. 2018. supplementation of stingless bee honey from heterotrigona itama improves antiobesity

parameters in high-fat diet induced obese rat model. *Evidence-Based Complementary and Alternative Medicine*. 6371582 (2018).
10. Song, J. et al. Anti-obesity effects of the flower of prunus persica in high-fat diet-induced obese mice. *Nutrients*. **11**, 2176 (2019).
11. Jung, C. H., Ahn, J., Jeon, T.-I., Kim, T. W., Ha, T. Y. *Syzygium aromaticum* ethanol extract reduces high-fat diet-induced obesity in mice through downregulation of adipogenic and lipogenic gene expression. *Experimental and Therapeutic Medicine.* **4** (3), 409–414 (2012).
12. Said, O., Saad, B., Fulder, S., Khalil, K., Kassis, E. Weight Loss in Animals and Humans Treated with "Weigh level", a Combination of Four Medicinal Plants Used in Traditional Arabic and Islamic Medicine. *Evidence-Based Complementary and Alternative Medicine.* 874538 (2011).
13. Anaya-Esparza, L. M., Ramos-Aguirre, D., Zamora-Gasga, V. M., Yahia, E., Montalvo- Gonzalez, E. Optimization of ultrasonic-assisted extraction of phenolic compounds from *Justicia spicigera* leaves. *Food Science and Biotechnology.* **27** (4), 1093–1102 (2018).
14. Ayoub, R. M. et al. The effect of anthocyanin-rich purple vegetable diets on metabolic syndrome in obese Zucker*. Journal of Medical Food*. (2017).
15. Go, R. et al. Effects of anti-obesity drugs, phentermine and mahuang, on the behavioral patterns in Sprague-Dawley rat model. *Laboratory of Animals Research*. **30** (2), 73-78 (2014).
16. Tan, P., Pepin, É., Lavoie, J. L. Mouse Adipose Tissue Collection and Processing for RNA analysis. *Journal of Visualized Experiments*. **13**, e57026 (2018).
17. Bjorndal, B., Burri, L., Staalesen, V., Skorve, J., Berge, R. K. Different adipose depots: Their role in the development of metabolic syndrome and mitochondrial response to hypolipidemic agents. *Journal of Obesity.* **2011**, 490650 (2011).

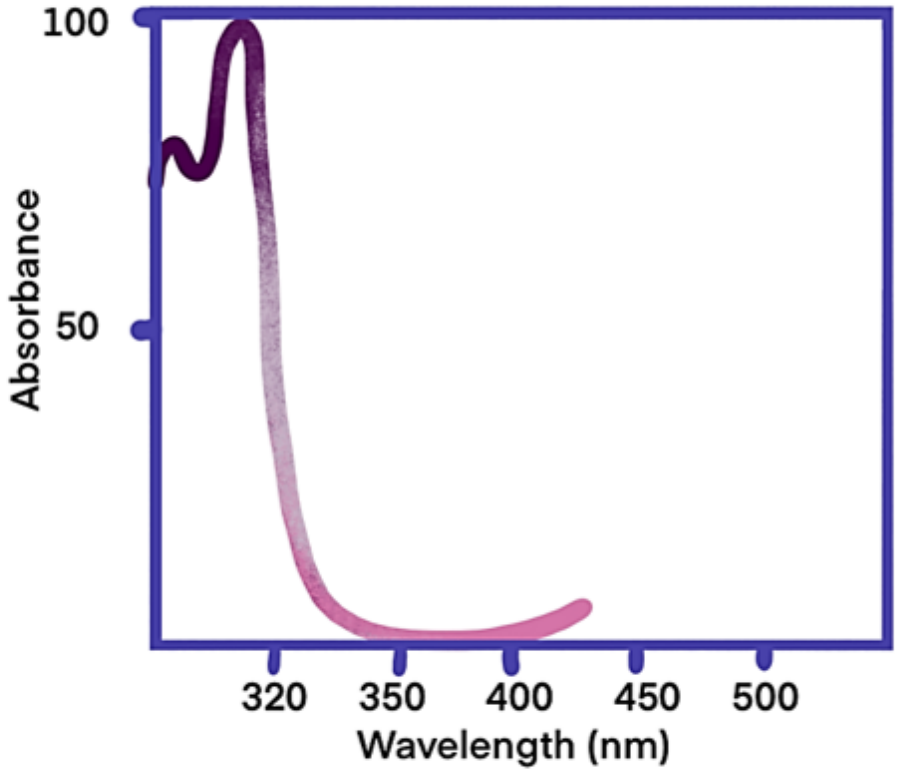

Figure 1. UV-Vis spectrum of clove and cumin extract (CC)

Figure

Click here to access/download;Figure;Fig 2.docx

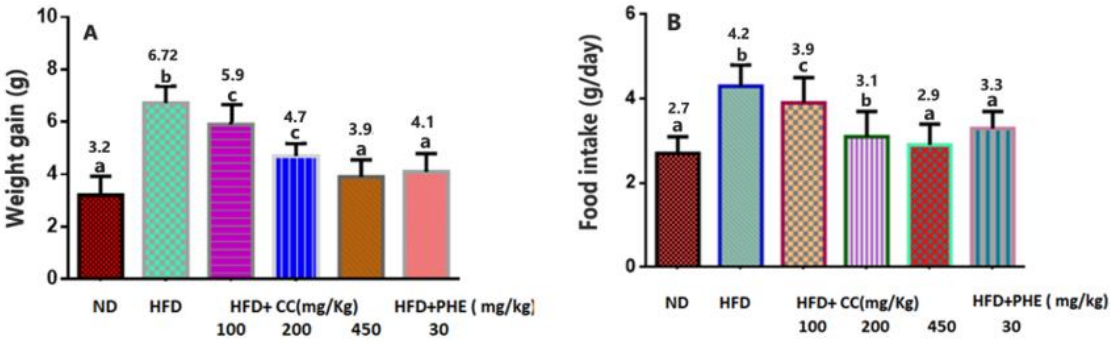

Figure 2. Effects of CC on: (A) body weight (g), (B) food intake in High-Fat Diet C57BL6/J mice at 5 weeks of treatment. Data are represented as mean ± SD. For each group, n=8. Different letter showed significant difference ($p<0.05$).

Figure

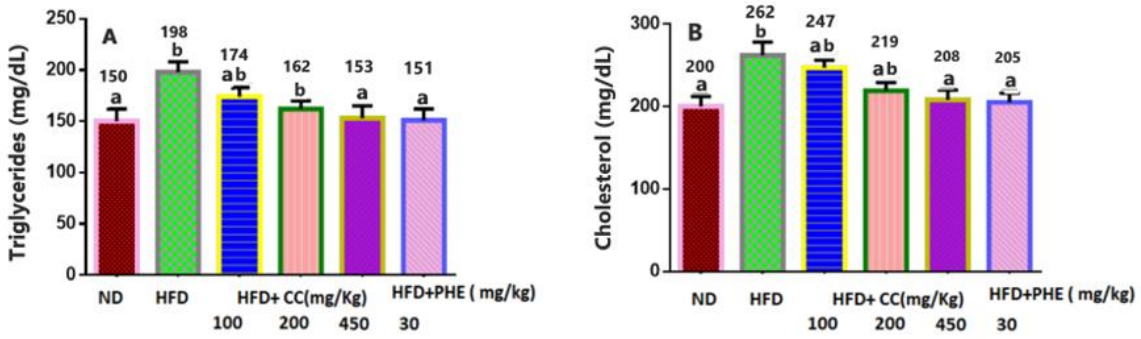

Figure 3. Effects of CC on: (A) Triglycerides, (B) Total cholesterol. All experimental data were Mean ± SD mice (n = 8). Different letters indicate significant differences among all the groups (P < 05).

Table 1

Ingredient	Quantity (g/kg)
Casein	140
L-Cysteine	1.8
lard	120
Soybean oil	40
Maltodextrin 10	150
Sucrose	450
Cellulose	50
Vitamins and Minerals	Tablet equivalent
Choline Bitartrate	2.5

Table 2

Power	370 Watts
Water temperature	30 °C (86 °F)
Extraction time	30 min
Solvent concentration	50% ethanol/50% distilled water
Number of extractions	2 extractions

Table 3

Group	Epididymal WAT (g)	Perirenal WAT (g)	Subcutaneous fat (g)	Fat/Body Weight (g/100)
ND	0.32 ± 0.04[a]	0.05 ± 0.06[a]	0.08 ± 0.01[b]	1.22 ± 0.09[a]
HFD	2.30 ± 0.09[b]	0.93 ± 0.07[b]	0.15 ± 0.04[a]	6.38 ± 1.02[b]
HFD + CC 100 mg/kg	1.26 ± 0.08[b]	0.50 ± 0.05[b]	0.12 ± 0.02[a,b]	5.12 ± 1.93[b]
HFD + CC 200 mg/kg	0.90 ± 0.05[b]	0.35 ± 0.04[b]	0.11 ± 0.04[a,b]	3.21 ± 0.32b
HFD + CC 400 mg/kg	0.42 ± 0.07[a]	0.16 ± 0.02[c]	0.09 ± 0.03[b]	2.30 ± 0.45[c]
HFD + PHE 30 mg/kg	0.49 ± 0.05[a]	0.19 ± 0.08[c]	0.10 ± 0.01[b]	2.41 ± 0.18[c]

Table 4

Group	Liver weight (g)
ND	1.01 ± 0.05[a]
HFD	1.48 ± 0.08[b]
HFD + CC 100 mg/kg	1.26 ± 0.09[b]
HFD + CC 200 mg/kg	1.12 ± 0.03[b]
HFD + CC 400 mg/kg	1.06 ± 0.06[a]
HFD + PHE 30 mg/kg	1.08 ± 0.07[a]

Click here to access/download
Table of Materials
Table of Materials.xlsx

Correction in the text

Titulo:

Experimental rapid model to determine the effect of Syzygium aromaticum (Clove) and cuminnum (Cumin) plants´extract on c57bl6/j mice fed high-fat diet as potencial anti-obesity therapeutic agents was change to

Rapid model to evaluate the anti-obesity potential of a combination of Syzygium aromaticum (Clove) and Cuminun cyminum (Cumin) on C57bl6/j mice fed high-fat diet

1.(Rafie et al., 2018) changed to 9

9

Protocol

The protocol was arranged for that both article and manuscript agree

2. Materials and methods

2.1. Preparation of the plant-based extract Was change to Change to

.Preparation of the extract of the combination of cloves and cumin

1.2.2. Filter the solids off was changed to

Extract was filtered using whatman number 2 filter paper under vacuum. Then, the UV-vis spectrum of the extract of both plants (CC) was measured.

Animals

2.1. Use C57BL6/J male mice of six weeks old with a body weight between 24-29 g at the beginning of the experiments was changed to

Male C57BL6/J mice of six weeks old (48 in total, body weight 24-29 g) were used for the study.

2.2. Acclimatize the mice under a 12 h light/dark cycle (lights on at 08:00 a.m.) in individual cages. Keep the animals at a humidity of 45–55% and a constant temperature of 20–22 °C in individual cages with metallic walls. Was changed to

The animals were housed in groups of eight in standard laboratory conditions of temperature (20-22 ± 1 °C), relative humidity (45-54 ± 2%), lighting (08:00–20:00 h) with food and water ad libitum. The mice were acclimatized in these conditions for a week before the experiment.

2.3. Provide ad libitum water and normal chow (Purina) for a week before the experiment. Equip their front wall with a drinking burette. This paragraph was deleted

2.3. **Experimental Procedure**

1.18 animals in each group?

The number of animals per group is indicated in the paragraph of Animals which effectively is 8

CC is extract of both plants

In Preparation of the extract of the combination of cloves and cumin

Is indicated that the clove and cumin extract is represented by CC

1.1. phentermine is a antiobesity drug used as positive control

Commented [A1]: CC is tested but when is *Syzygium aromaticum tested?*

Commented [A2]: What is this?

2.4. Sample collection

Please provide more details here to match the video protocol.

1.2. Use surgical means to remove the fresh liver and adipose tissue (epididymal WAT and perirenal WAT) and then weigh them.

Commented [A3]: Please provide more details here to match the video protocol.

The explanation of the surgical method was expanded

Others

Legend were included in the text

ACKNOWLEDGEMENTS:

This research was supported by Instituto Politécnico Nacional México

Commented [A4]: Please include an acknowledgement section specifying any funding.

DISCLOSURES:

Authors declare no conflict of interest

Commented [A5]: Please include an disclosure section specifying any conflicts of interest.

Editorial comments:

Changes to be made by the Author(s) regarding the written manuscript:

All changes indicated in the text were made
1. Please upload each figure separately as an image file.

Each of the Figures were loaded separately

2. Please upload each table separately as an xls/xlsx file.

Tables were loaded as an xls/xlsx file.

3. Additional details are needed in the written protocol. Please see comments in the attached manuscript.

Additional details were added in the text

4. Please revise the title for conciseness. Please reflect the revised title in the video as well.

Title was changed

Changes to be made by the Author(s) regarding the video:
1. All audio volume levels should be around the same values. There are some parts, such as the interview introduction and conclusion section, and this narration from 07:05 to 08:37 where the audio is too loud. Audio levels that are set during the narration of the Results section, starting at 08:40 are a good target to aim for.

The sound was unified throughout the video

2. Please increase the homogeneity between the video and the written manuscript. Furthermore, please revise the narration to be more homogenous with the written manuscript. Ideally, the narration is a word for word reading of the written protocol.

The manuscript and the narration in the video is the same in both

3. Please make sure the protocol details in the written manuscript and the video narration are the same: Written protocol says sonication is with 50% ethanol solution, video narration says it is with a 70% solution.

The correct volume of extraction was 60% was corrected

Additionally, please include the grinding of the seeds in the written protocol.

grinding of the seeds was including in the manuscript

The UV measurements of the sample is not in the written manuscript as well.

It was written

4. Please revise the numbering of the video protocol chapters to match the numbers in the written manuscript.

These indication were revised

5. Table 2 in the video is not the same as Table 2 in the written manuscript.

The mistake was corrected

6. 9:01 - Ethanol is spelled wrong.

The mistake was corrected

7. The same figures and tables in the video should be included with the written manuscript. Where are Tables 3 and 4 in the written manuscript?

The mistake was corrected