Research Article

Individual and Synergistic Anti-obesity Effect of Flax Seeds (*Linum usitatissimum*), Fenugreek Seeds (*Trigonella foenum*) and Black Seeds (*Nigella sativa*), *in-vivo* in *Drosophila melanogaster*

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Received: 02-08-2021; **Revised:** 18-09-2021; **Accepted:** 26-09-2021; **Published on:** 15-10-2021.

ABSTRACT

Obesity poses a major threat to global health, challenging the prevention of chronic diseases and health across the world. As synthetic medicines have their side effects, lately, there has been an inclination towards herbal remedies. The present study was conducted to evaluate the individual and syngeristic effect of *Linum usitatissimum* (flax seeds), *Trigonella foenum graecum* (fenugreek seeds) and *Nigella sativa* (black seeds) on obesity. In-vivo studies were performed in Drosophila as many tissues and organs related to obesity and its associated disorders are analogous in Drosophila and humans. Obesity was induced by supplementing lipid and high concentration of glucose in the media. The effect of the seeds on obesity was studied by quantitative estimation of lipid content and carbohydrate content in the larvae grown in different media preparations and also by assessment of physical activity of the flies. The study revealed the efficient ability of the seeds to reduce obesity, synergistically as well as individually proving it to be a potential strategy to combat the obesity naturally.

Keywords: Anti-obesity, Flax seeds, Fenugreek seeds, Black seeds, Drosophila.

QUICK RESPONSE CODE →

DOI:
10.47583/ijpsrr.2021.v70i02.021

DOI link: http://dx.doi.org/10.47583/ijpsrr.2021.v70i02.021

INTRODUCTION

Obesity is a complicated multifactorial disorder, which the World Health Organization (WHO) defines as an abnormal accumulation of fat that poses severe risk to health.[1] Obesity is characterized by a high body-mass index.[2] Body mass index (BMI), is a simple metric that indicates the sum total of fat in the body. It is calculated by dividing a person's weight in kilograms, by the square of his/her height in meters.[1] According to WHO a normal BMI ranges between 18.5kg/m^2 and 24.9kg/m^2, whereas a BMI greater than 25kg/m^2 is considered to be overweight, and a BMI greater than 30kg/m^2 is classified as obese, and BMI greater than 40kg/m^2 being severe obesity.[1]

Obesity is a result of chronic positive energy balance which means intake of dietary energy is greater than energy spent. Energy in excess gets converted into triglycerides.[3] Triglycerides are then deposited in fat cells that enlarge and/or increase in number. This hyperplasia and hypertrophy of fat cells result in the pathological lesions of obesity,[4] causing expansion of the adipose tissue, resulting in increased body fat and weight gain.[3] The enlarged fat cells are the cause of the comorbidities associated with obesity, either due to the increased weight/mass of the extra fat or due to the excessive free fatty acids and peptides secreted by enlarged the enlarged fat cells.[4] Genetic susceptibility, high-calorie diet and decreased physical activity are the major factors affecting obesity,[2] however factors such as physiological, environmental, psychological, social, economic, and political also contribute to the etiology of obesity.[5] Obesity can manifest at any point in life. Studies on the trends in obesity reveal that its prevalence has increased not only in adults of all ages, but also in children, irrespective of ethnicity, geographical topology and socioeconomic status.[1] Its global prevalence is such that, approximately one third of the world population now fall under the category of overweight or obese.[1] It is slowly replacing malnutrition and infectious diseases as the most significant cause of morbidity.[2]

The effects of over weight on health and longevity have long been known. Hippocrates identified the occurrence of sudden death to be more common in individuals who are naturally fat than in lean ones.[4] Obesity has adverse effects on almost all physiological functions of the body. The spectrum of diseased conditions includes diabetes mellitus, cardiovascular diseases, musculoskeletal disorders, several cancers and poor mental health. Obese individuals are at a higher risk for diabetes and other medical conditions than their healthy counterparts.[6] This in turn has a negative impact on work productivity, health care costs and the overall quality of living.[1] Their life expectancy is also short.[6] Obesity should not be considered as a mere cosmetic problem affecting few individuals, but a threat to global health.[2] Measures have to be taken to develop highly effective interventions that serve to nullify the obesogenic agents, ensuring healthy and happy living.[7]

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Linum usitatissimum commonly called flax is an annual herb with blue flowers. It produces tiny, flat, golden yellow to reddish brown colored seeds, which are crispy with a nutty taste.[8] It has been used for human consumption since ancient times.[9] Flax seeds are used for therapeutic purposes as well.[10] They are rich in antioxidants, proteins, soluble and insoluble fibers, phytoestrogenic lignans (secoisolariciresinol diglycoside-SDG), short chain polyunsaturated fatty acids (PUFA) and α-linolenic acid (ALA) an ω-3 fattyacid. Flax seeds serve as an excellent vegetal source of omega 3 fatty acid.[9] Flax seeds help alleviate cardiovascular diseases, osteoporosis, cancer of the mammary and prostate gland, inflammation, and menopausal symptoms.[11] All these properties make Flax seeds to stand out as a potential functional food, which is available in forms like whole seeds, ground meal and mucilage or extracted oil, which can be incorporated into ready to eat cereals, baked cereal products, bread, muffins, nutrition bars, salad toppings, meat extenders, juices, milk and dairy product.[9]

Trigonella foenum graecum commonly called fenugreek is an annual herb with yellow triangular flowers and pods containing hard, yellow brown, aromatic seeds. The leaves and seeds of fenugreek are used in cooking to enhance flavor and modify the color and texture of food as well as in therapeutic herbal preparations.[12] The phytochemical analysis reveal that polyphenols such as rhaponticin and isovitexin are the major bioactive compounds present in fenugreek seeds.[13] Fenugreek seeds also contain diosgenin, saponin (fenugrin B), several coumarin compounds and alkaloids namely trigonelline, carpaine and gentianine. On roasting, trigonelline is degraded to nicotinic acid and related pyridines.[14] It has a number of therapeutic properties. It serves as a blood purifier. It is diaphoretic which promotes sweating, thereby detoxifying the body. It cleanses the lymphatic system promoting its role to irrigate the cells with nutrients and to remove dead cells, trapped proteins and toxic waste from the body. Fenugreek acts as a throat cleanser and mucus solvent easing the urge to cough. It helps to maintain the mucus membrane in the respiratory tract, relieving chest congestion.[15] Apart from this fenugreek serves as a gastric stimulant, galactogogue, lactation aid, anti-bacterial, anti-diabetic, hypocholesterolemic, hepatoprotective and anti-cancer agent.[12] It is also used to treat indigestion, baldness, weakness, leg edema, hay fever, constipation, tuberculosis, pleurisy and emphysema.[15] It is used topically for wound healing, myalgia and cellulitis. It has also been acclaimed as a functional food and nutraceutical.[16]

Nigella sativa whose seeds are known as black seeds is an annual flowering plant. It has finely divided leaves, delicate flowers and a fruit which is a large and inflated capsule containing numerous seeds in 3-7 follicles.[17] The active compounds present in black seeds include thymoquinone, dithymoquinone, thymohydroquinone, p-cymene, sesquiterpene longifolene, α-pinene, thymol, carvacrol, 4-terpineol, t-anethol, etc. It contains two kinds of alkaloids, isoquinoline alkaloids namely nigellicimine and nigellicimine, and N-oxide pyrazol alkaloids or indazole ring bearing alkaloids including nigellidine and nigellicine.[18] Black seeds also contain carvone, limonene, citronellol, alpha-hederin, a water soluble pentacyclic triterpene and saponin, a potential anti-cancer agent in trace amounts. The seeds also contain a good amount of vitamins and minerals including iron, phosphorus, zinc, calcium, copper, niacin, thiamin, pyridoxine, and folic acid. They are a rich source of the precursor vitamin A, carotene.[19] It is shown to possess a wide spectrum of activities such as antioxidant, anti-hypertensive, anti-cancer, anti-diabetic, anti-inflammatory, immunomodulatory, anti-microbial, anthelminthic, spasmolytic, analgesic, diuretic, bronchodilator, gastroprotective, renal protective and hepatoprotective.[17] Most of the therapeutic properties of black seeds are attributed to quinine constituents, of which thymoquinone is the predominant one. Thymoquinone yields dithymoquinone and higher oligocondensation products.[17] They are used chiefly in the treatment of diseases like asthma, bronchitis, indigestion, diarrhea, skin disorders and rheumatism. It also serves as a liver tonic, appetite stimulant, immune booster, emmenagogue and lactation stimulant.[17]

Many practical and ethical obstacles hinder biomedical experimentation in humans, making the use of model organisms an important aspect of research. *Drosophila melanogaster* has served as a model organism for over a century now.[20] A comparison of the fully sequenced genome of Drosophila and the human genome revealed that about 75 % of known human disease genes have a recognizable match in the Drosophila genome of fruit flies making it a legitimate model organism for medical research.[21] Moreover, their small genome size, short life cycle, rapid growth rate, inexpensive breeding costs, minimal ethical and safety issues make it an ideal model organism. Drosophila is particularly useful in obesity related studies for various reasons. In addition to the genes associated with obesity being conserved in flies and humans, there exists many analogous tissues and organs in both that are responsible for obesity and its associated disorders.[22] Furthermore, obesity and its associated complications can be induced in Drosophila by incorporating calories in the diet.[23]

Obesity being a major epidemiological challenge in the world today and considering the fact that people are turning towards natural means to help overcome it. This study has been carried out to determine the synergistic anti-obesity effect of Flax seeds, Fenugreek seeds and Black seeds in-vivo in Drosophila melanogaster.

MATERIALS AND METHODS

Materials

For the present study, wild type flies of Drosophila were obtained from NCBS, Bangalore. *Linum usitatissimum* (Flax seeds), *Trigonella foenum-graecum* (fenugreek seeds) and *Nigella sativa* (black seeds) were purchased from a local super market in Vasanth Nagar, Bangalore. M224-500G

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Drosophila media was purchased from HiMedia Laboratories Pvt. Ltd, Bangalore. All the other chemicals used in the experiments were of analytical grade.

Preparation of growth media

Drosophila growth media was prepared according to the manufacturer's instruction (HiMedia Laboratories Pvt. Ltd). 10% w/w of individual seed powder (Flax seeds, fenugreek seeds, black seeds) and seed mix was incorporated into the media components separately. Media without any seed powder was used as control. 1% v/v olive oil was incorporated, to induce obesity in the Drosophila and to study the lipid accumulation. Similarly 1% w/v glucose was incorporated instead of olive oil to study the carbohydrate accumulation. Drosophila flies were transferred into each of the containers containing the prepared media. They were maintained at room temperature under standard laboratory conditions.

Measurement of lipid content in the larvae

Lipid content was estimated using the SPV method proposed by knight et al., in 1952 with a few modifications. Briefly, 10 drosophila larvae in the 3rd instar larval stage, were homogenized in chloroform to which equal volume of methanol was added. The homogenized mixture was centrifuged at 10000 rpm for 15 minutes. After centrifugation, the chloroform layer was aspirated and used for estimation of lipid content. Standard working solution was prepared by dissolving 10mg of olive oil in 10mL of chloroform. 20 to 100µL aliquots of the working solution were used as standard. In a fresh test tube 0.1mL of 98% concentrated H_2SO_4 was taken as blank. Appropriate aliquots of extracted test samples were taken in different test tubes. The tubes were incubated at 90°C for 10 minutes. To each tube 0.1mL of 98% concentrated H_2SO_4 was added. All the tubes were heated at 90°C for 10 minutes, and then cooled on ice for 5 minutes. After cooling 2.4 mL of phosphovanillin reagent was added, and the absorbance was measured at 530nm using a spectrophotometer.

Measurement of carbohydrate accumulation in the larvae

100mg of drosophila larvae in the 3rd instar stage, were homogenized in 5mL of 2.5 N HCl and then hydrolyzed in a boiling water bath for 3 hours. The hydrolysed sample was cooled and then centrifuged at 10000 rpm for 5 minutes. To the supernatant, sodium carbonate was added until there was no effervescence, neutralized sample was used for total carbohydrate estimation. Carbohydrate accumulation was determined by measuring the carbohydrate content in the larvae using Anthrone method proposed by Van Handel in 1985,[24] with a few modifications.

Climbing assay

The locomotory behavior of Drosophila was assessed using the climbing assay proposed by Crowther in 2006.[25] In which, ten Drosophila from each of the five different containers were taken in separate glass vials. A line was drawn 6cm from the bottom of the vial. The vials were tapped such that all the flies come to the bottom of the vial. The time taken by the flies to climb up the vial and cross the line was noted. Five trials were performed and the average was calculated.

RESULTS AND DISCUSSION

The present study was carried out to evaluate the anti-obesity property of flax seeds, fenugreek seeds and black seeds in Drosophila. The effectiveness of these seeds was screened by assessing the lipid content, carbohydrate accumulation and the locomotory behaviour of the different groups of Drosophila in the experiment.

Measurement of lipid content in the larvae

Excess lipid gets stored as lipid droplets in the fat body of Drosophila causing obesity.[22] Olive oil was supplemented into the standard growth media to induce obesity in Drosophila. The effect of the seed powder on lipid content, was studied by measuring the lipid content in the Drosophila larvae spectrophotometrically, by SPV method.

Results show that the larvae of the flies grown in the control media in the absence of any seed powder showed highest level of lipid content (12.752mg). Whereas those grown in the media containing the different seed powders, showed relatively lower levels of lipid content (Figure 1). Drosophila larvae grown in the media containing the seed mix showed the lowest level of lipid content (5.232mg), followed by those grown in the media containing flax seed (6.464mg), and then fenugreek (6.72mg) and black seed (8.976mg). The results indicate the effectiveness of the seed powders in reducing lipid content, thereby acting as anti-obesity agents.

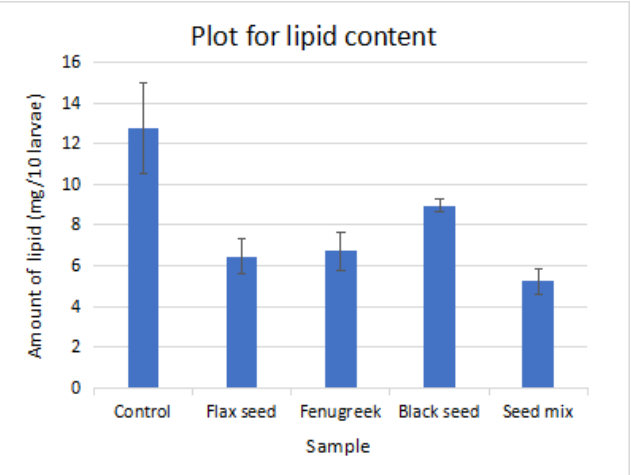

Figure 1: Plot for Lipid content

Measurement of carbohydrate accumulation in the larvae

Carbohydrates get deposited in the muscles in the form of glycogen serving as a metabolic reservoir.[22] High sugar diet consisting of glucose was incorporated into the growth media of the Drosophila, to induce hyperglycemia and obesity in them. The effect of the seed powder in

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carbohydrate accumulation, was studied by estimating the carbohydrate content in the Drosophila larvae spectrophotometrically, by Anthrone method. Results show that the larvae of the flies grown in the control media in the absence of any seed powder showed highest level of carbohydrate accumulation (50.55mg). Whereas those grown in the media containing the different seed powders, showed relatively much lower levels of carbohydrate accumulation (Figure 2), with the lowest level of carbohydrate accumulation in those growing in the media containing flax seed (17.55mg), followed by those in the media containing the seed mix (19.05mg), then black seed (25.85mg) and fenugreek seed (29.1mg). The results indicate the effectiveness of the seed powders, in reducing carbohydrate accumulation, thereby preventing the development of obesity.

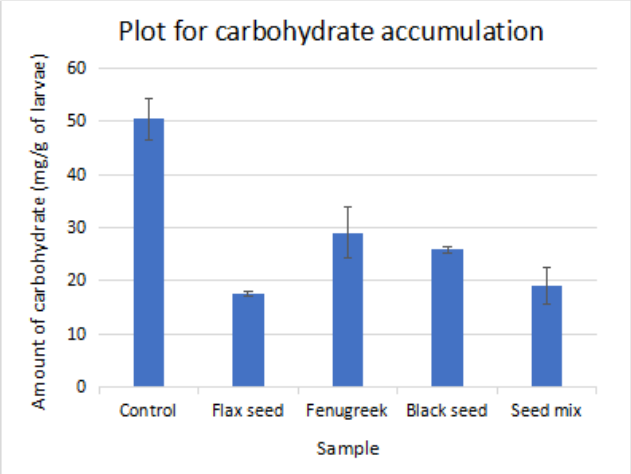

Figure 2: Plot for Carbohydrate content

Climbing assay

Change in locomotory behaviour is an important marker in certain diseases,[25] specially in obesity. Olive oil was supplemented into the growth media of the Drosophila, to induce obesity in them, which in turn would affect locomotion.

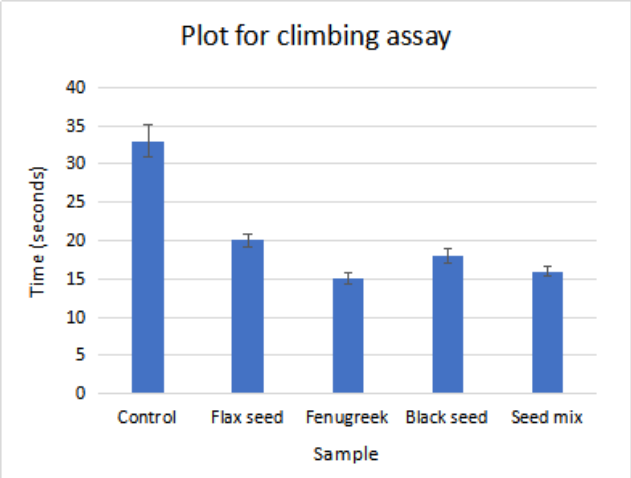

Figure 3: Plot for climbing assay

The climbing assay revealed that, the flies grown in the control media in the absence of any seed powder showed drastic decline in climbing behaviour taking the longest time (33s) to climb up the vial. Whereas the flies grown in the media containing the different seed powders, took relatively much lesser time to climb up (Figure 3). The shortest time was taken by those grown in media containing fenugreek (15s), followed by those in the media containing the seed mix (16s), and then black seed (18s) and flax seed (20s). Thus it can be inferred that the seed powders help alleviate the effects of obesity.

CONCLUSION

The present study concludes that the three seeds, flax seeds, fenugreek seeds and black seeds have anti-obesity property. Each of the seeds individually help reduce lipid content, which is enhanced by synergistic interaction. It also has beneficial effects on carbohydrate accumulation and on obesity influenced motility. Thus it provides scientific evidence for their anti-obesity property, and creates a potential interest for the development of anti-obesity therapeutics that are free from harmful side effects.

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Source of Support: The author(s) received no financial support for the research, authorship, and/or publication of this article.

Conflict of Interest: The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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