IJBPAS, April, 2022, 11(4): 1778-1793

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

EFFECT OF *MENTHA PIPERITA* AND *MUSA ACUMINATA* LEAVES ON BASIC DEMOGRAPHIC PARAMETERS OF ZEBRA FISH

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Received 20th April 2021; Revised 19th May 2021; Accepted 14th July 2021; Available online 1st April 2022

https://doi.org/10.31032/IJBPAS/2022/11.4.6009

ABSTRACT

Globally, obesity has reached wide-ranging proportions and is a major patron to chronic diseases and disabilities. The potential of natural products for treating obesity is under exploration which may be an excellent alternative for developing safe and effective anti-obesity drugs because of the dissatisfaction with unsafe side effects and pricey nature of the available drugs. Zebrafish is used as a model organism because they possess many structural similarities with humans. Zebrafish were first provided with commercially available diets for three weeks during the period of acclimatization and then with a fat induced diet. This Diet Induced Obesity (DIO) was treated with Peppermint (*Mentha piperita*) and Banana (*Musa acuminata*) leaves. Oral dosages as per their body weights were given. Weight and length were recorded to determine general obesity demographs. These demographs showed that *Musa acuminata* leaves were effective in decreasing the weight of Zebrafish as compared to

Mentha piperita leaves. Other obesity demographs were also studied for the efficacy of the plant extracts.

Keywords: *Mentha piperita*, *Musa acuminata*, demographs, Zebra fish, Obesity

1. INTRODUCTION

Obesity is defined as a phenotypic manifestation of abnormal or excessive fat accumulation that alters health and increases mortality **[1]**. The main reason for obesity is the positive balance of energy followed by excessive consumption of energy but there is an insufficient expenditure of energy. It is a complex neuroendocrine disorder. The main concerning factors are the environmental factors and their genotypes. Other than genotypes, the contributing components are the environmental and behavioral factors increasing the physical inactivity and calorie intake also **[2]**. The health issues related to obesity are serious commonly including Type 2 diabetes, pulmonary hypertension, cardiovascular disease and it is strongly linked to increased mortality risk **[3, 4]**. In 1997, World Health Organization (WHO) declared that obesity has reached wide-ranging proportions worldwide **[5]**. This statement is on the basis of data collected and the analysis of the collected data with respect to Body Mass Index (BMI). The Body Mass Index (BMI) or Quetlet Index is the value derived from the mass (weight) and height of an individual. The BMI is defined as the body mass divided by the square of the body height. BMI is mainly used as an attempt to quantify the amount of mass in the tissue.

Zebrafish (*Danio rerio*) are a very important model to study the development, genetics and diseases with respect to humans as they undergo rapid development and display genetic similarities to humans **[6]**. Even though rodent models have a great contribution in understanding obesity in humans **[7]** they require a notable amount of staff and infrastructural support and are quiet expensive. For example, the organs of the digestive system in zebrafish, the adipose tissues (AT) and skeletal muscles are arranged, physically, in a similar manner to humans **[8]**. Zebrafish is a tropical fresh water fish belonging to the Family Cyprinidae commonly known as the minnow family of the Order Cypriniformes.

A food field research has shown considerable interest in the usage of natural product to counteract on obesity recently **[9-11]**. These products contain dietary phytochemical which have a high potential to prevent diseases and also help in the promotion of health without having any side effects **[12-14, 26]**. The potential of nature products for treating obesity is under exploration which maybe an excellent alternative for developing safe and effective

anti-obesity drugs because of the dissatisfaction with unsafe side effects and pricy nature of the available drugs **[15, 16]**. Many natural products, including crude extracts or isolated compounds from plants and can induce the reduction in body weight and help in preventing diet induced obesity **[17, 18, 25]**.

Mentha piperita (peppermint) leaves are one of the species which contain biologically active compounds which are of great to scientists working on human diseases **[19]**. Biologists consider it as an astringent, antiseptic, antimicrobial, stimulant having anti-aging properties also **[20]**. The smell and flavor are very distinctive and is a characteristic feature of *Mentha piperita* which is due to the naturally occurring cyclic terpene alcohol known as methanol. This plant has been used to treat a wide range of diseases and is also consumed by a large population for different purposes, but not for treating diet induced obesity (DIO).

Musa acuminata, commonly known as banana is one of the most common agriculture crop across the world. Fruits of this plant is known for various health benefits and has high nutritional value. Stem and flower of *Musa acuminata* has also been reported for being anti diabetic, antiseptic, anti-ulcer etc. But the leaves of the plant have not been studied much for its health benefit specially DIO, though used traditionally for various purpose as wrapping and serving food, cooking etc.

Present study is an attempt to study the effect of water extract of the leaves of *Mentha piperita* and *Musa acuminata* on DIO in zebrafish. It will help us to provide a preliminary data to understand can these parts of plants having medicinal importance can be used as a common treatment for DIO and so as the cascading effect of the disease.

2. MATERIALS AND METHODOLOGY

Experimental system

The experimental system consisted of 5 rectangular tanks (30*15*15) (L*B*H) covered with tank lids with a capacity of 35 L, with 20 L of water in each. The water in each tank was dechlorinated by addition of chlorinating solution. 2drops (0.5ml) of anti-itch was also added each time the tank was cleaned. Each system was connected to an aerator. Temperature was monitored daily using a thermometer. Each tank contained a five inches long cylindrical plastic to provide a playful environment to the fish**.**

Fish

Adult Zebra fish from local pet store were taken and separated in five tanks according to their weight range with 5 fish in each tank. They were allowed to acclimatize in the new environment and fed with commercially available diet for fish (physical character of the fish did not affect

the fish). The average weight of the fish being 0.2g and average height of 4.5 cm.

Diet

Dietary treatments consisted of three different diets.

Diet 1: (Commercial Fish Food)

It consists of commercially available diet for a span of 21 days. All the five tanks were provided with the same commercial diet. The commercially available diet: TetraBits Complete pellets (protein 47.5%, oil 6.5%, fibre 2.0%, ash 10.5%, moisture 6.0%) was used. The quantity of food provided to each tank depended upon the amount of food the fish consumed within 5 minutes. The diet was provided twice a day at two specific time intervals with a time difference of 12 hours (6 a.m. and 6 p.m.). This span of 3 weeks was considered as the period of acclimatization. The length and weight were measured at specific time intervals of 7 days.

Diet 2: (Obesity inducing diet)

After 3 weeks, the fish were fed with live blood worms with their normal diet (Commercially Available). Live blood worms, with high fat content (2%) and the commercial diet were fed once a day each. They were fed at the same time as in diet 1 with the similar quantity. The zebra fish were kept on this diet for a period of 3 weeks. The length and the weight of each fish was measured regularly at a specific interval of 7 days.

Diet 3: (Commercial diet with treatment)

After the next 3 weeks, two tanks were given the normal commercially available diet with the same procedure as followed in the first diet. Along with the diet, two tanks were provided with the laboratory prepared liquid extraction of *Mentha piperita* and two other tanks were given liquid extraction of *Musa acuminata* leaves along with the diet. Dosage was given according to their weights and weight was recorded at specific time intervals of 7 days. This was carried out for 21 days.

Preparation of the laboratory liquid extracts:

Herbs were first taken, washed with normal tap water and then washed again with distilled water. These washed herbs were chopped. After cleaning, the herbs were first weighed. The herbs were then weighed using a weighing machine (Roy industry, Varanasi, India) and kept in an open tray for air drying for 48 hours after which their weight was noted. Now the air dried herbs were kept in the oven (Tempo Industrial Corporation, India) at 60ºC for 24 hours till the herbs were completely dried. After oven drying their weight was measured. The herbs were stored in the oven till the weight of herbs for two consecutive days was the same. Now they were crushed in the mixer grinder (Godrej) to get a powdery form and were then sieved by collecting the finer particles and stored in the air tight

container. This same procedure was followed for both *Mentha piperita* and *Musa acuminata* leaves. The prepared dry powders were now taken according to their required concentrations and were kept in water bath (Meta-lac Scientific Industries, Mumbai, India) for 15 minutes at 70ºC. They were then filtered with the help of vacuum filtration machine using Whattman filter paper No.1. The filtrate of both the extracts were collected in separated dark bottles and were then used for the following two purposes:

1. Phytochemical analysis.
2. Concoction for the Zebra fish. (In Diet 3)

3. METHODOLOGY

Acclimatization and maintenance of fish

Fish were brought and kept in fish tanks for 21 days. During these days fish were provided with normal fish food and were left for acclimatization. Normal activity was observed and weight was noted at the end of seventh day, fourteenth day and finally after three weeks. The mortality rate of the fish was noted.4 L (20%) of water from each fish tank was changed every three days and the whole tank was cleaned every 7 days.

Gavaging

1) Immobilizing the fish

Fish of each tank were taken on beaker with 200ml of dechlorinated water from its own tank. This beaker was then transferred into the lower portion of the freezer at 20 ºC for about 20 minutes. After 20 minutes, they were transferred to the upper freezer at 4 ºC. In the next 20 minutes, the fish got immobilized and could be used for gavaging. The sponge was kept in the freezer for 10 minutes. The sponge was placed on a flat surface and with the help of micropipette 5µL was drawn up and was gavaged by placing the tip of the micropipette on the tip of the mouth of the fish and was slowly released into the mouth. Now these fish was kept in the beaker in the freezer for 20 minutes and were then transferred to normal refrigerator at 4ºC for 20 minutes and was then transferred to the fish tank. This treatment was given for 3 weeks and weight was observed.

2) Preparation of the Gavage apparatus:

a. Variable Micro pipette having calibration range from 0.5 to 1000µL was washed with distilled water.

b**.** The moistened sponge was removed from the freezer and kept on a flat surface. A groove was cut into the sponge with a scalpel blade. Draw the dosage for the gavage into the micro pipette.

3) Gavage:

Checking for the righting reflex of the fish where it does not respond to the tail fin pinch but maintains opercular movements is done. Then the fish was removed from the beaker from its tail with the help of a blunt forcep and placed into the groove

made in the sponge with the head slightly protruding out from the sponge. Careful attention was given to the gills that are covered by the sponge. Move the space in a vertical position. The mouth of the Zebra fish was opened using the tip of the micropipette. Following, the tube having 5 µL of 2% water extract into the mouth of the fish was gently inserted but not forced. Any kind of resistance indicated that the tube is hitting the gill arch or the heart and if any resistance, gently the withdrawal of the apparatus was done and the process was repeated again. While injecting slowly the precautions were taken to ensure that the solution does not exit via the gills or the mouth. Then the fish was removed from the sponge and placed in the beaker. The fish was noted for its recovery when it started spinning upright and maintaining equilibrium. The fish was monitored for regurgitation shown by epilation of material from its mouth or no opercular movement. The fish was then kept back in the freezer at 4 ºC and then in the lower freezer at 20 ºC and then at normal room temperature to prevent it from getting any kind of shocks.

Phytochemical analysis of water extract of *Mentha piperita* &*Musa acuminate*

Phytochemical analysis of the two water extracts were tested for the presence of active principles such as Triterpenoids, Steroids, Glycosides, Saponins, Alkaloids, Flavonoids, Tannins, Proteins, Free Amino Acids, Carbohydrate and Vitamin C **[21, 22]**. Following standard procedures were used.

4. STATISTICAL ANALYSIS

All the data for the experiments were on the random basis. The BMI, Weight and Condition Index (CI) values were statistically compared using analysis of variance (ANOVA) to determine significant differences existed among the diets. Differences between BMI, Weight, (CI) between two different herbs within diet and control were statistically compared using the independent samples T-test ($p< 0.05$) and then compared by two different samples across diet and control using ANOVA. All data sets were further checked with Bonferroni correction. The Microsoft Excel 13 was used for the analysis.

5. RESULT AND DISCUSSION

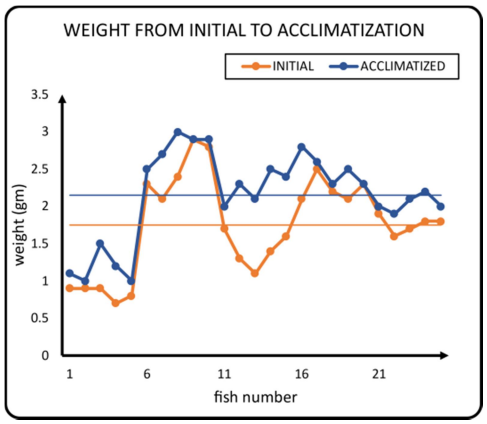
All the data were analysed post acclimatization of fish for a period of three weeks. The parameters under investigation were considered post acclimatization to study the changes. The fish after acclimatization of the mentioned period showed an increase in the weight, BMI and CI index. The average increase in the weight after acclimatization was 0.4 g, average increase in BMI was 0.24 and average increase in CI was 0.48. This increase in the various demographic parameters of the fish, suggests that the increase is due to growth of the fish and

settling into the new environment **(Figure 1a, 1b and 1c)**. During this time the regular fish feed was given. The mortality rate was noted to be in between 0.5 to 0.7%.

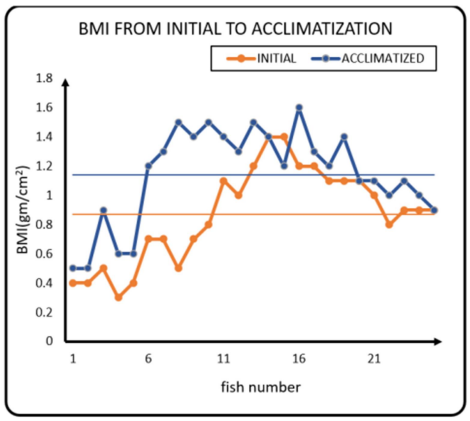
Figure 2a, 2b and 2c shows a significant increase (two-tailed paired t-test) in the weight, BMI and CI of the diet induced obese fish. The average increase in the weight after providing an obese diet for 21 days was 0.688g, increase in BMI was 0.30 and that of CI was 0.73.

The high fat diet has supported the induction of fat and increase in weight of the fish making it obese **[26, 28]**. The increase in weight also affected the health status of the fish so the BMI and CI is also affected. The effect on the mobility status of the fish was also monitored. The decrease in the activity of fish was noted, they were found to be relatively less active as compared to control.

Once the fish were obese with the treatment of fat diet, they were subjected to a preparation of leaf extracts of *Mentha piperita* and *Musa acuminate* for a period of three weeks to check for the efficacy of the selected plant extract on the obesity parameters under investigation. The treatment was given via gavaging for a period of three weeks. To find the significance of the effect of the water extract of leaf of *Mentha piperita* and *Musa acuminata* one-tailed unpaired t-test was used. The test used for the effect of *Mentha piperita* showed a significant decrease in the weight, BMI and CI of the obese fish. **Figure 3 A, 3B and 3C** shows that the mean decrease in weight was 2.10g, decrease in BMI was 1.02 and CI was 2.15. **Figure 4A, 4B and 4C** suggest the effect of the water extract of *Musa acuminata* on the parameters under investigation. The test showed significant decrease in the three parameters. The average decrease in the weight was 1.53g, in BMI was 0.76 and that of CI was 1.63.



(A)



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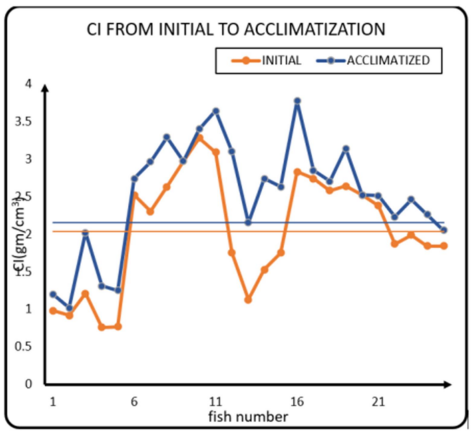
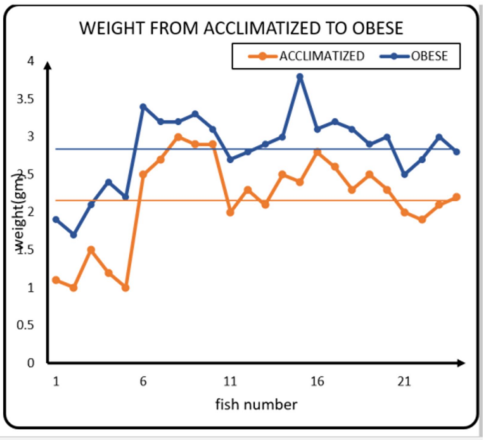
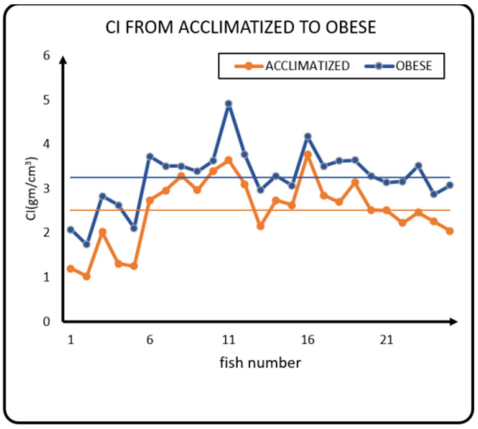


(C)

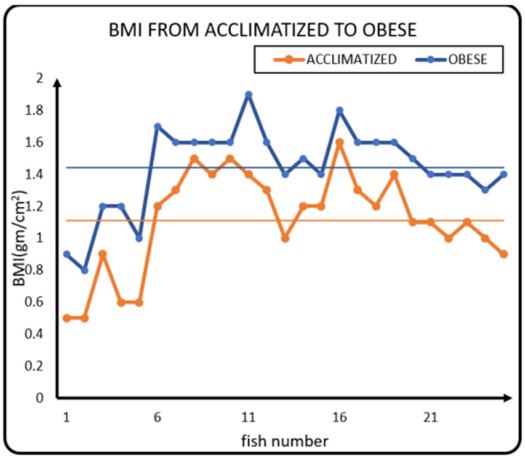
Figure 1A, 1B and 1C: Weight, BMI and CI of the fish after acclimatization



(A)

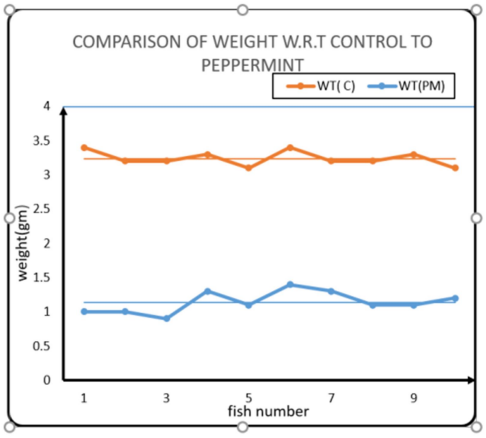


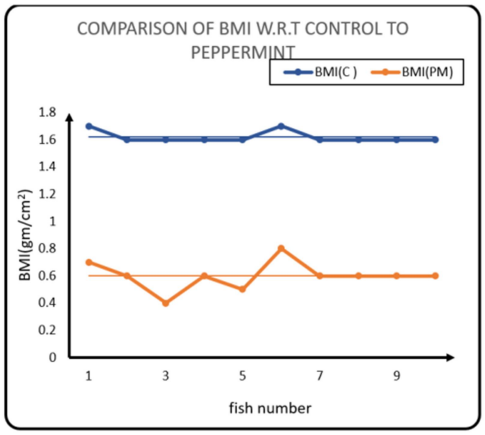
(B)



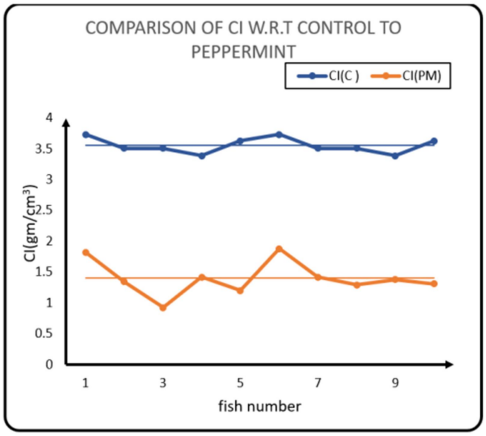
(C)

Figure 2A, 2B and 2C: Weight, BMI and CI of the fish post acclimatization



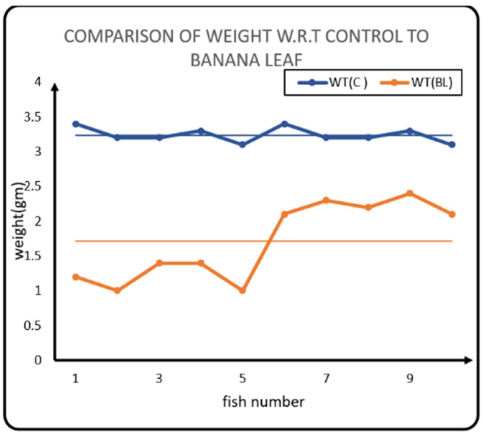


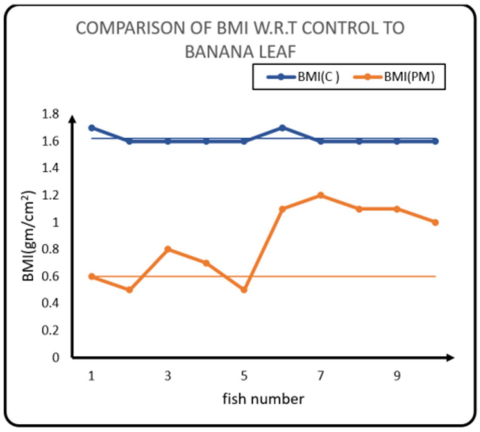
(A) **(B)**



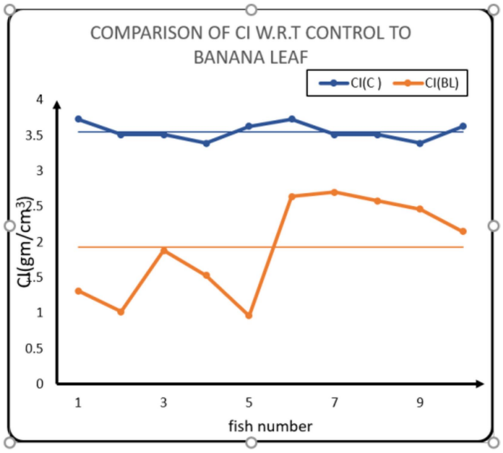
(C)

Figure 3A, 3B and 3C: Weight, BMI and CI of the fish Control Vs Treatment with *Mentha piperita* (PM)





(A) **(B)**



(C)

Figure 4A, 4B and 4C: Weight, BMI and CI of the fish Control Vs Treatment with *Musa acuminata* (BL)

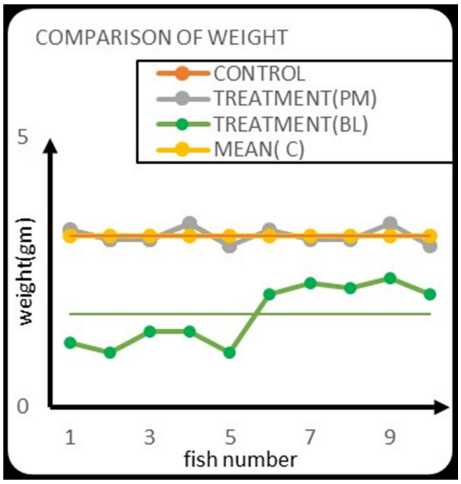


Figure 5 A: Comparison of Weight (Control Vs Treatment with Banana leaf (BL) extract and Peppermint (PM)

Table 1: ANOVA – Comparison of Weight following the treatment of extract of Peppermint and Banana leaf

Anova: Single Factor- Weight

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Column 1	**5**	**15.9**	**3.18**	**0.027**
Column 2	**10**	**31.8**	**3.18**	**0.024**
Column 3	**10**	**17.1**	**1.71**	**0.314333333**

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	**12.9654**	**2**	**6.4827**	**45.23292103**	**1.6E-08**	**3.443356779**
Within Groups	**3.153**	**22**	**0.143318182**			
Total	**16.1184**	**24**				

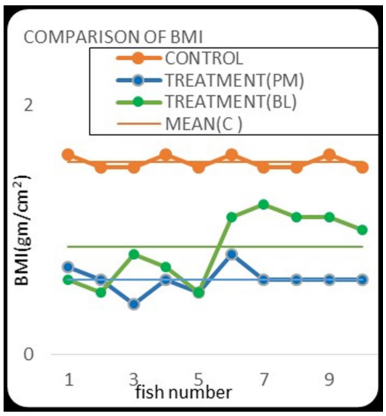


Figure 5B: Comparison of BMI (Control Vs Treatment with Banana leaf (BL) extract and Peppermint (PM)

Table 2: ANOVA – Comparison of BMI following the treatment of extract of Peppermint and Banana leaf

Anova: Single Factor= BMI
SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Column 1	5	7.7	1.54	0.003
Column 2	10	6	0.6	0.011111111
Column 3	10	8.6	0.86	0.073777778

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	2.9624	2	1.4812	41.99278351	3.08E-08	3.443356779
Within Groups	0.776	22	0.035272727			
Total	3.7384	24				

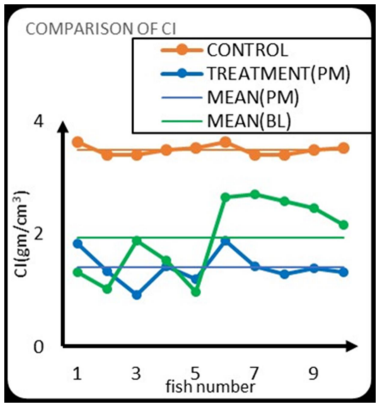


Figure 5 C: Comparison of CI (Control Vs Treatment with Banana leaf (BL) extract and Peppermint (PM)

Table 3: ANOVA – Comparison of CI following the treatment of extract of Peppermint and Banana leaf

Anova: Single Factor CI
SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Column 1	5	17.43	3.486	0.00848
Column 2	10	13.98	1.398	0.07801778
Column 3	10	19.23	1.923	0.46269

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	14.7079	2	7.353963	33.0158391	2.37477E-07	3.443356779
Within Groups	4.90029	22	0.22274045			
Total	19.6082	24				

Analysis of Variance (ANOVA) was used to statistically compare the three parameters of *Mentha piperita* and *Musa acuminata* leaves.

The current investigation evaluated the relative effectiveness of the liquid extract of *Mentha piperita* and *Musa acuminata* leaves on diet induced obesity (DIO) in Zebra fish **[27]**. The effect of plant extracts on the weight of the obese fish suggest that Banana leaf extract is more effective in comparison to Peppermint. The concentration and dosage published for both the liquid extracts is the same but the quantity of the decrease in the three parameters viz., weight, BMI and CI is different. The effect of *Mentha piperita* is found to be effective on all the obesity demographs studied.

In this study the demographic parameters studied were Weight, BMI and CI of the zebrafish. It was noted that the effect of peppermint extract was not able to reduce in weight much as compared to banana leaves extract. This is contrary to an investigation on green tea extract studied by Takahiro Hasumura *et al*., (2010). The reduction in all the three parameters has been observed in Mentha leaves when compared with banana leaves. This suggest that green tea which has high content of peppermint many times, can be considered to have a positive effect on DIO which is found to be rich in flavanoids **[13]**. The reduction in weight following the treatment with banana leaf extract was found to be significant. This reduction in weight could be due to high flavonoids, polyphenols and other secondary metabolites **[24]**. Flavonoids are suggested to have an effect in reduction of visceral fat and increase in lipid metabolism **[17]**. The mechanism of action of the banana leaf extract needs to be understand further, the potential treatment could be via increased hepatic expression of the lipid catabolism genesor decreased the visceral fat expression of SOCS3 which is a suppressor of cytokine signalling **[23, 27]**. The diet induced obesity can be reduced by treating with banana leaf water extract and peppermint decoction. The anti-obesity effects of banana leaf extract suggest a novel application to achieve complete use of the leaf which was normally used in the Indian tradition for steaming the food under cultural practice. This study also provides a potential for use of various traditional cooking methods to be explored for their benefit in maintenance of health of the people. This study provides and insight that there could be potential herbal extract which has low economical value and is available easily for treating diet induced obesity. Both *Mentha piperita* and *Musa acuminata* leaves need to be investigated for their mechanism of action with a dose dependent study.

6. CONCLUSION

The need to find an alternative for drugs which help in controlling obesity, which are made of natural materials, has increased over the years. The two alternatives used in the experiment are from natural sources as well. The demographics of zebra fish after treatment with the alternatives has showed a significant difference. The effect of *Mentha piperita* is more than the effect of *Musa acuminata* leaves for all the three parameters, thus forming the basis for plant-based food which can play a role in weight loss and combating obesity. The mechanism of action for the effect of the extract used need to be elucidated. The importance of our tradition cultural practices of cooking has lot of health benefits which required to be explored in detail and must be encouraged to follow with modern lifestyle.

7. FUTURE PROSPECTS

The other important parameters such as lipid profiling, cholesterol level analysis and blood glucose levels and three-dimensional microcomputed tomography analysis can further validate the study. The concentrations of the active components and their mechanism of action are to be studied along with their role in reducing the obesity parameters which requires analysis. Since type 2 Diabetes is related to obesity, the effect of these leaves can also be studied upon. The pathways of action at cellular level need to be established.

8. ACKNOWLEDGEMENT

We are grateful to University Grant for Minor Research Project for funding this research study.

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