Administration of *Momordica charantia* Alcoholic extract for the treatment of hypothyroidism induced obesity in mice

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Abstract

Hypothyroidism is marked by increased levels of thyroid stimulating hormone and decreased levels of T3 and T4. Hypothyroidism reduces the basal metabolic rate leading to slowed down metabolism which is a result of imbalance in calorie consumption i.e. more calories being consumed than expended, this imbalance in turn leads to weight gain resulting in hypothyroid obesity more prevalent in woman above 30 years of age. Alteration in mice body weights were recorded after administering them Methimazole (0.025% hypothyroid inducing drug) orally in their drinking water for 21 days then for the next 21 days, group 3 was administered standard drug (Thyroxine), and group 4 was administered *Momordica Charantia* alcoholic extract and rest both the groups were administered normal food and water. Every group contained 6 mice and mice were divided into 4 groups. Mice were weighed at the starting of the experiment and reweighed every 7th day and water and food intake was observed throughout the study period. At the end of the experiment the weight changes on final day were analyzed by comparing with the initial day. Weight alterations were recorded to be significant and $p^* < 0.01$ in the experimental mice. The mice were sacrificed at the end of the experiment and their liver was dissected out and histopathological studies were done on it to study the hypothyroid obesity linked changes in it. The experiment lead to the conclusion that the body weight of the experimental mice had increased due to induction of hypothyroid obesity and the *Momordica Charantia* alcoholic extract was effective in treating it and decreasing the mice body weight. The histological studies revealed the appearance of fatty vacuoles throughout the liver in the disease group and the *Momordica Charantia* alcoholic extract was able to reverse these changes successfully to a great extent.

Keywords: Body weight, Hypothyroid obese, Swiss albino mice, Methimazole, Alteration

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Introduction

Thyroid is a butterfly shaped endocrine gland located at the base of the neck and it releases thyroid hormones (THs) T3 (Tri-iodothyronine) and T4 (Tetra-iodothyronine) which are primarily responsible for maintaining the basal metabolic rate (BMR) of the body (Dahiya et al., 2022). Thyroid hormones affect the cardiovascular system, metabolism, nervous system, energy consumption, lipid profile and body weight and control total and basal energy expenditure (Trexler et al., 2014), (Roef et al., 2012), (Fox et al., 2008), (Biondi and Wartofsky, 2012). Thyroid dysfunction is linked with transient tachypnea, apnoea, respiratory distress syndrome, sepsis (Roef et al., 2012), (Männistö et al., 2013) Weight loss and gain have been associated with hyperthyroidism and hypothyroidism respectively (Trexler et al., 2014), (Roef et al., 2012), (Milionis and Milionis, 2013) . Hypothyroidism is marked by increased levels of thyroid stimulating hormone (TSH) and decreased levels of T3 and T4 resulting in decreased weight and metabolism. The normal TSH level should be 5-6µl (Dahiya et al., 2022). Hypothyroidism reduces the basal metabolic

rate leading to slowed down metabolism which is a result of imbalance in calorie consumption i.e. more calories being consumed than expended, this imbalance in turn leads to weight gain resulting in hypothyroid obesity. Synthetic drugs used for treatment of hypothyroidism have resulted in side effects like headache, fever, sensitivity to heat, diarrhea, vomiting, hair loss, joint pain etc.; therefore there is necessity for finding suitable herbal drugs which have proved to be comparatively safer for its treatment (Dahiya et al., 2022), (Dahiya et al., 2020).

Hypothyroidism nearly affects 5% of the population (many suffer from it without even knowing it). Hashimoto's disease which is an autoimmune disease is mostly responsible for hypothyroidism. Other causes comprise of lack of iodine in the diet, some medications, cancer history, radiation treatment and genetics. Women are 10 fold more susceptible to hypothyroidism when compared to men and their risk is elevated with growing age, following pregnancy and menopause. 1st Degree family history makes an individual more susceptible to it (Robinson M, 2022). Elderly and the women are found to be most affected. X 4)5 Hypothyroid patients can show the signs of mood alteration, dyslipidemia, cognitive deficit, osteoporosis, cardiac dysfunction, fractures and prominent weight gain (Biondi and Wartofsky, 2012) (Warner et al., 2013). Unreasonable weight gain accompanied by lowered food intake and appetite loss was noticed among men (Cakic-Milosevic et al., 2004). However hypothyroidism does not disturb food absorption and energy consumption. Most of the people suffering from hypothyroidism encounter some amount of weight gain. Mostly people add on 5-10 lbs but in severe hypothyroidism some women may add on more weight. Whilst mostly weight gain is due to increase in retention of salt and water, some amounts of fat gain may as well be responsible for it (Robinson M, 2022).

Hypothyroidism is a common condition that affects up to 5% of people (although more probably suffer from it without even realizing it). In hypothyroidism, your thyroid gland isn't producing enough thyroid hormones, which are needed to maintain many of your body's systems, including your weight and metabolism. The most common cause of hypothyroidism is an autoimmune disease called Hashimoto's thyroiditis, where your immune cells attack the thyroid gland, keeping it from working properly. Some other causes include a history of cancer, radiation treatment, certain medications, and not enough iodine in the diet. Women are up to 10 times more likely to have hypothyroidism than men, and their risk increases with age, during and after pregnancy, and during menopause. Additionally, people with a first-degree family member with hypothyroidism may have an increased risk of developing it. (Robinson M, 2022)

Obesity results from imbalance between energy intake and energy expenditure, and sometimes due to hormonal and metabolic disturbances like hypothyroidism; mostly it manifests into several other metabolic complications like hypertension, and cardiovascular disturbances, diabetes in addition to other ailments like respiratory difficulties, stroke and sleep apnea. Recent studies have revealed the potential of natural substances like herbal extracts, herbal teas and tinctures to combat obesity (Dahiya et al., 2020). It is a disease of concern around the world especially in the developed countries like USA, around 42% of adults there were counted as obese in 2018 and the number is steeply rising (Hales CM, 2020). Several researches have been conducted to study promotion of energy expenditure to counteract obesity via stimulation of adaptive thermogenesis (Chouchani et al., 2019). Body weight and energy utilization can be controlled by Brown Adipose Tissue (BAT) in human and mice. BAT triggers heat production by non-shivering mechanism and via releasing surplus energy in form of heat. BAT plays an important role in managing obesity via the regulating the energy homeostasis (Dahiya et al., 2020).

Hypothyroidism is mostly linked to weight gain in humans due to decreased metabolism, in turn decreasing thermogenesis (Chaker et al., 2017). This study is an attempt to study the body weight changes in Mtz induced hypothyroidism in body weight of the mice and to administer the test plant extract to the hypothyroid obese mice and to study the reversal of the disease. This study also focusses on the histopathological

changes in the liver of hypothyroid obese mice, and their comparison with the group treated with the test plant extract to study whether the test drug was able to restore the hepatic damage caused in the hypothyroid obese group. Levothyroxine (Thyroxine) has effectively been used treatment for hypothyroid obese individuals. Thyroid treatment often results in weight loss in hypothyroid obese individuals' (Ríos-Prego et al., 2019). In today's world, it becomes necessary to diagnose, prevent and cure obesity prone people. This may be achieved by studying the altered thyroid function that maybe leading to weight gain (Krotkiewski, 2002).

Material and Methods

Collection, Authentication and Preparation of Plant parts

Fruits from *Momordica Charantia* Linn. were collected during the months of September-November in 2015-2016 from countryside of Hisar, Haryana 125001. The fruits were authenticated at NISCAIR, New Delhi, India via (NISCAIR/RHMD/Consult/2015/1620/218) specimen numbering. Voucher specimen was deposited at the Department of Pharmaceutical Sciences herbarium of Guru Jambheshwar University of Hisar, India. The fruits were washed by spraying fresh water on them and they were split into two halves and shade dried (in order to avoid destruction of thermolabile substances) for 15 days, later the fruits grinded and converted into coarse powder and stored suitably for use in the future (Wallis, 1985).

Preparation of Plant Extract

The *Momordica Charantia* alcoholic extract was prepared using 95% ethanol for extraction of 500g of coarse powder of fruits from the plant. Soxhlet extractor was used for extraction and the extract collected (yield 6.675g) was evaporated and concentrated using Rotavapor, followed by lyophillization. The extract was refrigerated at -20°C for future use (Agrawal et al., 2012) (Goel et al., 2016).

Hypothyroid Obesity Induction

This research was carried out in the animal house of GJUS&T, Hisar and IAEC approval was obtained via Endst. Number IAEC/2021/10-19. Female Swiss albino mice were procured from the animal house of GJUS&T, Hisar. It was ascertained that all the animals chosen were healthy and they were weighed before beginning of the experiment. The mice were kept under controlled conditions of humidity (50±10%), temperature (22±0.5%) and 12 hours light and dark cycle.

Animals were acclimatized for one week period and were fed with chow diet and drinking water ad libitum. Body weight was noted down in the beginning and then every 7th day and amount of water and food intake was also recorded. Health status of the animals was measured during the study. Swiss albino mice were separated in 4 groups of 6 mice in each group; Group 1 was taken as control and group 2 was taken as the disease control group and group 3 was the treatment group. Hypothyroidism was induced in the animals by administering them with 0.025% w/v Methimazole (Mtz) in drinking water for 21 days. Daily, fresh Mtz solution was prepared.23 Control group was administered placebo in form of distilled water. On 21st day, the experimental mouse were weighed and then for the next 21 days group 3 was administered standard drug (Thyroxine 0.25µg/100gm) by i.p. (intraperitoneal) injections and group 4 was administered MC Al Extract, and rest both the groups were administered normal food and water. $p^*<0.05$ was assumed as statistically significant.

Histopathological studies

10% Buffered formalin solution was prepared and liver from every group was stored in it. A section of liver was cut and was fixed in paraffin mould. 5µm thick sections of the liver tissue were cut using a microtome and stained with haematoxylin and eosin. Same procedure was repeated for every group. These sections were fixed on slides and observed under light microscope for observing histological alterations in the liver tissue taken from various groups.

Results

Hypothyroid Obesity linked weight gain

Mtz administration greatly affected the body weight of the mice. Significant changes were observed in the final and initial body weight of mice of the experimental and control group of animals, p. <0.01. The average body weight of group 1 animals was 90.10±1.08g at the starting of the experiment and 101.70±1.23 at the finishing of the experiment; the average weight gain was 11.60±0.015g, and the average body weight of group 2 was 90.20±1.06g at the starting of the experiment and 120.40±1.11 at the finishing of the experiment; the average weight gain was 30.20±0.05g. Whereas, the average body weight of group 3 was 90.24±1.13g at the starting of the experiment and 105.34±1.25 at the finishing of the experiment; the average weight gain was 15.10±0.12g and the average body weight of group 4 was 90.13±1.10g at the starting of the experiment and 108.630±1.20 at the finishing of the experiment; the average weight gain was 18.50±0.10g (**Table 1.**). The amount of water and food intake was also found to almost same in various groups.

Table 1. Body weight alteration by Methimazole induced hypothyroid obesity in Swiss albino mice

Group	Initial body weight (g) Mean±SD (n=6)	Final body weight (g) Mean±SD (n=6)	Weight change (g) (Initial body wt–Final body wt) Mean±SD (n=6)	p value
Group 1- Control	90.10±1.08	101.70±1.23	11.60±0.015	0.001
Group 2- Disease	90.20±1.06	120.40±1.11	30.20±0.05	0.01
Group 3- Standard (T3)	90.24±1.13	105.34±1.25	15.10±0.12	0.001
Group 3- MC Al Extract	90.13±1.10	108.63±0.20	18.50±0.10	0.01
*p value < 0.05 is statistically significant				

Histopathological studies

The control **(Fig 1.)** and standard **(Fig 1.)** groups exhibited a normal histo-architecture in appearance with hepatocytes distributed radially. Sinusoids converging towards central vein (without any connective tissue around it) are clearly visible. Hepatocytes have normal architecture with cytoplasm and nucleus (round, violet colored). Kaupfer cells (violet, spindle shaped macrophages) are also seen at some places. Nucleus to cytoplasm ratio is normal. There are no signs of fat and infiltration in the control group and some signs of small sized fatty vacuoles in the standard. Whereas in the disease group **(Fig 2.)**, the normal histo-architecture of the liver appears to be clearly disturbed when compared with the normal control group and fatty morphosis appears predominantly, but there are no signs of cirrhosis. The liver has a lot of vacuoles denoting presence of fats and almost half of the portion of liver is vacuolated and hollow instead of predominant pink color, the liver appears to be whitish mainly. The hepatocytes are filled with fat and appear to be macrovascular type (fat cells are larger than hepatocytes). There are signs of infiltration towards the top-center. Lastly in the MC al Extract group **(Fig 2.)**, fatty vacuoles have decreased to a great extent and normal histo-architecture of the liver has almost been restored. Liver appears more pinkish and filled with hepatic cytoplasm than whitish and hollow. More fat is present when compared to standard group and less than the disease group. Little amount of infiltration can still be traced in the cells.

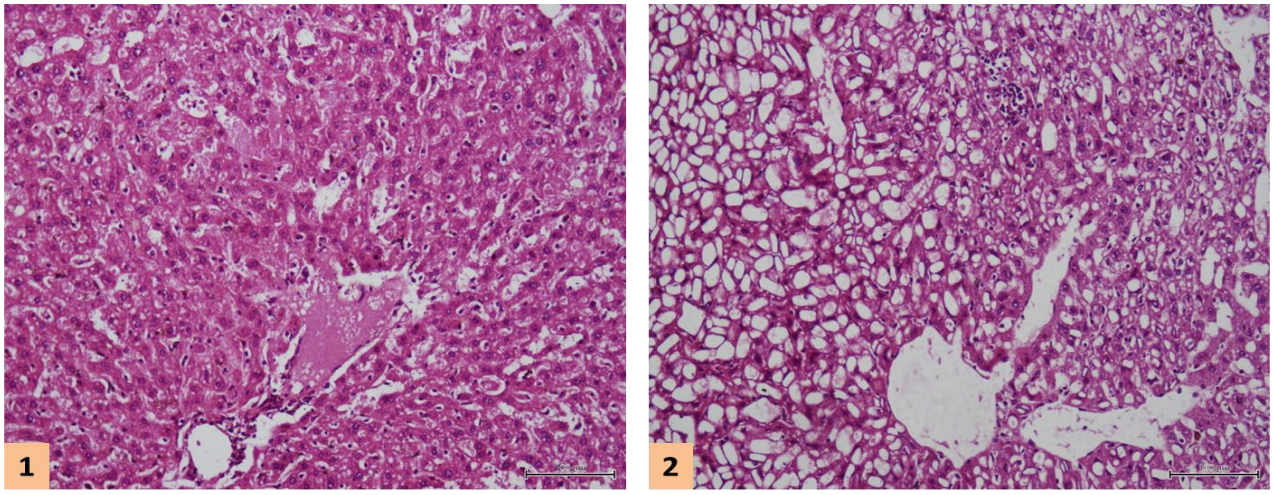

Fig 1. 1 is Control group and shows normal histo-architecture with sinusoids converging towards central vein and healthy hepatocytes with violet colored nucleus, whereas 2, the Disease group has fat cells (macro-vesicular) present all over the area and shows infiltration in the top-center region

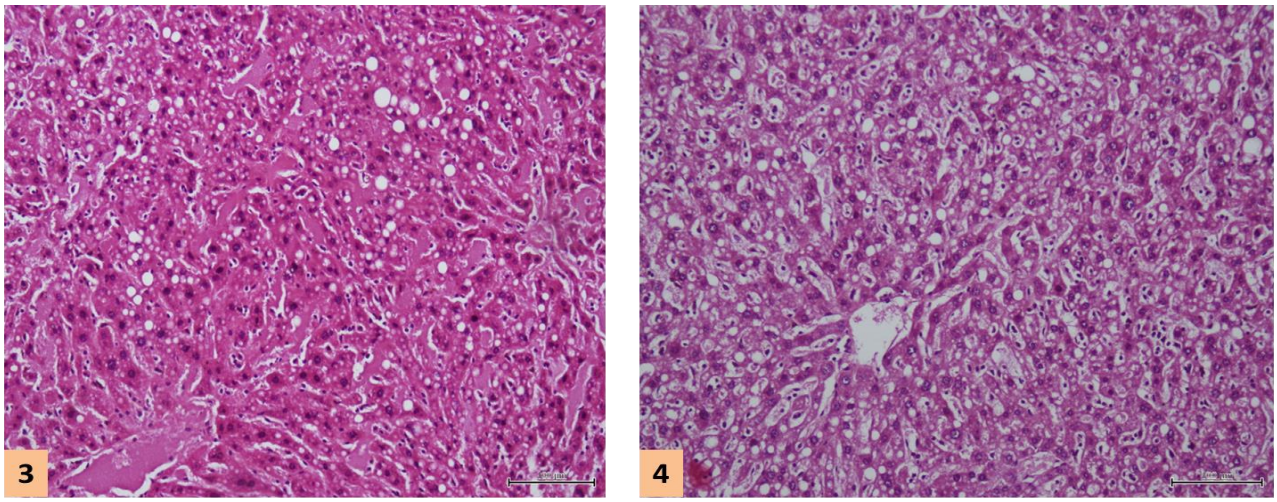

Fig 2. 3 is Standard group and shows normal histo-architecture whereas with some fatty vacuoles here and there 4, MC Al Extract group has some traces of fat cells, although much less than disease group and has almost attained back the normal histo-architecture

Discussion

This study showed that mice administered with Mtz were found to have gained more body weight than control group mice; which was found to be similar to reports in previous studies (Karmisholt et al., 2011). Several reasons related to hypothyroidism related weight gain have been proposed one of them links this weight gain to decreased peristalsis which leads to chronic constipation further yielding in weight gain. This gain in weight is primarily due to retention of water and not due to increased body fat mass (Biondi, 2010). Other studies say that this gain in weight is mostly due to retention of water and salt but some amount of gain in fat may also be accountable for it (Robinson M, 2022).

Several studies have shown that when the hypothyroid obese patients were treated with Thyroxine, as the treatment progressed, weight loss was observed in the patients and this weight loss has mostly been accredited to

surplus body water excretion related to a general condition related to hypothyroidism known as myxedema (characterized by weight gain, mental dullness and increased sensitivity to cold) as overall body energy equilibrium is maintained during the treatment. Treatment of hypothyroid obesity has been linked to increased thermogenesis and increased BMR both resulting in moderate weight loss. Apart from this, this treatment results in more amount of energy lost via REE (Resting Energy Expenditure) is also responsible for weight loss. An average increase in REE following 1 yr of treatment was 215 kcal/24 h and if it is effective for another 6 months therapy period, this will overall result in burning of 4.4kg of tissue fat (Karmisholt et al., 2011).

Hypothyroidism in humans has been generally linked to decreased activity, increased lethargy and weight gain. The decrease in body weight upon treatment with thyroxine has also been observed in mice. Various studies tend to show slightly different results upon Mtz treatment possibly due to the differences in animal age, drug dose, mode of drug administration and treatment duration (Karmisholt et al., 2011). The amount of water and food intake by the control and test group was almost same therefore we can deny the chances of any effects produced by water and food. Thyroid hormones have a crucial role in maintaining homeothermy (keeping the body warm) and hypothyroidism results in decrease in thermogenesis which supposedly generates several altered mechanisms of BAT metabolism (Dahiya et al., 2020). Leptin is also responsible for neuroendocrine regulation of the pituitary-thyroid axis. It is a regulator of energy homoeostasis by giving feedback to the central nervous system regarding tissue reserves of adipose (Biondi, 2010). In a study conducted on humans in 2008, it was concluded that moderate increase in TSH concentrations were related to weight gain (Fox et al., 2008).

Conclusion

Thyroid dysfunctions have commonly linked to weight changes i.e. gain of weight in hypothyroidism and loss of weight in hyperthyroidism has mostly been observed in patients. Increase in TSH has been linked with subsequent weight gain and its decrease has been related to weight loss. Thyroid dysfunction and obesity have been found to be interlinked; therefore physicians should keep in mind the chances of occurrence of hypothyroidism in obese patients. Whereas treatment with thyroxine has been found to show decrease in weight of the hypothyroid obese people, but it is not recommended for weight loss in euthyroid people. The MC Al Extract was found to show significant results for the reversal of the hypothyroidism induced obesity in mice. The histopathology of the liver revealed that hypothyroid obesity induction lead to the formation of fat vacuoles all over the liver which accounted for about 50% weight of the liver and the MC Al Extract was able to reverse these changes significantly. Further *in vivo* studies can be done on MC extracts for establishing their use as potent drugs for treating hypothyroidism linked obesity. Studies can also been done for establishing the role of leptin and BAT in thyroid dysfunctions related obesity.

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Conflict of Interest

No conflict of interest was declared by the authors.

Human and Animal Rights

Guidelines of CPCSEA were followed (Registration No. 0436).

Abbreviations

TSH- Thyroid Stimulating Hormone

T3- Tri-iodothyronine

T4- Tetra-iodothyronine

BMR- Basal Metabolic Rate

Mtz- Methimazole

MC Al Ex- *Momordica Charantia* Alcoholic Extract

BAT- Brown adipose tissue

REE- Resting Energy Expenditure

Lbs- pounds

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