summary
SUMMARY

➢ In the last few decades the prevalence of obesity is higher than compared to other diseases. Obesity is most prevalent in children, adolescents as well as middle age people due to life style changes. Obesity is one of the most widespread metabolic disorders in contemporary society.

➢ Obesity has a high degree of socioeconomic burden for many developing countries; hence the rate of mortality and morbidity is panicking. Obesity is associated with the development of type II diabetes mellitus, coronary heart disease, cancer, respiratory complications and osteoarthritis. The high cost of synthetic drugs and its toxic side effects in treating obesity and obesity associated complications leads to alternative medicine.

➢ Hence in an ancient time, herbs have been used as a valuable source of medicinal agent. They have been used for healing and to promote longevity of life. Recently, natural and alternative antiobesity agents in the form of beverages or teas, have been used for the treatment of obesity. These herbs and herbal products are considered safe with no toxic compounds and without causing any side effects and are also less cost effective compared with chemical antiobesity agents. People consider herbs as a source of alternative or complemenary medicine. Researchers have turned towards the natural resources for their research for the development of drugs for obesity.

➢ To investigate the anti-obesity potential of Benincasa hispida fruit and its active fraction, its therapeutic potential to prevent or suppress the obesity and obesity associated complications with high fat diet fed obesity induced animal model and inhibits lipogenesis via down regulation of AMPK and PPARγ in 3T3-L1 cells.

The outcome of the study has been summarized below,

CHAPTER I

➢ Microscopic examination of Benincasa hispida fruit showed the presence of homogenous parenchymatous tissue, ground tissue, xylem and phloem were arranged in collateral position. The xylem elements are thick walled and sparcely seen in the ground tissue. The calcium oxalate crystals were observed in the ground tissue.
The results obtained in the physico chemical analysis revealed that total ash, acid insoluble ash and water soluble extract contents of Benincasa hispida was within the limits as prescribed by the Ayurvedic pharmacopeia of India.

The results of pharmacognostic evaluation and physico chemical analysis may help in identifying Benincasa hispida in powdered form and serve as a standard drug to maintain its quality control. This study was used for the identification and authentication of Benincasa hispida fruit.

Extracts of Benincasa hispida fruit was prepared using soxhlet apparatus. The n-hexane, ethyl acetate, ethanol and water were used for the preparation of extracts. Then the extracts were evaporated using the rotary evaporator and the lyophilized powder was used for further analysis.

The Qualitative phytochemical analysis was helpful in identifying the phytochemicals present in the extract of Benincasa hispida and found phenols, flavonoids, tannins, terpenoids, quinones, glycosides, carbohydrates, proteins, coumarins, alkaloids, saponins, amino acids and phytosterols to be present. Comparing all the extracts n-hexane has less phytochemicals so quantitative analysis was done with the extracts such as ethyl acetate, ethanol and aqueous extract.

The Quantitative phytochemical analysis showed that ethanolic extract of Benincasa hispida has large quantity of phytochemicals such as total phenols, flavonoids, tannins, terpenoids and saponins.

The quantitative phytochemical analysis revealed that phytochemicals concentration was found to be higher in the ethanolic extract. So further study was performed with ethanolic fruit extract of Benincasa hispida.

The alkaloids present in the EEBH were 3.9 ± 0.25mg/g, total protein was 4.11 ± 0.18 mg/g and total sugars was 30.33 ± 2.51mg/g.

Then the EEBH was subjected to HPLC analysis. The HPLC analysis showed the presence of phenols such as gallic acid, coumaric acid and flavonoids present were rutin and quercetin.

The above results showed that the extracts of Benincasa hispida were rich in phytochemicals. These phytochemicals have specific biological activities.
CHAPTER II

- *In vitro* antioxidant study viz., DPPH, reducing activity, superoxide anion radical scavenging assay, nitric oxide scavenging assay, Hydroxyl assay and hydrogen peroxide radical scavenging assay revealed the strong antioxidant capacity of EEBH. EEBH showed better scavenging activity against superoxide radical followed by DPPH, H\textsubscript{2}O\textsubscript{2} and hydroxyl radical. *Benincasa hispida* had less nitric oxide radical scavenging activity. The reducing capacity indicated that it has better antioxidant activity.

CHAPTER III

- The fractionation of EEBH by silica gel column chromatography showed 84 fractions. The fractions that showed same R\textsubscript{f} values were pooled into five fractions. Then the fractions were dried using rotary evaporator.
- All five fractions were subjected to quantitative phytochemical analysis. The phenols, flavonoids, tannins and terpenoids present in all the fractions were quantified and it was found that fraction IV of ethanolic fraction showed higher concentration of these phytochemicals, so further study was carried with this fraction.
- The HPTLC analysis of fraction IV of EEBH revealed the presence of compound β-Sitosterol that has anticancer, antimicrobial, antiulcer, antiarthritic and antihypolipidemic activity.
- The GCMS analysis of fraction IV showed the presence of compounds like 5 hydroxy methyl furfural, 3-chlorophenyl isothiocyanate, 1H-1,3 Benzimidazole, 2 (methoxy methyl)-1-2 propynyl, Glycerin, 4H- pyran-4-one 2,3-dihydro-3,5-dihydroxy-6-methyl, 1,2,3-propanetriol, 1-acetate, N-methoxy carbonyl methyl-N-ethyl nitrosamine, n-hexadecanoic acid, 1,2,3,4 tetrazola (1,5-b) (1,2,4) triazine 5,6,7,8 tetrahydro, 9,12 octadecadienoic acid, Hexadecanoic acid ethyl ester, Linoleic acid ethyl ester, Phenol, 4,4'-1-methyl ethylidene bis and Diethyl phthalate.
- These compounds showed biological activity such as antioxidant, anti inflammatory, antimicrobial and hypocholesterolemic activity. The phytochemicals present in EEBH may serve as a natural source of antioxidant.
- Phytochemicals were rich in fraction IV of EEBH and was identified by quantitative phytochemical analysis. HPTLC analysis showed the presence of β-
Sitosterol in fraction IV of EEBH and GCMS analysis showed the presence of volatile compounds. These chemical compounds exert many biological activities.

CHAPTER IV

➢ All five fractions from column chromatography were subjected to MTT assay using 3T3-L1 cell line. This assay results showed that fraction IV had highest cell viability of 3T3-L1 preadipocyte cells. Since fraction IV of EEBH showed the better result, it is considered as active fraction of *Benincasa hispida* (AFBH). The MTT assay suggested that AFBH was non-toxic. Hence further study was performed with AFBH.

➢ 3T3-L1 pre-adipocyte cell line was used for *in vitro* analysis. 3T3-L1 pre-adipocyte cell line was converted into adipocyte cell using dexamethasone by differentiation process.

➢ The inhibiting effect of AFBH on lipid accumulation in 3T3-L1 adipocyte was measured using Oil red O staining. AFBH at various concentrations (12.5, 25, 50 and 100µg/ml) of sample was used for oil red O staining. The concentration of 100µg/ml of AFBH showed better lipid inhibitory effect in 3T3-L1 adipocyte by preventing adipogenesis.

➢ The effect of AFBH on Glyceraldehyde 3 phosphate dehydrogenase activity was determined on 3T3-L1 cells. The concentrations of 12.5µg, 25µg, 50µg and 100µg were used for the assay of glyceraldehyde 3 phosphate dehydrogenase activity. 100µg concentration of AFBH showed better inhibiting activity of glyceraldehyde 3 phosphate dehydrogenase. The result suggested that AFBH inhibited the Glyceraldehyde 3 phosphate dehydrogenase activity in 3T3-L1 cell line.

➢ The activity of HMG-CoA reductase was assayed using various concentrations of AFBH concentrations (12.5µg, 25µg, 50µg and 100µg). 100µg of AFBH showed better inhibition of HMG-CoA reductase activity.

➢ The gene expression of the peroxisome proliferator activated receptor (PPARγ), CCAAT/enhancer binding protein (C/EBPα), Sterol regulatory element binding protein (SREBP-1), Stearoyl CoA desaturase (SCD-1), Lipoprotein lipase (LPL) and Fatty acid synthase (FAS) gene with AFBH at different concentration (12.5µg, 25µg, 50µg and 100µg) can reduce the adipocyte differentiation and it was performed using RT-PCR. 100 µg of AFBH showed better result in down
regulating the gene expression of PPARγ, C/EBPα, SREBP-1c, SCD-1, FAS and LPL in 3T3-L1.

- The protein expression of PPARγ, C/EBPα, AMPK, P-AMPK, FAS and LPL were studied using western blot analysis at different concentrations of 12.5µg, 25µg, 50µg and 100µg of AFBH. 100µg of AFBH showed better activity in suppressing PPAR γ, C/EBPα, FAS and LPL in 3T3-L1 adipocyte cell line. AFBH reduces the AMPK protein level by increasing the P-AMPK activity.
- The results of this phase suggested that AFBH exerts a beneficial effect on lipid metabolism and preventing obesity by attenuating adipogenic genes such as PPAR γ, C/EBPα, FAS and LPL.
- AFBH attenuate adipogenesis by attenuating genes and protein expression of lipogenic genes and also transcriptional factors, which are involved during adipogenesis. AFBH at 100µg concentration showed better result in attenuating adipogenesis.

**CHAPTER V**

- This phase of study was carried out in obesity induced wistar albino rats by using high fat diet fed rats to evaluate the antiobesity potential of AFBH and EEBH. The results of the animal study are given below,
- The occurrence of obesity was observed after 8 weeks with high fat diet fed rats induced obesity and was confirmed by the visceral body mass index and body weight. The high fat diet fed animals showed significant increase in their body weight. AFBH and EEBH showed a significant decrease in body weight.
- Body weight gain and feed consumption was increased in obesity induced animals. AFBH, EEBH and orlistat treated animals showed better result in reducing body weight and feed consumption.
- The anthropometric determination was also significantly increased in high fat diet fed animals. The Thoracic circumference and abdominal circumference was significantly increased in high fat diet fed animals (group II). AFBH and EEBH treated animals showed reduced level of Thoracic and abdominal circumference and active fraction, AFBH showed better result.
- Body mass index was significantly increased in high fat diet fed animals (Group II). When compared with AFBH, EEBH and orlistat treated animals. AFBH treated animals showed reduced BMI values.
AFBH, EEBH and orlistat treated animals maintained the organ weight such as brain, kidney, liver, pancreas, spleen, pancreas and spleen when compared with obese rats. No significant change was observed in the weight of brain. Organ weight of liver, heart, spleen, kidney and pancreas of high fat diet fed animals was increased significantly. AFBH, EEBH and orlistat treated animals showed better result in maintaining the organ weight. Comparing all the groups AFBH showed better result in maintaining organ weight.

The weight of the fat pad such as perirenal, mesenteric, epididymal and gonadal fat weight was increased in high fat diet induced obesity animals. On treatment with AFBH and EEBH there was a significant reduction of fat pad weight. Comparing all the groups AFBH showed better result in reducing fat pad.

The high fat diet induced obese animals showed significant increase in serum lipid parameters like total cholesterol, triglycerides, LDL, VLDL and decrease in HDL level. AFBH treated high fat diet fed animals showed a decrease in total cholesterol, triglycerides, LDL, VLDL and increase in the level of HDL than EEBH and orlistat treated rats.

Animals fed with high fat diet showed significantly increased changes in the biochemical parameters such as glucose, insulin, insulin resistance, total protein and albumin in serum. Treatment with AFBH, EEBH and orlistat attenuated these levels. Comparing all the groups AFBH showed better result in maintaining these levels to near normal.

The levels of free fatty acids and phospho lipids also increased in high fat diet fed obesity animals and on treatment with AFBH, EEBH and orlistat showed significant reduction. When compared to all the groups AFBH treated group showed better result in reducing free fatty acids and phospholipids.

The activities of enzymatic markers such as serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), alkaline phosphatase (ALP), Lactate dehydrogenase (LDH), creatinine kinase (CK) and lipase levels were significantly increased in high fat diet fed animals due to hepatic steatosis or fatty liver. AFBH, EEBH and orlistat treated animals showed significant reduction in activities of enzymes in serum. Comparing all the groups AFBH showed better result in reducing the activities of these enzymes than EEBH.
The level of homocysteine in plasma sample of high fat diet fed animals showed significant increase while AFBH treated animals showed significant reduction.

The adiponectin level of high fat diet fed animals showed significant reduction while AFBH, EEBH and orlistat treated animals showed significantly increased level of adiponectin and decreased level of leptin in serum sample. AFBH showed better result in increasing the adiponectin level and decreasing the leptin level in experimental animals.

The levels of serum apolipoprotein B is increased in high fat diet induced obesity animals when compared with control animals and AFBH, EEBH and orlistat treated animals showed significantly reduced level of apolipoprotein and AFBH showed best result in reducing the apolipoprotein B.

The pancreatic lipase activity showed significant increase in high fat diet fed animals when compared with AFBH, EEBH and orlistat treated animals. AFBH treated animals showed reduced activity of pancreatic lipase. Comparing AFBH and EEBH treated animals, AFBH treated animals showed significant reduction of pancreatic lipase activity.

The levels of lipid peroxidation in liver and serum were significantly increased in high fat diet fed animals than AFBH, EEBH and orlistat treated animals. Comparing all the groups AFBH showed significant reduction in lipid peroxidation.

Non enzymatic antioxidant levels such as Vitamin C, E and glutathione level are significantly decreased in high fat diet fed animals. AFBH, EEBH and orlistat treated animals showed significantly increased levels of the non-enzymatic antioxidants.

Activities of Enzymatic antioxidant such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX) in liver and serum were significantly reduced in high fat diet fed animals. AFBH, EEBH and orlistat treated animals showed significant increase in these antioxidants. AFBH showed more significant increase in the level of these enzymatic antioxidant.

These results revealed that AFBH is a potent antiobesity agent in modulating physical, biochemical changes by attenuating adipogenesis process. Orlistat was used as a drug control for all biochemical and physical parameters.
Histological examination of adipose tissue of high fat diet induced animals showed increase in the adipocyte size. AFBH and EEBH treated animals showed decreased adipocyte size. AFBH treated animals showed better result in reducing the adipocyte size.

Hepatic steatosis was observed in liver of high fat diet fed animals which was caused due to lipid accumulation. The level of hepatic steatosis was significantly reduced by EEBH and AFBH treatment. Comparing AFBH and EEBH treated animals, AFBH showed better result in reducing lipid content in liver. Lipid accumulation in liver was observed by Oil red O staining. Both the methods showed that AFBH showed better results.

Organs like spleen, heart and pancreas were not affected by high fat diet fed as well as AFBH and EEBH treatment.

Histopathological examination of adipose tissue and liver showed that high fat diet fed animals cause changes in adipocyte size as well as lipid accumulation in liver. This was reduced by AFBH and EEBH treatment. AFBH showed significant decrease in the adipocyte size.

The results obtained from in vivo experimental studies of high fat diet fed animals treated with AFBH showed better results in ameliorating high fat diet induced obesity when compared with EEBH. AFBH showed better results in reducing lipid accumulation.