DISCUSSION
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Obesity impact on healthcare systems is becoming unsustainable and urgent advances in treatment are needed. Obesity is still widely decried as a lifestyle ‘choice’, but even determined changes in lifestyle have very limited long-term impact on it and, pragmatically, the need for medical intervention is now clear. Surgical or endoscopic bariatric procedures are effective therapies for obesity, but bariatric surgery in particular carries significant peri-operative morbidity and mortality. Moreover, it is very expensive, recommended for severe obesity and thus restricted in many healthcare systems. Therefore, there is clearly an important role for pharmacotherapy as an adjunct to lifestyle changes\textsuperscript{(154)}.

In this study we used Cafeteria diet induced obesity (DIO) model. It is widely accepted model for induction of obesity. High fat diet inevitably causes hyperphagia resulting in increased body weight. It simulates clinical obesity and shares many features with human obesity. This gain in body weight is largely due to increased fat mass as a result of pre-adipocyte proliferation and differentiation and, accumulation of lipids in the liver, to some extent\textsuperscript{(155,156)}.

Some reports have attributed obesity induced by high-fat diets to their high food efficiency (g body-weight gain per kJ food consumed). Energy from fat has a larger effect on body-weight than has energy from non-fat sources\textsuperscript{(157-159). Diet-induced thermogenesis is the energy for digesting, absorbing and storing nutrients. It leads to loss of energy from the body which is 2–3\% for fats, 25–30\% for proteins and 6–8\% for carbohydrates. Therefore, the efficiency of nutrient utilisation differs among macronutrients and fats have an efficiency of 97–98\%, whereas efficiency is 70–75\% for proteins and 92–94\% for carbohydrates\textsuperscript{(158-161)}. In addition, it costs energy to build long-chain fatty acids from glucose or amino acids, whereas dietary fat contains long-chain fatty acid pre-formed. Some studies have shown that a fat-rich diet induces obesity by increasing energy intake.
Some studies have reported that not all fats are obesogenic and the dietary fatty acid profile rather than the amount of energy from fat is an important variable in developing dietary obesity\(^{(158-162)}\), but there is some controversy on this matter since there are reports showing non-significant differences in final body weight and/or body-weight gain of the animals consuming various fatty acids\(^{(167-169)}\).

Other factors that may contribute to obesity induced by a diet rich in fat include failure to adjust oxidation of fat to the extra fat in the diet\(^{(170)}\), increase in adipose tissue lipoprotein lipase activity\(^{(171)}\), increased meal size and decreased meal frequency\(^{(172)}\), as well as overconsumption of energy attributed to high energy density of the diet\(^{(173-175)}\), orosensory characteristics of fats and poorly satiating properties of the high-fat diets. Reviews of dietary obesity describe potential mechanisms of body weight and food intake regulation involving the central nervous system mainly the hypothalamus neuropeptides such as ghrelin and neuropeptide Y, and hormones such as insulin and leptin\(^{(176,177)}\). Adipose tissue per se is considered to be an endocrine organ that secretes cytokines such as IL-6 and TNF\(\alpha\); thus obesity could possibly be regarded as a chronic inflammatory disease\(^{(177-179)}\).

Obesity occurs when energy uptake surpasses energy expenditure in the individual animal and so the stores of energy in body fat are enlarged, particularly in adipose tissues. Obesity involves both or either an increase in the number of adipocytes (hyperplasia) and their size (hypertrophy)\(^{(156,180,181)}\).

Drug treatment for obesity is an evolving branch of pharmacology, burdened by severe side effects and consequences of the early drugs, withdrawn from the market, and challenged by the lack of long-term data on the effect of medications on obesity-related morbidity and mortality, first of all cardiovascular diseases.

Current pharmacological intervention in obesity is limited by the availability of a few licensed drugs. Available drugs have limited efficacy and many untoward effects.

Orlistat is reversible inhibitor of gastric and pancreatic lipases that acts in the lumen of stomach and small intestine, preventing absorption of fat significantly. Bakris et al.,\(^{(182)}\)
showed weight loss of 5.4 kg, Broom et al.,(183) showed mean weight loss of 5.8 kg, Lindgarde et al.,(184) found mean weight loss of 5.6 kg while Swinburn et al.,(185) reported 4.7 kg mean weight loss. Effect on BMI in orlistat-treated group in present study is comparable to the previous trials; Bakris et al.,(182) found mean reduction in BMI by 1.9 kg/m² while Krempf et al.,(186) showed reduction by 2.3 kg/m².

Orlistat remains the sole medication licensed for the treatment of obesity in the UK. Although numerous studies have shown that orlistat can produce desired weight loss in the short term, maintained reduction of weight remains challenging. This is further compounded by NICE guidelines(187) that recommend the use of orlistat for a maximum of two years. Moreover the efficacy of orlistat on obesity-related morbidity is ambiguous as cardiovascular outcomes and progression of type 2 diabetes remain largely unaffected by the treatment.

Sibutramine (usually in the form of the hydrochloride monohydrate salt) is an oral anorexiant. The review by the European Medicines Agency’s (EMEA) Committee for Medicinal Products for Human Use (CHMP) was initiated because data from Sibutramine Cardiovascular Outcomes Trial (SCOUT) showed an increased risk of serious, nonfatal cardiovascular events such as stroke or heart attack with sibutramine compared with placebo. The CHMP noted that the use of sibutramine was not in accordance with the prescribing information for most of the patients enrolled in SCOUT, as sibutramine is contraindicated in patients with known cardiovascular disease. The treatment duration in the study was also longer than normally recommended(188). Until 2010 it was marketed and prescribed as an adjunct in the treatment of exogenous obesity along with diet and exercise. It has been associated with increased cardiovascular events and strokes and has been withdrawn from the market in countries and regions including Australia, Canada, China, the European Union(EU), Hong Kong, India, Mexico, New Zealand, the Philippines, Thailand, the United Kingdom, and the United States.

Rimonabant, an antagonist of cannabinoid type-1 (CB-1) receptor, was withdrawn (2009) from the market because of adverse psychiatric side effects, including a negative
affective state\textsuperscript{189}. It was indicated for use in conjunction with diet and exercise for patients with a body mass index (BMI) greater than 30 kg/m\textsuperscript{2}, or patients with a BMI greater than 27 kg/m\textsuperscript{2} with associated risk factors, such as type 2 diabetes or dyslipidemia.

Lorcaserin is a serotonin 2C receptor agonist and is thought to aid weight loss by reducing appetite and promoting satiety\textsuperscript{190}. The FDA approved lorcaserin in 2012, although it initially denied approval because of concerns that the potential risks of the drug outweighed the benefits. Nonselective serotonergic agonists, such as fenfluramine and dexfenfluramine, carry an increased risk of serotonin-associated cardiac valvular disease. Theoretically, lorcaserin should not have the same cardiac effects because it is a selective agonist of serotonin receptor 2C. However, there are currently few long-term safety data. Although lorcaserin was approved in 2012, as of now it was not available pending a decision to designate lorcaserin as a Schedule IV controlled substance\textsuperscript{191-193}.

BLOOM (Behavioral modification and Lorcaserin for Overweight and Obesity Management), the phase-III clinical trial of lorcaserin, was conducted over two years. The BLOOM trial enrolled 3,182 overweight or obese patients (with at least one co-morbidity, though diabetes was excluded) who received 10 mg lorcaserin or placebo twice a day, alongside lifestyle modification\textsuperscript{194}. At the end of year one, 47.5\% of those receiving lorcaserin compared to 20.3\% of those receiving placebo (p<0.001) lost >5\% of their baseline body weight, and 22.6\% of those receiving lorcaserin compared to 7.7\% of those receiving placebo (p<0.001) lost >10\%. Additionally, lorcaserin significantly reduced waist circumference, BMI, fasting glucose, fasting insulin, glycosylated hemoglobin levels, total cholesterol, LDL cholesterol, triglyceride levels, C-reactive protein, fibrinogen levels, and systolic and diastolic blood pressure. More profound weight loss was observed in year two. Possible side effects of lorcaserin included headache, dizziness and nausea, but these were not significantly different to the side effects reported by the placebo group.

Phentermine-Topiramate ER, the combination of phentermine and topiramate extended-release is another recent addition to the approved medical options for chronic weight
management. Phentermine is an appetite suppressant and topiramate is an anticonvulsant thought to act as an appetite suppressant\textsuperscript{195}. Phentermine-topiramate ER appears to be slightly more effective than orlistat and lorcaserin. However, concerns about phentermine-topiramate ER’s effect on heart rate limit its use in patients with cardiovascular disease.

The development of new drugs is impeded by both the complexity of systemic pharmacotherapy and variables such as ethnicity and age. The varied distribution of metabolically active adipose tissue in different ethnic groups can affect a potential drug’s efficacy, perhaps ultimately rendering it ineffective. The complex interplay of genetics, environmental factors and psychosocial issues makes successful drug treatment an extremely challenging venture. In addition, as the prevalence of obesity is rising amongst children and adolescents, the demand for effective, sustainable treatments increases. At present, these groups are under-represented in drug development trials, compounding our ability to ascertain drug suitability across the patient spectrum.

The story of anti-obesity pharmacotherapy is littered with initially promising drug candidates that were eventually withdrawn because of safety concerns. This fate could engulf the new generation of drugs being trialed, but it seems likely that some will succeed.

Herbs contain a wide variety of active phytochemicals, such as flavonoids, terpenoids, lignans, polyphenols, saponins, plant sterols and carotenoids, and there is now a lot of interest in herbs that possess hypolipidemic, antiplatelet, anti-tumour and immune stimulating properties\textsuperscript{196}.

*Caralluma fimbriata* is an edible cactus from the Asclepiadaceae family. The plant vary from thin, recumbent stems from ½ to 1½ inches thick to erect growing clumps up to 8 inches high. The star-shaped, fleshy flowers of these plants are some of the worst smelling of the succulent plants. Ordinarily borne in late summer, the foul-smelling blossoms are usually colored purple, black, yellow, tan, maroon, red or dark brown. In the wild, these blossoms are pollinated by flies which are greatly attracted to the plant. It
is commonly used as a vegetable in several regions of India. It is eaten raw or cooked with spices. It is also used in pickles and chutneys\(^{(197)}\).

*Caralluma fimbriata*, which grows widely in India, is associated with folklore of appetite reduction, and it is of interest to verify this effect through controlled studies. There are several varieties of *Caralluma* that grow in India although these species are botanically and phytochemically similar. The key phytochemical ingredients in *Caralluma* are pregnane glycosides, flavone glycosides, megastigmane glycosides, bitter principles, saponins and various other flavonoids\(^{(129)}\). The appetite suppressing action of *Caralluma* could be attributed to the pregnane glycosides, which are particularly rich in plants belonging to the Asclepiadaceae family. It is unclear as to how pregnane glycosides or its related molecules may suppress appetite, and it is thought that they amplify the signaling of the energy sensing function in the basal hypothalamus. A similar appetite suppressing action has been observed in the South African cactus-like plant Hoodia, in which a steroidal glycoside was isolated, which demonstrated anorectic activity in animals\(^{(198)}\).

The first clinical trial performed in India consisted of 50 overweight/obese subjects (BMI >26) 25 received active compound and 25 received a placebo. The study, under the purview of the Institutional Ethics Review Board of St John’s National Academy of Health Sciences, Bangalore, India, was randomized, double-blind, and placebo controlled. Over eight weeks, the subjects were tested for weight-loss, anthropometry, body fat composition, BMI, net weight and systemic functions. During the study, no changes were made in diet; and all subjects were advised to walk 30 minutes in the morning and evening. The adverse events were minor and limited to mild upset of the gastrointestinal tract. Importantly, they were present equally in the active and placebo groups. Constipation and flatulence subsided within a week and were attributed to the gelatin capsules more than the ingredients from the cactus present in the capsules. Examination of fasting and post-prandial sugar, total cholesterol, LDL, HDL, triglycerides, serum creatinine, BUN, total protein, serum albumin, total bilirubin, conjugated bilirubin, SGOT and SGPT, and alkaline phosphatase and hemoglobin failed to reveal any overall toxicity from the extract. Blood pressure and ECG also showed no toxic reactions secondary to ingesting *Caralluma fimbriata*. Compared to placebo group,
individuals receiving the extract showed no significant change in body weight, body mass index, hip circumference, body fat or energy intake; however, both appetite and waist circumference were reduced\(^{(199)}\).

The second study performed in California at the Western Geriatric Research Institute consisted of 26 overweight patients, 19 on active compound and 7 on placebo. Over 60\% of those taking the extract lost 6 pounds or more for the month. This study is suggestive of a positive effect of the *Caralluma fimbriata* extract on weight loss. Importantly, it reaffirmed the safety of the extract, as no serious adverse events occurred\(^{(200)}\).

In this study our results showed that CFE given as pretreatment led to significant (p<0.001) reduction in the gain in body weight, while its administration after induction of obesity also resulted in significant (p<0.001) weight loss in this experimental model of obesity (DIO in rats). This reveals that concurrent administration of CFE with cafeteria diet reduces the development of obesity in rats. Previous studies have shown that concurrent administration of CFE with cafeteria diet prevented rats from becoming obese\(^{(147)}\).

**Proposed mechanisms of action (also described in detail in page No.61)**

The exact mechanism of action of this effect is not well established. Pregnane glycosides present in CFE may act via multiple mechanisms. There is also evidence that they act directly on adipose tissue, by inhibiting adipocyte proliferation and differentiation\(^{(201-203)}\). *Caralluma fimbriata* contains pregnane glycosides which are believed to block the activity of citrate lyase, an extra mitochondrial enzyme involved in the initial step of *de novo* lipogenesis\(^{(204)}\). By blocking this enzyme, *Caralluma fimbriata* may block the formation of fat by the body. Further, *Caralluma fimbriata* also blocks another enzyme called Malonyl Coenzyme A. By blocking this enzyme, fat formation is further blocked and the body is forced to burn its fat reserves. This might accelerate the rate of fat loss by the body.

There was significant (p<0.001) decrease in food intake after treatment with CFE suggesting its appetite suppressant effect. Food consumption in humans is regulated
through a number of complex biological mechanisms which ensures that body weight is relatively constant over long periods. Appetite regulates the body’s desire for food through a complex biological process designed to satisfy the body’s need for energy, protein, fat, carbohydrates and other nutrients\(^{(205)}\). Appetite therefore plays an important role in weight regulation. Obese individuals have been shown to have an increased appetite. Eating disorders such as binge eating, night eating or compulsive overeating disorders are also developing in obese persons\(^{(206)}\). Thus measures to reduce appetite of overweight and obese individuals could help in preventing further weight gain and in enhancing weight reduction.

The decline in food intake may reflect direct intervention in appetite control at the level of the hypothalamus, where the pregnane glycosides present in CFE are known to act\(^{(207)}\). An alternative hypothesis is that CFE may down regulate ghrelin synthesis in the stomach and subsequently neuropeptide-Y in the hypothalamus, with ultimately the same effect of appetite suppression\(^{(208,209)}\). Neuropeptide-Y is a 36-amino acid neuropeptide that acts as a neurotransmitter in the brain and in the autonomic nervous system of humans; slight variations of the peptide are found in many other animals. In the autonomic system it is produced mainly by neurons of the sympathetic nervous system and serves as a strong vasoconstrictor and also causes growth of fat tissue. In the brain, it is produced in various locations including the hypothalamus, and is thought to have several functions, including: increasing food intake and storage of energy as fat, reducing anxiety and stress, reducing pain perception, affecting the circadian rhythm, reducing voluntary alcohol intake, lowering blood pressure, and controlling epileptic seizures\(^{(210)}\).

There were favorable effects on serum lipid profile viz. significantly (p<0.001) reduction in TC, LDL, VLDL, TG and significant (p<0.001) increase in HDL levels. Similar findings were also reported by Soundararajan K et. al., 2010\(^{(147)}\) and Jagtap et. al., 2013\(^{(198)}\).

Treatment with CFE also had favorable effect on the blood sugar levels in these animals. In folklore medicine, plants of the *Caralluma* species have been used to treat diabetes. In a study using streptozotocin diabetic mice, acute or subacute treatment with *Caralluma*
arabica caused a statistically significant lowering of circulating blood glucose levels\textsuperscript{(133)}. Similar to Caralluma arabica extracts from Caralluma attenuate were found to be antihyperglycemic in alloxan diabetic rats\textsuperscript{(134)}.

Alterations in liver enzyme levels and kidney function tests induced by the cafeteria diet were reduced by CFE. Consumption of cafeteria diet led to decrease in blood urea levels significantly (p<0.001). T. Barberet al, also found decreased urea levels and reduced activity of several enzymes involved in urea cycle on cafeteria diet feeding\textsuperscript{(211)}. In our results, pretreatment with CFE prevented reduce in blood urea levels, while its administration after induction of obesity also resulted in significant (p<0.001) increase in blood urea levels.

In addition to the long history of safe ingestion of the cactus as a food, further evidence of safety of its extract is evident through an acute oral toxicity study on rats and two clinical studies\textsuperscript{(152,200)}. The former was carried out by the Department of Pharmacology, St John’s Medical College in Bangalore, India. Doses of 2g/kg body weight and 5g/kg body weight were gavaged to rats. All animals survived until the scheduled necropsy at the end of the study period of 14 days. Histology revealed no abnormalities in the various organs. Accordingly the LD\textsubscript{50} for the rats exceeded 5g/kg.

In histopathology study, excessive accumulation of fat and sinusoidal congestion were observed in liver tissues of obese group of animals compared to liver tissues of control group of animals. However less fat accumulation and no sinusoidal congestion were observed in the livers of CFE treated obese group of animals compared to untreated obese group of animals. This effect is probably by inhibiting adipocyte proliferation and differentiation\textsuperscript{(201-203)}.

No significant changes were observed in kidney and aorta in all the groups.