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Effects of low molecular weight galactomannans based standardized fenugreek seed extract in subjects with high fat mass: A randomized, double-blind, placebo-controlled clinical study

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ABSTRACT

The present investigation evaluated the effects of low molecular weight galactomannans-based standardized fenugreek seeds extract (LMWGAL-TF) on human subjects with high-fat mass for 8-weeks using a prospective, randomized, double-blind, placebo-controlled design. Twenty-four subjects with percent body fat were randomized to ingest a capsule of LMWGAL-TF (500 mg, once a day) or the matching placebo at a 1:1 ratio for 8 weeks. The outcome measurements were recorded at baseline, week-4, and week-8 (end of the treatment). The efficacy outcome included fat mass (absolute, non-fat mass and %) by skinfold thickness method (along with triceps, suprailiac, and abdominal) and bioelectrical impedance analysis method, body weight, body mass index, and abdominal girth. The standard safety parameters were measured, such as adverse events, vital signs, hematology, and biochemistry. Eight weeks of LMWGAL-TF supplementation showed significant reduction in suprailiac skinfold thickness (v/s baseline) and abdominal skinfold thickness (v/s baseline and v/s placebo), and percent fat mass, (v/s baseline). The LMWGAL-TF supplementation was found to be safe and well-tolerated. In conclusion, LMWGAL-TF supplementation showed safety and efficacy in reducing skinfold thickness (abdominal and suprailiac) and percent body fat in subjects with a high fat mass.

INTRODUCTION

Overweight and obesity are the major risks of global deaths (Stevens *et al*., 2012). Obesity has become a major public health problem both in developed and developing countries (Ng *et al*., 2014). Obesity arises as the result of an energy imbalance between calories consumed and the calories expended, creating surplus energy and a state of positive energy balance resulting in excess body weight (Hruby and Hu, 2015). Exercise plays a major role in targeting increased energy expenditure via cardiovascular and/or resistance training result in small weight losses. It results in marginal improvements in weight loss in combination with dietary interventions (Jakicic *et al*., 2001; Shaw *et al*., 2006). Very low energy diets (< 800 kcal/day or < 3,350 kJ/day) (Anderson *et al*., 2007) and the high-protein, low-carbohydrate diets (Sumithran and Proietto, 2008) are used to reduce fat for a short time such as 8–12 weeks. However, for long-term use, strict medical supervision with contraindication has been recommended.

Some pharmacological agents are approved to improve weight loss parameters (Franz *et al*., 2007; Padwal *et al*., 2004). These agents act centrally to increase levels of satiety or act on the gastrointestinal tract to restrict nutrient absorption. However, their use is limited due to many side effects (Chan *et al*., 2013; Franz *et al*., 2007). Even surgical interventions are becoming popular for substantial weight loss, but they also carry the most risk (Colquitt *et al*., 2014; Grima and Dixon, 2013). Therefore, there is a need for safe intervention for a population with high fat mass.

Historically, dietary fibers have been reported to reduce body fat mass and prevent the body weight gain in clinical studies (Bano *et al*., 2015; Tucker and Thomas, 2009). The low molecular

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weight galactomannans-based standardized fenugreek seed extract (LMWGAL-TF) has been reported to decrease percent body fat in resistance-trained males after 8 weeks of treatment (Poole *et al*., 2010). LMWGAL-TF has also been found safe during 90-day oral and prenatal exposure studies conducted as per The Organisation for Economic Co-operation and Development (OECD) guidelines (Deshpande *et al*., 2016a; 2016b). Furthermore, the efficacy of oral LMWGAL-TF supplementation to high fat diet-induced obese mice was reported to be mediated through multiple pathways via down-regulation of fatty acid synthase, leptin, and transcriptional regulator interacting with the transcriptional regulator interacting with the PHD-bromodomain 2 (TRIP-Br2) (Kandhare *et al*., 2018). LMWGAL-TF holds a promise to provide safe intervention for fat mass reduction to address the current need of the obese population. Therefore, the present study evaluated the potential efficacy and safety of LMWGAL-TF supplementation on fat mass related parameters in subjects with high fat mass using randomized, double-blind, placebo-controlled design.

MATERIALS AND METHODS

Recruitment and randomization

Twenty-seven subjects were screened, and 24 subjects were recruited from the existing patient databases of Obesity clinic, Department of Clinical Nutrition, Shivaji Peth, Kolhapur-416012, Maharashtra, India. The sample size of 12 per group was followed as a recommended rule of thumb for such pilot studies (Julious, 2005). The study was performed according to the Declaration of Helsinki. The consort flow diagram of the study is presented as Figure 1. The study protocol was approved by the Drushti Independent Ethics Committee for the human ethics requirement (study code – IBHM19/2008). This study is registered on the Clinical Trial Registry of India (CTRI), New Delhi, India (Registration No. CTRI/2013/10/004050). The inclusion criteria consisted of subjects with body fat mass > 25% for males and > 30% for females willing to sign informed consent with the age of 18–35 years. The exclusion criteria were as follows: subject has an elevated resting heart rate (> 100 beats per minutes) or blood pressure (BP) (systolic BP > = 140 or diastolic BP > = 90 mm Hg), Serum glutamic oxaloacetic transaminase and serum glutamic pyruvic transaminase > = 2 times the upper limit of normal, serum creatinine > = 2 times the upper limit of normal, haemoglobin < 10 g/dl, subject with history of medical or surgical events that may affect the study outcome or place the subject at risk, including cardiovascular disease, gastrointestinal problems, metabolic (including but not limiting to diabetes), renal, hepatic, neurological, or active musculoskeletal disorders, subject with history of orthopaedic injury or surgery, which can interfere

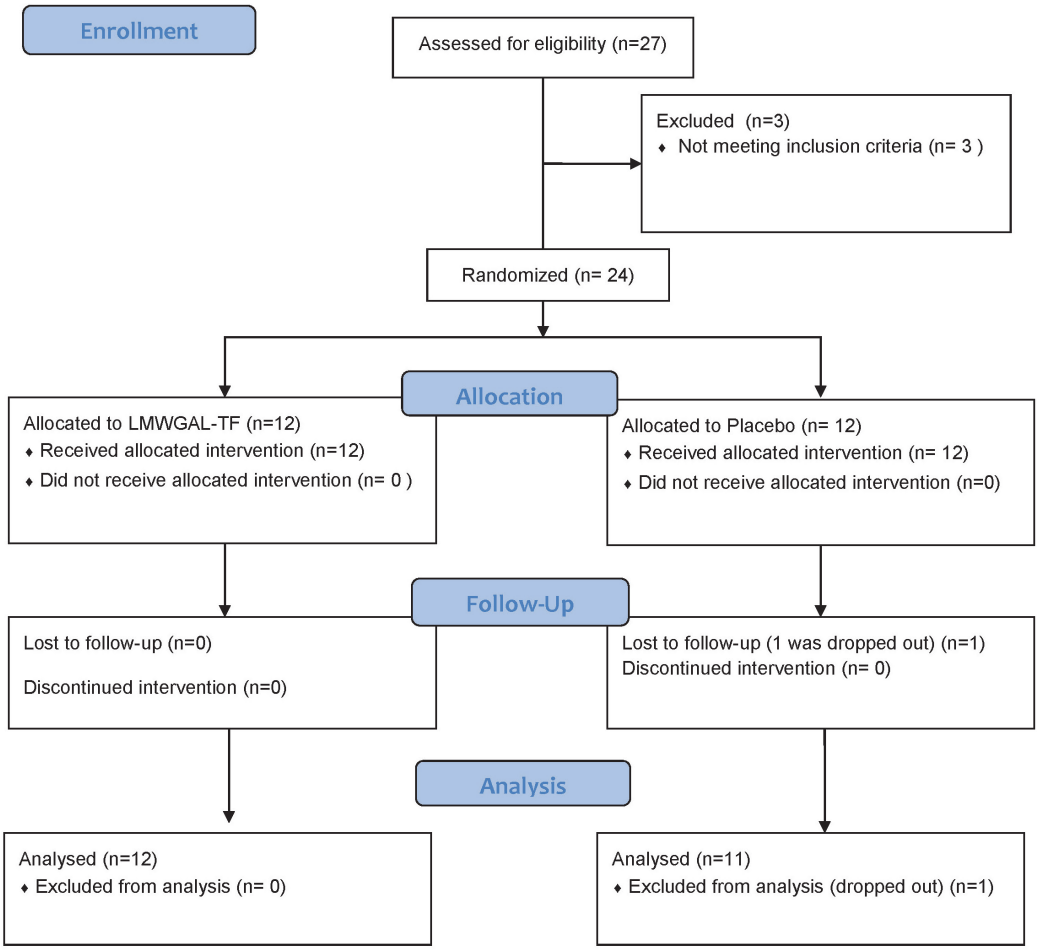

Figure 1. Study flowchart.

in activity schedule, known hypersensitivity to herbal drugs/ nutritional supplement/ foods, postmenopausal females, subjects who had received any anti-obesity or fat reducing therapy during last 2 months, subjects received any performance enhancing medication, subjects undergoing any additional weight loss or diet plan during the study period, chronic alcoholics, drug abusers, pregnant, willing to get pregnant or not ready to use contraceptive measures during the study period, participation in any other clinical studies during last 30 days, simultaneous participation in another clinical study.

Twenty four subjects were randomly allocated into two arms of equal numbers of subjects (12 subjects in each group) using a random number table to receive either LMWGAL-TF or placebo. Subjects were allocated with a unique randomization number at the randomization visit. The study flowchart is presented in Figure 1.

The supplementation

The investigational product, LMWGAL-TF, was supplied by Indus Biotech Private Limited (Pune, India). LMWGAL-TF is being marketed as a dietary ingredient as Torabolic®. The capsules containing 500 mg of LMWGAL-TF and matching placebo (Dicalcium Phosphate) treatments were prepared, packaged, and labeled in generic bottles for double-blind administration. Each subject ingested one capsule from the allocated bottle once a day for 8 weeks of duration.

Outcome measures

Outcome measures were recorded at baseline, at 4-weeks, and 8-weeks (end of treatment). The primary outcome measure of the study was a change in percent fat mass and absolute fat mass (kg) from baseline and after 4 and 8 weeks. The change was calculated based on skinfold thickness and bioelectrical impedance analysis (BIA).

The skinfold thickness was measured with Lange skinfold Calliper (Beta Technology, Santa Cruz, CA) (Jackson *et al*., 1980). The skin was pinched for the fold measurements. The skinfold measurement was carried out at three sites, namely, abdominal, suprailiac, and tricep on the body of subjects. The percent body fat mass, absolute body fat mass, and absolute non-fat mass was calculated by the following equations (Jackson *et al*., 1980; Siri, 1961):

- The percent Body Fat Mass = [495/Body Density] – 450 where,

- Body Density = [1.089733] – [0.0009245 × sum of skin folds] + [0.0000025 × square of sum of skin folds] − [0.0000979 × age]

Secondary outcomes included a change in absolute non-fat mass (kg) from baseline and after 4 and 8 weeks. The change was calculated based on skinfold thickness and BIA. The BIA was carried out using body composition monitor, Tanita BC-545 (Tanita India Private Limited, Mumbai, India). Instructions regarding hydration were given 24 hours before assessment. The subjects were called for a visit in the morning, after 8–10 hours of fasting and without exercise on the previous day. Percent Body fat was measured by direct readings from BIA. The absolute fat mass and non-fat mass were derived from the calculations as follows:

- Absolute Body Fat Mass = % Body Fat Mass × Body weight

- Absolute Non- Fat Mass = Body weight − Fat mass

Other secondary outcomes changed in body weight (kg), body mass index (BMI) (kg/m^2), abdominal girth (cm), and absolute skinfold thickness (triceps, suprailiac, and abdominal) (mm) from baseline and after 4 and 8 weeks. The BMI was calculated as follows: BMI = weight in kg / (height in m)^2. The abdominal girth was recorded at the time of the BIA assessment. Each subject was allowed to stand upright with the shoulder-width distance between his feet. A non-flexible measuring tape was placed at the level of the iliac crest on both sides, keeping it parallel with the floor. The subjects were asked to inhale once and then exhale. The measurement was taken with the exhaled state with an abdomen not held tight.

Safety analysis

The vital signs, namely BP (systolic and diastolic) (mmHg), heart rate (beats per minutes), respiratory rate (breaths per minute), and body temperature (°C) were measured at the baseline, 4-week,, and 8-weeks (at the end of study visit).

Haematological investigations including haemoglobin (g/dl), red blood cells (million/mm^3), platelets (×10^4 /mm^3), erythrocyte sedimentation rate (mm/h), total white blood cells (×10^2 /mm^3), and differential white blood cells (eosinophils, neutrophils, lymphocytes, monocytes, and basophils) were measured. Liver function tests were comprised of aspartate transaminase (AST) (IU/L), alanine transaminase (ALT) (IU/L), alkaline phosphatase (ALP) (IU/L), bilirubin (mg/dl), protein (g/dl), creatinine (mg/dl), blood urea nitrogen (BUN) (mmol/l), fasting blood sugar level (BSL) (mg/dl). The laboratory estimations were performed at baseline, 4 weeks, and 8 weeks. Adverse events were monitored for the date of occurrence, severity, relationship with treatments and action taken throughout the study. Medication compliance was monitored during subject visits at the end of 4 weeks and 8-weeks of treatment. Subjects were encouraged not to skip scheduled medication intake, or alter dosages on their own. Investigator judged the treatment compliance by interviews during the study period and by measuring unused medication on visits. The measurement of compliance was done as per formula: % Compliance = (Number of capsules consumed/Number of capsules advised) × 100. Less than 70% consumption of the total prescribed medication by the subject was considered as noncompliant.

Statistical analysis

The parametric data were expressed as the mean ± standard deviation (SD). The statistical analysis was done on the per-protocol (PP) population (anthropomorphic and efficacy parameters) and modified intent to treat (mITT) population (safety parameters). Normality testing was done using a Kolmogorov-Smirnov test for parametric data. Demographic and baseline characteristics (Age, Weight, Height, BMI, heart rate, respiratory

rate, body temperature, systolic BP, skinfold thickness measurement of abdominal/suprailiac/triceps/% fat mass and compliance) were analyzed by unpair '*t*' test (between the group comparison). Each parameter of anthropomorphic and efficacy parameters (BIA and skinfold thickness method) was analyzed for within the group comparison for repeated measure analysis of variance (ANOVA) and between the group comparison by unpaired '*t*' test. The safety parameters were analyzed for between the group comparison by unpaired '*t*' test. The analysis was performed using SPSS for Windows (SPSS Inc., Chicago).

RESULTS

Demographics and baseline characteristics

Twenty-four subjects were enrolled and randomized between the LMWGAL-TF group (n = 12) and the placebo group (n = 12). All the subjects were found female. As shown in Table 1, the population was found homogenous (no significant difference between the groups) with respect to demographic and baseline parameters, such as age, height, weight, BP (systolic and diastolic), heart rate, respiratory rate, and body temperature. Out of 24 patients, 23 patients (12 in LMWGAL-TF group and 11 from the placebo group) completed the study and were included for analysis for the efficacy measure PP population. None of the subjects from the LMWGAL-TF group and one subject from the placebo group was dropped out of the study. The compliance to study medication was excellent and did not significantly different in LMWGAL-TF and placebo groups.

Effect of treatments on anthropomorphic parameters

The data of anthropomorphic parameters are presented in Table 2. The anthropomorphic parameters values of body weight, BMI, abdominal girth, heart rate, respiratory rate, body temperature, and BP did not have a significant difference within the group (Week 4 or Week 8 v/s baseline) or between the groups (LMWGAL-TF v/s placebo).

Table 1. Demographic and baseline characteristics of subjects.

Parameters	Placebo (n = 11)	LMWGAL-TF (n = 12)
Gender (Male: Female)	0:12	0:12
Age (years)	24.27 ± 5.12	27.42 ± 5.38
Weight (kg)	75.37 ± 11.02	73.58 ± 8.27
Height (cms)	155.00 ± 4.53	157.67 ± 5.02
BMI (kg/m^2)	30.47 ± 5.26	28.02 ± 3.83
Heart rate (bpm)	79.27 ± 5.00	76.83 ± 6.35
Respiratory rate (breaths per minute)	18.45 ± 1.57	17.92 ± 1.78
Body temperature (°C)	36.82 ± 0.41	36.96 ± 0.45
Systolic BP (mmHg)	116.55 ± 11.10	111.17 ± 7.41
Diastolic BP (mmHg)	77.45 ± 5.45	76.83 ± 5.62
Skinfold thickness—abdominal (mm)	43.09 ± 6.04	39.00 ± 6.18
Skinfold thickness—suprailiac (mm)	35.73 ± 7.60	32.75 ± 5.19
Skinfold thickness—triceps (mm)	36.64 ± 6.07	34.58 ± 5.81
Skinfold thickness—Fat mass (%)	37.87 ± 2.57	36.43 ± 3.07
Percent IP compliance	97.04 ± 3.34	95.55 ± 5.16

Data are reported as mean ± SD. Note: Per-protocol (PP) population. Data of each parameter was analysed by separate unpaired '*t*' test (Between the group). No significant difference between the groups. BMI: Body Mass Index, IP: Investigational product.

Effect of treatments on efficacy parameters - skinfold thickness method

The data obtained from skinfold thickness measurement is presented in Table 3. The significant reduction was found in the LMWGAL-TF supplementation group for abdominal, and suprailiac skinfold thickness and % body fat mass at 8-weeks as compared to corresponding baseline values (within the group). The abdominal skinfold thickness at week-4 in LMWGAL-TF supplemented group showed significant reduction as compared to corresponding placebo group values (between the group). All other values between the groups or within the groups were found not-significant.

Effect of treatments on efficacy parameters - BIA

The data obtained from BIA is presented as Table 4. All values between the groups or within the groups were not found significant.

Effect of treatments on safety parameters – biochemistry and haematology

The data on safety parameters obtained from biochemistry and hematology are presented in Tables 5 and 6, respectively. There was no significant difference observed in any of the haematology or biochemical safety parameters at week-4 as well as week-8 with an exception to creatinine levels which were significantly lowered in LMWGAL-TF ($p < 0.01$) and placebo group ($p < 0.05$) at week-8 as compared with baseline values (between the group) However, these values were within normal physiological limits and were not of clinical significance. Nineteen adverse events were reported in the LMWGAL-TF group, whereas the placebo group showed 20 adverse events. All reported adverse events were mild and temporary in nature and unrelated to treatment.

DISCUSSION

Obesity causes a cluster of non-communicable diseases and creating an enormous socioeconomic and public health burden (Meharda *et al*., 2017). Historically, dietary fibers have been reported to reduce body fat mass and prevent the body weight gain in clinical studies (Bano *et al*., 2015; Tucker and Thomas, 2009). LMWGAL-TF supplementation has been shown to decrease % body fat in resistance-trained males after 8 weeks of treatment (Poole *et al*., 2010). In the present study, evaluation of 8-weeks of LMWGAL-TF supplementation for body fat mass reduction in obese non-exercising subjects was evaluated in a double-blind placebo-controlled clinical study.

The inclusion criteria was based on percent fat mass. Percent body fat mass in Indians is higher than that in Western populations for a given age and BMI (Marwaha *et al*., 2014). Percent total body fat mass > 25% in males and 30% in females is being suggested cut off for the determination of overweight in India population (Marwaha *et al*., 2014) and followed in the present study. Skinfold thickness measurement is a good predictor of body fat percentage although it is considered as an indirect measure of the total body fat mass (Sarria *et al*., 1998).

Obesity negatively impacts the health of women. Obesity in women is associated with changes in the reproductive cycle with a reduction in fertility, as well as an increased risk of polycystic ovarian syndrome (PCOS) and infrequent or no ovulation (Dennett and Simon, 2015). Overweight women with

Table 2. Effect of treatments on anthropomorphic parameters.

Parameters	Placebo (n = 11)			LMWGAL-TF (n = 12)		
	Baseline	4 weeks	8 weeks	Baseline	4 weeks	8 weeks
Weight (kg)	75.35 ± 11.07	73.77 ± 11.04	73.08 ± 11.91	73.65 ± 8.32	71.04 ± 8.41	69.55 ± 8.93
BMI (kg/m^2)	31.39 ± 4.69	30.84 ± 4.88	30.47 ± 5.26	29.66 ± 3.65	28.62 ± 3.72	27.92 ± 4.01
Abdominal girth (cms)	101.09 ± 11.55	97.73 ± 10.49	94.55 ± 10.61	95.24 ± 9.60	91.50 ± 9.94	88.33 ± 10.22
Heart rate (bpm)	79.82 ± 4.51	74.18 ± 4.85	75.82 ± 4.05	77.17 ± 5.42	76.50 ± 6.83	76.50 ± 5.20
Respiratory rate (breaths per minute)	18.09 ± 1.51	18.82 ± 1.40	18.73 ± 0.65	18.25 ± 1.36	18.75 ± 1.55	19.00 ± 2.45
Body temperature (°C)	37.00 ± 0.00	37.09 ± 0.30	37.00 ± 0.00	36.92 ± 0.29	37.08 ± 0.29	37.00 ± 0.00
Systolic BP (mmHg)	116.36 ± 8.80	106.36 ± 8.09	108.00 ± 6.00	113.50 ± 5.40	110.00 ± 11.28	113.33 ± 7.79
Diastolic BP (mmHg)	77.27 ± 5.39	75.27 ± 5.08	76.55 ± 4.57	77.33 ± 4.77	75.17 ± 6.69	75.50 ± 6.22

Data was represented as Mean ± Standard deviation (SD). Note: Per protocol (PP) population, BMI: Body Mass Index.

Table 3. Effect of treatments on efficacy parameters - Skinfold thickness method.

Skinfold thickness Parameters	Placebo (n = 11)			LMWGAL-TF (n = 12)		
	Baseline	4 weeks	8 weeks	Baseline	4 weeks	8 weeks
Abdominal (mm)	42.91 ± 6.04	39.91 ± 5.43	35.18 ± 6.74*	38.83 ± 6.18	34.67 ± 5.82$^{\#}$	31.00 ± 5.61**
Suprailiac (mm)	36.00 ± 7.52	33.73 ± 6.44	31.09 ± 6.12	32.67 ± 5.31	29.92 ± 4.72	27.00 ± 4.82*
Triceps (mm)	36.45 ± 6.06	34.27 ± 6.29	31.00 ± 6.63	34.67 ± 5.79	32.83 ± 5.73	29.83 ± 6.21
Absolute fat mass (kg)	28.74 ± 5.91	27.23 ± 5.91	25.50 ± 6.56	26.96 ± 4.85	25.18 ± 4.71	22.94 ± 5.03
Absolute non-fat mass (kg)	46.66 ± 5.43	46.55 ± 5.52	47.58 ± 5.74	46.63 ± 4.06	45.87 ± 4.04	46.61 ± 4.52
Percent fat mass	37.87 ± 2.57	36.62 ± 2.70	34.48 ± 3.45	36.39 ± 3.08	34.86 ± 3.13	32.68 ± 3.42*

Data was represented as Mean ± Standard deviation (SD). Data was evaluated by repeated measures ANOVA (between the group) and unpaired *T*-test (within the group). Note: Per protocol (PP) population, *$p < 0.05$, **$p < 0.01$ v/s baseline, #$p < 0.05$ v/s Placebo group.

Table 4. Effect of treatments on efficacy parameters—BIA.

BIA Parameters	Placebo (n = 11)			LMWGAL-TF (n = 12)		
	Baseline	4 weeks	8 weeks	Baseline	4 weeks	8 weeks
Absolute fat mass (kg)	30.98 ± 7.62	29.69 ± 8.08	28.45 ± 9.24	29.10 ± 6.07	27.69 ± 5.80	26.09 ± 6.42
Absolute non-fat mass (kg)	43.63 ± 3.99	43.87 ± 3.42	44.06 ± 3.27	43.91 ± 2.86	43.20 ± 3.31	42.73 ± 3.34
Percent fat mass	41.01 ± 4.48	39.73 ± 5.43	38.35 ± 6.68	39.57 ± 4.26	38.71 ± 4.13	37.33 ± 5.02

Data was represented as Mean ± Standard deviation (SD). Data was evaluated by repeated measures ANOVA (between the group) and unpaired *T*-test (within the group). Note: Per protocol (PP) population.

Table 5. Effect of treatments on safety parameters—biochemistry.

Parameters	Placebo (n = 11)			LMWGAL-TF (n = 12)		
	Baseline	4 weeks	8 weeks	Baseline	4 weeks	8 weeks
BSLs (mg/dl)	82.19 ± 12.16	72.11 ± 6.15	73.57 ± 9.64	78.55 ± 5.78	74.21 ± 5.48	72.29 ± 4.82
ALP (IU/L)	164.65 ± 23.52	176.78 ± 24.90	164.93 ± 23.12	148.47 ± 36.40	178.95 ± 27.24	179.53 ± 31.05
ALT (IU/L)	18.00 ± 4.54	19.64 ± 7.72	18.10 ± 6.77	19.18 ± 10.71	22.17 ± 12.16	20.91 ± 10.59
AST (IU/L)	25.83 ± 4.19	22.56 ± 5.40	25.29 ± 4.25	24.61 ± 9.69	23.70 ± 8.42	28.62 ± 10.09
Total Bilirubin (mg/dl)	0.81 ± 0.09	0.82 ± 0.07	0.82 ± 0.07	0.86 ± 0.09	0.83 ± 0.09	0.86 ± 0.06
BUN (mmol/l)	11.20 ± 1.11	10.38 ± 0.96	10.52 ± 1.64	11.17 ± 1.91	11.18 ± 1.98	10.46 ± 1.80
Creatinine (mg/dl)	0.90 ± 0.10	0.83 ± 0.22	0.74 ± 0.09*	0.90 ± 0.08	0.81 ± 0.17	0.71 ± 0.08**
Protein (g/dl)	6.10 ± 0.51	6.02 ± 0.63	6.10 ± 0.51	6.08 ± 0.42	6.11 ± 0.59	5.87 ± 0.48

Data was represented as Mean ± Standard deviation (SD Data was analysed for between the group by unpaired *T*-test, Modified intent to treat (mITT) population. *$p < 0.05$, **$p < 0.01$ V/s baseline.8

PCOS tend to develop insulin resistance and diabetes, particularly in later life (Sam, 2007). Furthermore, the tendency toward menstrual and ovarian disturbances associated with obesity may predispose to an increased risk of ovarian, breast and endometrial cancer (Bhaskaran *et al*., 2014). Obesity has been correlated to the development and progression of low back pain and knee osteoarthritis in women (Kulie *et al*., 2011). In this study, all the recruited subjects were found to be females.

The percent body fat mass was measured for all the subjects using both methods, namely, skinfold thickness, and BIA

Table 6. Effect of treatments on safety parameters—haematology.

Parameters	Placebo (n = 11)			LMWGAL-TF (n = 12)		
	Baseline	4 weeks	8 weeks	Baseline	4 weeks	8 weeks
Hemoglobin (gm/dl)	11.80 ± 0.77	11.91 ± 0.52	11.66 ± 1.06	12.20 ± 0.70	12.27 ± 0.98	12.30 ± 1.17
ESR (mm/hr)	20.91 ± 12.65	17.09 ± 9.42	20.60 ± 11.42	19.42 ± 11.88	20.67 ± 10.33	18.00 ± 9.67
RBC (million/mm^3)	4.67 ± 0.39	4.69 ± 0.28	4.64 ± 0.33	4.74 ± 0.69	4.74 ± 0.58	4.82 ± 0.63
WBC (/mm^3)	8,463.64 ± 1,876.31	7,818.18 ± 1,674.41	7,900.00 ± 2,201.01	8,141.67 ± 1,722.82	7,883.33 ± 2,308.42	7,609.09 ± 1,698.50
Eosinophils (%)	3.18 ± 0.41	3.18 ± 0.41	3.20 ± 0.42	3.25 ± 0.45	3.58 ± 0.52	3.18 ± 0.60
Basophils (%)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Neutrophils (%)	65.91 ± 6.98	65.36 ± 5.43	65.60 ± 5.15	68.33 ± 4.25	67.50 ± 5.14	67.73 ± 4.56
Lymphocytes (%)	25.18 ± 6.94	25.64 ± 5.54	25.30 ± 5.08	22.92 ± 3.78	23.75 ± 4.56	23.45 ± 4.01
Monocytes (%)	5.73 ± 0.65	5.82 ± 0.75	5.90 ± 0.74	5.50 ± 0.80	5.17 ± 0.84	5.64 ± 0.92
Platelets (/mm^3)	305,818.18 ± 82,072.92	289,545.45 ± 80,415.63	272,600.00 ± 96,638.16	287,416.67 ± 66,156.09	265,000.00 ± 61,495.01	253,727.27 ± 75,081.41

Data was represented as Mean ± Standard deviation (SD). Data was analysed for between the group by unpaired *T*-test. Modified intent to treat (mITT) population. ESR - Erythrocyte Sedimentation Rate, RBC – Red blood cells, WBC -White blood cells

Table 7. Summary of adverse events recorded.

Adverse event	Total	LMWGAL-TF	Placebo	Subject Discontinued (Yes/No)	Treatment related?
Increased scalp hair density	4	2	2	No	Not related
Loose motion	3	2	1	No	Not related
Constipation	1	0	1	No	Not related
Fever	6	1	5	No	Not related
Rhinorrhoea	5	4	1	No	Not related
Heartburn	3	2	1	No	Not related
Darkness around eyes	1	0	1	No	Not related
Vomiting	4	3	1	No	Not related
Bodyache	2	1	1	No	Not related
Common cold	1	0	1	No	Not related
Headache	2	2	0	No	Not related
Hot sensation	1	1	0	No	Not related
Giddiness	1	1	0	No	Not related
Skin rash	2	0	2	No	Not related
Cough	1	0	1	No	Not related
Stomatitis	1	0	1	No	Not related
Leg pain	1	0	1	No	Not related

method. The recorded data showed that all recruited subjects had baseline values of percent body fat > 35% in skinfold thickness data. A systematic review and meta-analysis screened articles reported the relationship between obesity and percent body fat as a diagnostic criterion with assessment methods as a gold standard (Okorodudu *et al*., 2010). BIA is a non-invasive and commonly used method for body composition measurements, especially for fat mass and fat-free mass (FFM). (Khalil *et al*., 2014). The percent body fat (> 30% (Frankenfield *et al*., 2001) or > 35% (Ko *et al*., 2001; Romero-Corral *et al*., 2008)) using the BIA method is referred as reliable diagnostic assessment criteria for obesity. LMWGAL-TF-treated group showed significant ($p < 0.05$) reduction in percent body fat mass after 8 weeks of treatment from baseline in obese subjects. The results of the present study are in agreement with the earlier reports of a significant reduction in percent body fat by LMWGAL-TF in resistance-trained males with 8 weeks of treatment (Poole *et al*., 2010).

Measurement of subcutaneous fat thickness using a skinfold caliper is a simple and inexpensive technique for body composition assessment (Eston *et al*., 2005; Garcia *et al*., 2005). Although measurement error in skinfold thickness tends to become greater with increasing obesity level, the influence of an increase in subcutaneous fat thickness on the measurement error was smaller at the abdominal and suprailiac skinfolds as compared with other sites (Demura and Sato, 2007). In this study, LMWGAL-TF showed a significant reduction at suprailiac site ($p < 0.05$) and at the abdominal site ($p < 0.01$) after 8 weeks as compared with baseline values (skinfold thickness method).

LMWGAL-TF showed a trend for a decrease in absolute fat mass or non-fat mass as compared with baseline during skinfold or BIA analysis. The increased awareness during initial counseling about food and exercise in all subjects might result in the observed trend of decrease of absolute fat or non-fat mass (regardless of treatment). However, the reduction in fat mass or

not-fat mass was not found significant either between or within the treatment groups. The target of weight loss in obese subjects is a loss of fat mass (Chaston *et al*., 2007). Loss of FFM may be undesirable if excessive as non-adipose tissues are responsible for the majority of resting metabolic rate, regulation of core body temperature, preservation of skeletal integrity, and maintenance of function and quality of life as the body ages (Marks and Rippe, 1996). Hence, LMWGAL-TF supplementation does not have the loss of FFM for obese subjects.

Taken together, the results of a present study on fat mass in subjects are in support to past clinical research reports of lipid-lowering and fat mass reducing properties by the fenugreek seeds (a source of raw material) and active bioactive component (a soluble fibre content or low molecular weight galactomannans) (Mukthamba and Srinivasan, 2015; Venkatesan *et al*., 2003; 2007). The animal studies also reported having antihyperlipidemic properties (Kassaian *et al*., 2009; Kumar *et al*., 2014) and inhibition of fat accumulation in high-fat diet-induced obese rats (Kumar *et al*., 2014) and mice (Knott *et al*., 2017).

In the present study, LMWGAL-TF was found to be well-tolerated and safe during 8 weeks of treatment, as evident from the results of laboratory investigations. (Table 5 and Table 6) All the subjects that are recruited in the study were female. Therefore, the present results need to be viewed with the limitation of gender, i.e., females with high fat mass. In addition, the results of the present study support the excellent safety profile of LMWGAL-TF as reported in previous reports in rats (Deshpande *et al*., 2016a; 2016b) and clinical studies in human subjects (Poole *et al*., 2010). The reported adverse events (19 in LMWGAL-TF and 20 in Placebo group) are listed in Table 7. No serious adverse events were reported in the study. All events were temporary and mild in nature. Furthermore, none of the subjects discontinued the study due to adverse events, and therefore, confirmed to be unrelated to the treatment and clinically non-significant.

CONCLUSION

LMWGAL-TF supplementation showed a significant reduction in abdominal skinfold thickness as compared with placebo during the double-blind placebo-controlled study in female subjects with a high-fat mass within 8 weeks. Eight weeks of LMWGAL-TF but not placebo supplementation showed a significant reduction in % fat mass and skinfold thickness (suprailiac) as compared to baseline values in female subjects with high-fat mass. The LMWGAL-TF was well tolerated and safe.

CONFLICT OF INTEREST

The two authors (PD and PT) are emplyee of Sponsor (Indus Biotech Private Limited, Pune) have declared that no competing interests exist.

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