ABSTRACT
An experimental study to evaluate the molecular mechanisms by which *Momordica charantia* (Bitter gourd) alters lipid levels and imparts anti-diabetic effects

BACKGROUND
Obesity is contributing to substantial morbidity and mortality due to increased adipogenesis and down regulation of lipolysis resulting in insulin resistance in the peripheral organs. Bitter melon (*Momordica charantia*, MC) is effective in reducing weight gain due to reduced adipose hypertrophy, inhibition of lipogenic genes and increased plasma catecholamine through enhancement of energy utilization and oxidative phosphorylation finally improving the sensitivity of insulin.

OBJECTIVES
To determine the total phenolic content (TPC) and anti-inflammatory activity (AIA) in different percentage of ethanolic extract of *Momordica Charantia* (EEMC) and determine the cytotoxic concentrations of EEMC during 3T3-L1 preadipocyte differentiation. Also, to study the effect of varying concentrations of 50% EEMC, on adipogenesis and adipolysis, during and after differentiation of 3T3-L1 preadipocyte cell lines. To study of expression level of PPARγ and SREBP1 (proteins regulating the accumulation of lipid-droplets) in pre-adipocytes cell line treated with different concentration of ethanolic extracts of EEMC and also to study the lipid lowering effect of MC extract in animal models

MATERIALS AND METHODS
3T3-L1 preadipocytes were procured from National Center for Cell Sciences, Pune, and cultured in DMEM supplemented with 20% FBS and 1mM L-glutamine. EEMC was prepared by graded ethanol fractionation method and the TPC determined using Folin-Ciocalteau assay and the AIA was determined by Human Red Blood Cell Membrane Stabilisation assay. The cytotoxicity dose was determined by Sulforhodamine-B assay. The adipogenesis and adipolysis assay (Cayman chemicals, Ann Arbor, USA) were performed as per manufacturers standard operating procedures. Protein and mRNA levels of PPARγ and SREBP1 in the cell lysate was analysed by western blot and real time quantitative PCR. Diabetes was induced in albino rats of Wister strain by intraperitoneal injection of streptozotocin. Glucose levels were estimated on day 0, 7, 14 21 and 28 days to assess the efficacy of bitter melon juice (BMJ) and pioglitazone. mRNA levels of PPARγ and SREBP1 in the dissected adipose tissue was analysed by western blot and real time quantitative PCR.

RESULTS
The TPC (p=0.0001) and AIA (p=0.01) of EEMC decreased with increasing ethanol concentration from 50% to 100% .The preadipocytes treated with varying concentrations of EEMC during (p=0.012) and after (p=0.026) differentiation demonstrated significant reduction in lipid content. Also there was significant increase in glycerol release with increase in concentration of EEMC both during (p=0.018) and after (p=0.0007) differentiation. However, effect of EEMC on adipogenesis and adipolysis was more during differentiation when compared to after differentiation of 3T3-L1 preadipocytes. The expression of PPARγ and SREBP1 decreased in the preadipocytes treated with EEMC. The decrease in expression was more during differentiation when compared to after differentiation of the preadipocytes. BMJ significantly reduced blood glucose levels in rats when compared to diabetic controls (p<0.001). Total cholesterol and triglycerides and also the expression of PPARγ and SREBP1 significantly reduced in the BMJ treated rats.

CONCLUSION
The data showed that the 50% EEMC contain high percentage of TPC and AIA and also potent inhibitor of lipogenesis and stimulator of lipolysis activity in 3T3-L1 preadipocytes. It decreases the accumulation of lipid droplets by decreasing the expression of PPARγ and SREBP1. BMJ also has hypoglycaemic and lipid lowering effect in diabetic animal models. Further studies will be done to know the exact mechanism of action of EEMC on adipogenesis and adipolysis.

KEYWORDS
Diabetes mellitus, *Momordica charantia*, 3T3-L1 preadipocytes cell lines, PPARγ and SREBP1.