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# Rutin, a bioactive compound derived from plant affect lipid synthesis genes in yeast

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**Abstract**
Rutin a flavonoid and natural compound found in certain plants, fruits, vegetables, buckwheat, Japanese pagoda tree, and Eucalyptus. Rutin is known to have biological activity antioxidant, nephroprotective, anti-inflammatory, neuroprotective, anti-diabetes and hepatoprotective. Atorvastatin, a commercial drug is used for treatment of hyperlipidemia which leads to some side effect in human like myopathy, weakness, myalgia, headache, dyspepsia, arthralgia and liver injury. In the present study we have evaluated rutin in reducing the hyperlipidemia genes TAG (LRO1 and DGA1) and SE (ARE1 and ARE2). These results suggest, the rutin can act as anti-hyperlipidemia effect in *Saccharomyces cerevisiae*.

**Keywords:** bioactive compound, *Saccharomyces cerevisiae*, hyperlipidemia

## Introduction
Rutin is bioflavonoid (vitamin p) abundant in fruits, vegetables. Rutin possess antioxidant, anti-inflammation, anti-angiogenic and anti-tumor properties [1, 2]. JOEN-RONG SHEU *et al* 2004 reported rutin has antiplatelet activity [3]. Rutin was reported to have *in vivo* anticancer activity by the decrease in tumor volume, CEA level (Carcinoembryonic antigen), cholesterol content, FAS, [4]. Rutin protect copper-induced brain damage via antioxidative and anti-inflammatory mechanisms [5]. *In vivo* antioxidant and anti-inflammatory activity by rutin was reported in *Hypericum capitatum* [6]. Rutin showed anti-cancer effects on 786-O human cancer cells at 50 µM [7].
Hyperlipidemia caused by smoking, obesity, sedentary lifestyle [8]. Hyperlipidemia is identified by increasing levels of lipids and it can be caused by a variety of genetic disorders. Hyperlipidemia is important risk factor by developing cardiovascular disease. Cardiovascular disease (CVD) affects many people that lead to mortality s and 38% of adults affected by CVD and it is likely to raise serum lipid levels, high blood pressure and diabetes [9]. Hyperlipidemia and hypercholesterolemia can be reduced by using commercialy available drug atorvastatin and ezetimibe which lowers lipid level [10]. Atorvastatin reduce the fatty substance but it can induce of side effect for breakdown of skeletal muscle tissue in type 2 diabetes mellitus [11]. Statin can cause myopathy and weakness, myalgia, headache, dyspepsia, arthralgia and liver injury [12, 13].
Yeast genome is genetically similar to human genomic system [14]. The yeast has lipid particles in organelle consists of triacylglycerols (TAGs) and sterol esters with surface membrane consist of phospholipids and proteins [15]. Triacylglycerols (TAGs) is storage form of energy and it hydrolysis fatty acids; this fatty acid required to membrane biosynthesis in eukaryotic cells. Most prominent enzyme in TAG biosynthesis are LRO1 and DGA1.This biosynthetic pathway is acyl-coenzyme A (CoA): diacylglycerol acyltransferase [16, 17]. In yeast, sterol ester is storage form of energy by help of enzymes AREl and ARE2, which is encoded by acyl-coenzyme A (CoA): cholesterol acyl transferase (ACAT). It is homologous yeast genes to human gene (isoforrn) [18]. The present study evaluated the anti-hyperlipidemic property of rutin in *Saccharomyces cerevisiae*.

## Materials and Methods
### Materials
Rutin (R5143-50G), Nile red, kanamycin and thin layer silica plates were purchased from sigma. Yeast extract, peptone, bacterial agar and solvents, and other chemicals were purchased from Himedia (Bengalore, India) unless specifically mentioned. Trizol (RNA isolation reagent), PCR Master Mix, cDNA synthesis kit, PCR Master Mix, and ethidium bromide were purchased from Invitrogen (Bangalore, India).

### Growth condition and treatment
Wild type BY4741 (BY4741 MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0) was grown in aeration at 30°C in YEPD medium (1% yeast extract, 2% peptone and 2% glucose) up to mid-log phase. The cells were harvested at 0.1 OD (A 600) and regrown in SC (synthetic complete) medium containing 0.67% yeast nitrogenous base, supplemented with the amino acids and 2% glucose. And then add either Atorvastatin 5mM or rutin (0.125 mM, 0.25 mM) with or without 0.5 mM oleic acid for 24 h.

### RNA isolation and qRT-PCR analysis
The cells grown in YEPD medium up to mid log phase was harvested and treated with rutin, atorvastatin and oleic acid with 15 ml SC-D media and grown for 24 hours with aeration at 30°C. Total RNA from cultured cells will be isolated according to the manufacturer's instruction (Trizol, one step RNA isolation kit, Medox Biotech Pvt Ltd.). Briefly, cells were homogenized in 1 ml of one step RNA reagent. The homogenate was centrifuged for 10 min at 12,000 x g and the resulting supernatant was transferred into a 1.5 ml new micro centrifuge tube followed by the addition of 500 µl of chloroform to the lysate and shaken well for 15-

25 sec and incubated at 15-30°C for 3-5 min and then centrifuged for 15 min at 12,000 x g. The aqueous layer transferred to 1.5 ml micro centrifuge tube and 500 µl of ice cold isopropanol added. The tubes were kept for precipitation of RNA at -20°C for 1-2 h and centrifuged for 10-15 min at 12,000 x g. Then, the RNA pellet was washed twice by adding 1 ml of 75% ethanol and centrifuged at 7,500 x g for 5-10 min. The pellet was allowed to air dry, and dissolved in 50-100 µl RNase free water. The RNA was stored at -80 °C. The purity was measured by the absorbance at 260 nm and 280 nm (Absorbance ratio of 260/280 ranges from 1.6-1.8 will be taken for further reaction). Complementary DNA (cDNA) was synthesized from the total RNA. Primer sequences used in this study listed in table 1. For qRT analysis, 1 µl of diluted cDNA (1:20) sample was amplified using Applied Biosystems machine Step One Plus ™ Real- Time PCR machine with the power SYBR Green PCR master mix (Applied Bio system). Samples were evaluated $^{\Delta\Delta}$Ct method (Livak and Schmittgen, 2001). The samples were analyzed in triplicate, and the results were analyzed using relative quantification. The qRT-PCR data are presented as the mean and were analyzed using Student's t-test. The relative mRNA expression was determined by qRT-PCR analysis which measured in triplicate. Gene expressions were normalized using ACT1 as the endogenous control. The mRNA expression data are denoted as the fold- change with the respective control.

**LD Staining (Neutral lipid confirmed by using Nile red staining)**

Lipid droplet number and size was examined by growing the cells on selection media up to stationary phase, and 1 ml of the yeast cells were harvested and resuspended in 2% para formaldehyde for fixation. The cells were washed with distilled water: Nile Red ≈552/636 nm (0.5µg/ml) was added to the cell suspension and incubated for 15 min at room temperature. Cells were washed thrice with PBS, and resuspended in 50 µl of PBS and observed under laser scanning confocal microscope (Zeiss LSM710, with 100 x oil objectives).

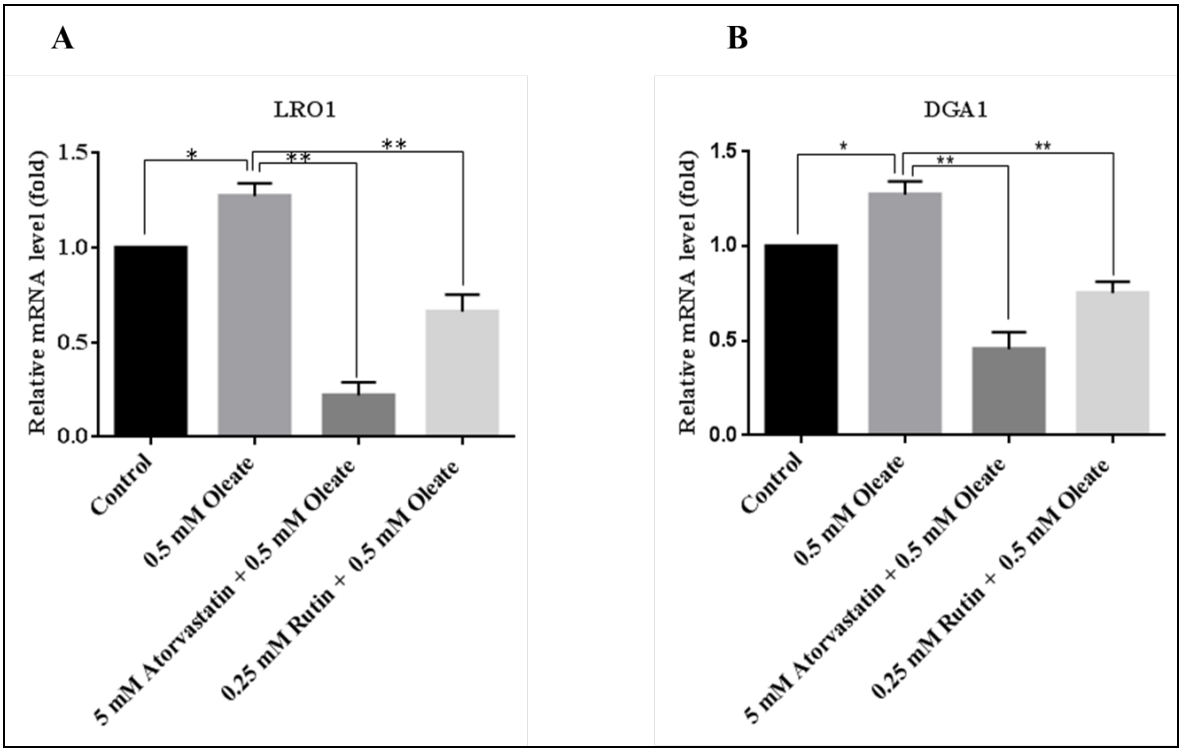
**Statistical analysis**

Experimental Quantitative data were analyzed using Student's t-test, and the difference were considered statistically significant when *p<0.05 and **p<0.01. Each experiment was repeated at least thrice independently. Data are presented as the average ± standard deviation (SD). Statistical significance among groups were analyzed by using two-way ANNOVA.

**Results and Discussion**

**Expression of neutral lipid genes in *Saccharomyces cerevisiae* treated with rutin**

The lecithin cholesterol acyltransferase (*LRO1*) and diacylglycerolacyltransferase (*DGA1*) are the essential genes which are responsible for triacylglycerol synthesis. The *LRO1* gene was ~ 1.3 fold up-regulated in oleic acid induction condition. Treatment with Atorvastatin and rutin reduced the LRO1 expression compared to oleic acid induction (Fig.1A). The *DGA1* gene expression was elevated ~ 1.2 fold in oleic acid induction conditionand in the Atorvastatin and rutin treatment the DGA1 gene expression level was reduced significantly when compared to oleic acid treatment (Fig. 1B).The expression of sterol acyltransferases synthesizing gene (*ARE1* and *ARE2)* levels were up-regulated in oleate treatment. The Atorvastatin treatment partially restored the *ARE1* expression compared to control. The rutin restored *ARE1* expression to normal (Fig. 1C). The *ARE2* gene was ~ 3.2 fold up-regulated on oleic acid induction, and the atorvastatin or rutin treatment with oleic acid reduced the ARE2 gene expression compared to oleic acid treatment alone (Fig. 1D).



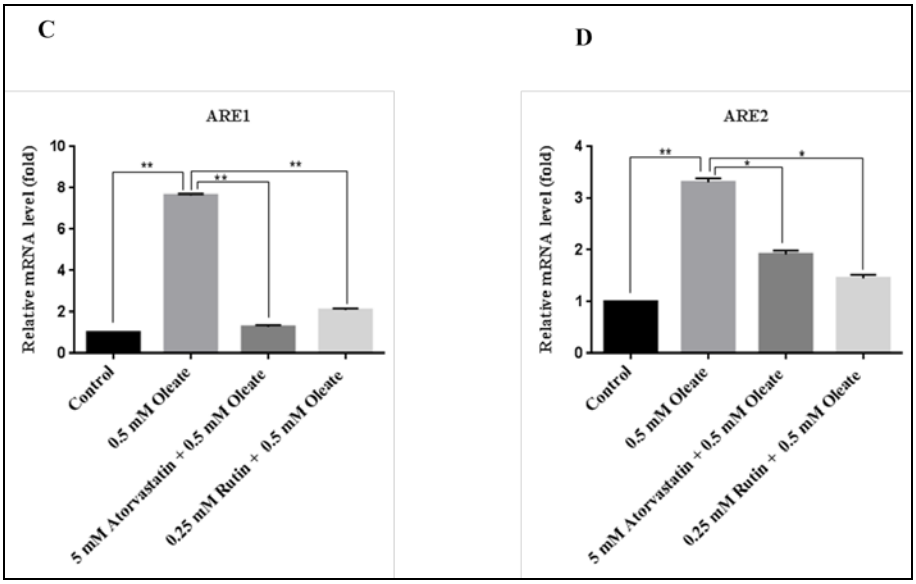



**Fig 1:** Expression of neutral lipid genes by oleate induced hyperlipidemia in *Saccharomyces cerevisiae* treated with rutin.

Equal OD ($A_{600nm}$ = 0.1) of yeast cells were treated with 0.5 mM of oleic acid and with 5 mM of atorvastatin or 0.25 mM rutin and cells were grown in the SC medium for 24 h at 30 $^0$C. Equal amount of cells were taken and total RNA was isolated. The mRNA expression of neutral lipids genes namely (a) *LRO1* (b) *DGA1* (c) *ARE1* and (d) *ARE2* were measured in wild-type control cells, atorvastatin or rutin-treated. β-actin was used as an internal control. Values are expressed as mean ± SD of three separate experiments, Control cells were compared with 0.5 mM of oleate induced cells and this group was compared with rutin treated groups and 0.25 mM of rutin is significantly reduced. Values have been statistically significant at **p<0.01, *p<0.05.

**Effect of rutin on increasing LD formation by oleic acid induced hyperlipidemic in *Saccharomyces cerevisiae*.**

Storage of triacylglycerol and sterol esters in an intracellular organelle called as the lipid droplets (LDs), Nile red is a lipophilic dye used for staining lipid droplets (LDs) and observation of yeast cells were done by using laser scanning fluorescent microscopy. Oleic acid induction increased LD numbers. However the treatment of atorvastatin and rutin to oleic acid induction significantly reduced the LD number (Fig.2).

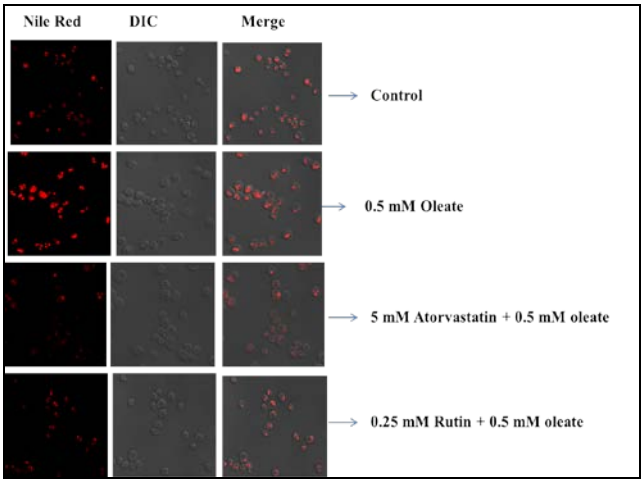



**Fig 2:** Effect of rutin on increasing LD formation by oleic acid induced hyperlipidemic in *Saccharomyces cerevisiae*.

Equal OD (0.1 OD) of yeast cells were treated with 0.5 mM of oleic acid. These cells were treated again with 5 mM atorvastatin or 0.25 mM rutin and cells were grown in the SC medium for 24 h at 30 $^0$C. Cells were collected and stained with lipophilic dye nile red Iimage were taken under scanning confocal microscope (laser scanning fluorescent microscope) (excitation/emission at 483/503 nm).

Rutin was used for the treatment of antioxidant, anti-inflammatory, anti-cancer activities [6, 8, 4]. In yeast lipid droplets formation, increasing TAG and SE synthesis is responsible by the enzymes LRO1, DGA1, ARE1 and ARE2. In mammals, increase in LDs accumulation can lead to lipid metabolic disorder, and many number diseases, they are obesity, diabetes and artherosclerosis in human and increasing lipids number in yeast is known as hyperlipidemia [19]. Here we report rutin at 0.25 mM concentration treatment significantly reduced the elevated lipid synthesizing genes such as *LRO1*, *DGA1*, *ARE1*, and *ARE2* (Fig.1). LDs are synthesized by lipid (*LRO1*, *DGA1*, *ARE1* and *ARE2*) genes. The down regulations of neutral lipid synthesizing genes are responsible for the reduction of stored TAG and SE level and lipid droplet number (Fig.2). Since the Lipid pathway in yeast model system is similar to mammalian system it can be used to develop therapeutics for dysfunction lipid metabolisms in human. Therefore, these results suggest that the decreased level of lipid droplets formation genes, leads to the prevention of hyperlipidemia and obesity.

## Conclusion

This study demonstrated that the rutin supplementation improves dyslipidemia or hyperlipidemia by reducing the neutral lipid gene expression and lipid droplets size decreasing observed by rutin, and it's comparable to standard drug Atoravastatin. Rutin lowers the lipid levels and LD numbers equal to the standard drug atorvastatin. From this result we conclude that the rutin has strong anti-hyperlipidemic natural drug.

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