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Lowering of oleic acid induced hyperlipidemia using a plant bioactive compound (Rutin) in yeast model system

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Abstract
Hyperlipidemia is an abnormal increase of fats (lipids) in the blood and it can cause cardiovascular disease, diabetes and stroke worldwide. Hyperlipidemia induced by the oleic acid, increased the level of triacylglycerol and sterol esters. Atorvastatin is commercialy available drug, used to cure hyperlipidemia which cause side effect like diarrhea, stomach upset, muscle and joint pain. Rutin a flavonoid found in some fruits and vegetables have biological activity including antioxidant, anti-inflammatory, anti-diabetes, hepatoprotective, neuroprotective and nephroprotective. Hence, we have used rutin for hyperlipidemia treatment in a model system *Saccharomyces cerevisae*. Rutin does not affect cell growth at 0.5 mm, 1 mm concentrations. These results conclude that rutin can be used to reduce hyperlipidemia in *Saccharomyces cerevisiae*.

Keywords: rutin: hyperlipidemia: yeast

Introduction
Hyperlipidemia (high triglyceride and cholesterol) is important risk for coronary heart disease (CHD) link to cardiovascular disease, inflammation, dyslipidemia, and atherosclerosis [1, 2, 3]. It is a major risk responsible for increased cardiovascular mortality and it can leads to myocardial infarction, stroke etc [4]. It is an important issue for children's health, such as genetic predisposition, familial hyperlipidemia, renal disease and diabetes. They are at higher risk problem and with childhood obesity [5]. Commercial available drugs such as rosuvastatin and atorvastatin are used for dyslipidemia patients [6]. Atorvastatin is a potential therapeutic mediator for hearing impairment through the PI3K-pAKTNrf2 pathway [7]. It cause neurological symptoms, such as tingling, pain, numbness, and tremor in the hand and feet [8]. High dose of statins (40mg and 80 mg atorvastatin) can leads to side effect like new onset of diabetes mellitus and hepatotoxicity [9]. Hyperlipidemia drugs cause many side effects to human body, hence we have focused our study on natural drug for hyperlipidemia treatment.
Rutin a common flavonoid (vitamin p) is found in fruits, vegetables and plant derived beverages. Rutin has many biological function and properties like antioxidant, cardioprotective activity, anti-inflammatory, anti-diabetes, anti-cancer, heptoprotective, neuroprotective and nephroprotective [10, 11, 12]. Rutin showed increase in the activity/concentrations of all ossification markers and proliferative activity in bone cells [13]. Rutin can enhance memory and learning performance through reducing the oxidative stress [14]. Shaoqi Qu [15] reported that rutin reduces Vancomycin - induced toxicity by inhibiting oxidative stress, apoptosis, and mitochondrial dysfunction and prevention of nephrotoxicity. Rutin can act to be an immunomodulatory molecule. Moshahidkhan *et al*., (2011) reported the neuroprotective effect of rutin against the neurological disorder such as Parkinson's disease [16].
In this study, we have used *Saccharomyces cerevisae* as a model organism since it is genetically similar to human genomic system. The metabolic pathways and cellular mechanism are similar with human metabolisms [17]. Previous work from our lab reported that hyperlipidemia was induced by oleic acid in yeast [18]. In the present study rutin has been evaluated for the reducing of hyperlipidemia in yeast cells.

Materials and methods
1. Materials
Rutin, thin layer silica plates and kanamycin were purchased from sigma. Yeast extract, bacterial agar, peptone, and solvents, and other chemicals were purchased from Himedia (Bengalore, India) unless specifically mentioned.

2. Spot test analysis
The wild stain was grown in YPD medium 30◦C up to mid-log phase. Cells were harvested, and cell density was adjusted to 1 at $A_{600.}$ The cells were serially diluted in 1:10, 1:100, 1:1000, and 1:10000 was made and 3 µl of cells were spotted onto SC-D agar plates with or without rutin supplementation (0.5 mm, 1 mm) and incubated at 30◦C for two days.

3. Growth condition, treatment and lipid extraction
Wild type BY4741 (BY4741 MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0) was grown with aeration at 30°C in YEPD medium (1% yeast extract, 2% peptone and 2% glucose) up to mid-log phase. Then from that 0.1 OD (A_{600}) of cells harvested and regrown in SC (synthetic complete) medium containing 0.67% yeast, supplemented with the amino acids and 2% glucose. And then add either Atorvastatin 5 mm or rutin (0.125 mm, 0.25 mm) with or without 0.5 mm oleic acid for 24 h. The wild type cells were grown to mid-log phase at 30°C in synthetic complete medium. The cells were pelleted, and lipids were extracted

using the Bligh and Dyer method [19]. Briefly, chloroform and methanol were added to the cell pellet in 2:1 (v/v) ratio and vortexed, and an equal volume of acidified water (2% phosphoric acid) was added again vigorously vortexed. The lipid containing organic layer was dried, and neutral lipids separated by thin layer chromatography on Silica Gel TLC plates using petroleum ether: diethyl ether: acetic acid (70: 30: 1, v/v) as the solvent system. A plate was exposed to iodine vapor to visualize the lipids from the TLC plate.

4. 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 analyzed by using two-way ANNOVA.

Results and Discussion

1. Effect of rutin on growth and cell viability of *S. cerevisiae*

The WT strain was serially diluted and spotted onto SC agar plates supplemented with or without 0.5 mm oleate. Rutin (0.5 mm, 1 mm) was added and cells were grown for 2 days. Addition of oleate and rutin had no effect on cell growth and were similar as in the cells of the control plate (Fig.1).

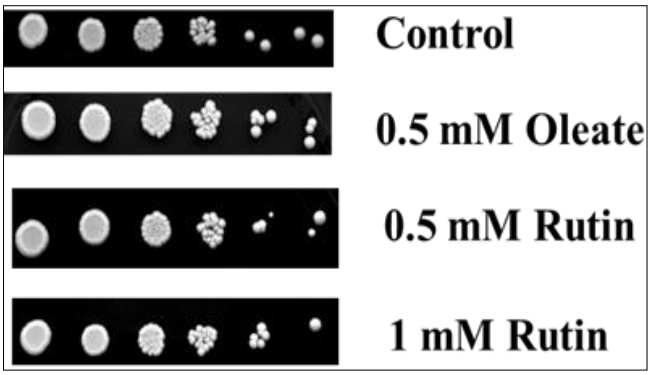

Fig 1: Effect of rutin on growth and cell viability of *S.cerevisiae*.

2. Effect of rutin on oleate induced hyperlipidemia in *Saccharomyces cerevisiae*

Yeast cells were induced to hyperlipidemic with 0.5 mm oleic acid and treated with rutin (0.125 mm, 0.25 mm). Rutin (0.25 mm) treated cells significantly reduced the yeast TAG level equal to atorvastatin (5 mm) against oleic acid induction (Fig.2).

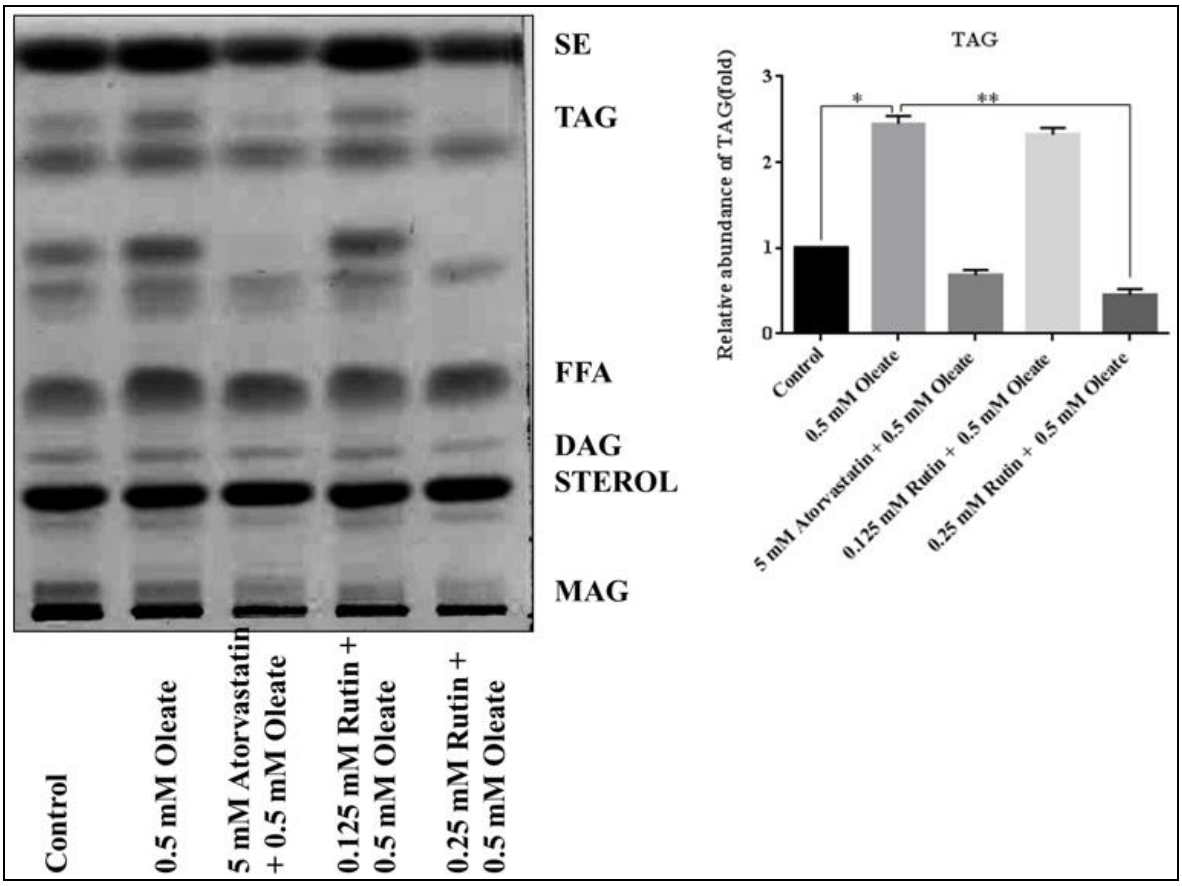

Fig 2: Effect of rutin on oleate induced hyperlipidemia in *Saccharomyces cerevisiae*.

Cells were grown in YPD medium in the presence of glucose as a carbon source to mid-log phase. An equal OD of cells (A_{600}=0.1 OD) were taken and treated with 0.5mM of oleate and different concentration of rutin (0.125 & 0.25 mm) and cells were grown in the SC medium for 24 h at 30 °C. An equal amount of cells were taken and lipid extracted. The lipids were separated by silica- TLC using petroleum ether: diethyl ether: acetic acid (70:30:1, v/v) as a solvent system. Experiments were repeated thrice and values are expressed as mean ± SD. 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.

Excess Sterol ester and TAG depository in lipid droplets form of yeast is known as hyperlipidemia. Our lab previously reported that the treatment of oleic acid induces hyperlipidemia by increasing lipid droplets in *Saccharomyces cerevisiae* (18). The TAG and sterol ester accumulation was observed in oleic acid treated cells. The hyperlipidemia induced yeast cells were tested for the anti-hyperlipidemic effect using rutin compound. Rutin has been widely used for the treatment of antioxidant, anti-inflammatory, anti-diabetes, anti-cancer, hepatoprotective, cardioprotective activities. Here we have analyzed the effect of rutin on cell growth and viability. The cell viability was not affected by rutin Fig (1). Treatment of atorvastain 5 mM reduced TAG level in hyperlipidemia induced yeast cells Fig (2). Rutin at 0.25 mm significantly reduced TAG and SE level (fig (2)). Results of the present study showed rutin possess potent anti-hyperlipidemic activity and it was observed at a dose of 0.25 mM in hyperlipidemia induced yeast cells.

Conclusion

Hyperlipidaemia or dyslipidemia a condition affect many people to death. It is more amounts of lipid accumulation (increased LDL and decreased HDL cholesterol levels) in serum. Our study showed that the rutin act as equal to commonly used lipid lowering drug of Atoravastain, so it can act as anti-hyperlipidemic activity by attenuates the oleic acid induced hyperlipidemic of neutral lipids accumulation, lipid droplet in yeast cells. Rutin did not affect the cell growth.

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