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Antidiabetic effects of marine brown algae *Spatoglossum asperum* J. Ag. ethanolic extract on 3T3-L1 Pre-Adipocytes activity an in vitro approach

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Abstract---Marine algae have emerged as good alternative source of medical, pharmaceutical, and food industries, it's used for anticancer, anti-inflammatory, antioxidant, antimicrobial, etc., In this study, anti-diabetic effect of brown seaweed *Spatoglossum asperum* was focused, We aimed systematically to examine the ethanolic extract effects of *S. asperum* (EESA) as an anti-diabetic agent using 3T3-L1 *in vitro* assay, focused through molecular works; protein extraction, DNA isolation, gene analysis, western blot, ROS measurement, senescence associated β -galactosidase and deoxy glucose uptakes. In results for p21/ β -actin H_2O_2 +EESA treated cells showed very good potential results showed (0.44), same combination reduced the toxic level (0.60) in p53/ β -actin, awfully superior results showed (0.39) in p16/ β -actin. In adiponectin (1.16), $tnf-\alpha$ (0.68), $il6$ (0.42), RFU (211.5), same combination in COMET assay observed that DNA damage (4.95), glucose uptake noticed (68.45%), mRNA expression in p53 showed (0.07), p21 (1.8), adiponectin (3.25), $tnf-\alpha$ obtained (1.95), $il6$ expression obtained (2), p16 expression (2). From the results concluded that the capability to supplement glucose absorption in 3T3-L1 pre-adipocytes and augment glucose consumption activity through the appearance of glucose transporter type 4 (GLUT 4). From the work confirmed that EESA might be a prospective anti-diabetic material for the handling of diabetic patients and can avoid inflammatory or oxidative connected diseases in humans.

Keywords---anti-diabetic activity, hyperglycemia, inflammatory, type-2 diabetes, western blot.

Introduction

Marine algae have been used for human consumption; industrial productions like agar, algin, and seaweeds can serve as a source of minerals, vitamins, amino acids, poly unsaturated fatty acids, paints, adhesives, hydro-mulching paper sizing, bioethanol, dyes, textile printing, cosmetics, paper coatings, gels, drilling, carrageenan, toothpaste, food products; cheese, jam, tea, wine, noodles and soup in many countries (Chandini et al., 2008). The 3T3-L1 is a cell line consequent from the mouse, it's used in biological investigate on adipocyte morphology, adipose tissue increase the combination and accretion of triglycerides and obtain the signet circle manifestation cells of adipose tissues (Lertpatipanpong et al., 2020).

The p53 plays in the development of diabetes, pancreatic function, glucose, and homeostasis and insulin resistance, ROS are standard merchandise of cellular metabolism, both stressed and unstressed cells in chloroplasts, plasma membrane, peroxisomes, mitochondria, endoplasmic reticulum (ER) and cell walls. Extreme production of ROS causes oxidative smash up and eventually cell death, p21 is a regulatory molecule augmented in β -cells in reaction to mitogenic encouragement, p21 acts as inhibitors in the pancreatic β -cell to conclude the practical position of p21 molecules *in vivo* in cell lines (González-Casimiro et al., 2021). Adiponectin is secreted from adipocytes connected with fatness, insulin

resistance, cardiovascular and type 2 diabetes diseases, 3T3-L1 cell lines communicate adiponectin have a speedy expansion stage contrast with the control cells. Also, adiponectin augmented insulin's capability to highest encourage glucose uptake more throughout glucose transporter 4 (GLUT 4) gene expression and augmented conscription of GLUT4 in the plasma membrane (Ilavenil et al., 2016).

The resistance of insulin is a chief imperfection in fatness and obesity connected with tumor necrosis factor- α (TNF- α) and type 2 diabetes, concerned as a causative of insulin confrontation connected with the type-2 diabetes throughout its appearance prototype as well as by challenging *in vivo* and *in vitro* models (Adam et al., 2012). TNF- α is articulated in adipose tissue and its protein levels and mRNA are considerably augmented in adipose tissue of animals and humans. Long-term exposure of 3T3-L1 pre-adipocyte cultured cells TNF- α persuade resistance to insulin, while TNF- α neutralization in cultured cell increases insulin sensitivity, thus, TNF- α obviously supply to the expansion of insulin resistance in the cultured cells (Antonella et al., 2015).

In humans Interleukin 6(IL-6) is an interleukin that perform as a pro-inflammatory cytokine and anti-inflammatory myokine, prearranged through the IL6-gene, in accumulation osteoblasts secrete IL-6 encourage osteoclast formation, IL-6 modify compassion and accomplishment, extended expression incubation with IL-6 increased basal glucose transport in 3T3-L1 adipocyte, whereas acute inspiration had no consequence, IL-6 up-regulated in chubby cells from insulin unwilling subjects, 3T3 IL-6 impairs both insulin action and signaling alleyway (Victoria Rotter et al 2003). A brown alga *Spatoglossum asperum* J. Agardh, the presence of active metabolites such as alkaloids, flavonoids, phenols, steroids, saponins, polysaccharides that are carboxyl, hydroxyl, and amino functional groups (Abdel-Raouf et al., 2012).

In the current study ethanolic extract of *S. asperum* J. Ag. (EESA) of marine brown seaweed tested in 3T2-L1 adipocytes cell line, focused through molecular works; protein extraction, DNA isolation, gene analysis, western blot, ROS measurement, senescence associated β -galactosidase and deoxy glucose uptakes. Determine the effects of anti-diabetic on adipocytes differentiation and changes in the expression of actin, p21, p53, p16, adiponectin, TNF- α and il6 genes.

Materials and Method

Chemicals and Cell line

The chemicals and reagents were received from Sigma-Aldrich (Louis MO, USA), Serum from Bio Whittaker-Lonza (Verviers, Belgium), 3T3L1 cell line - National Centre for Cell Science (NCCS), Pune, India, antibodies Santa Cruz Biotechnology (Santa Cruz CA), senescence β -Galactosidase Staining Kit, Cell Signaling Technology, Inc.

Extract preparation

The *S. asperum* collected from Ramanathapuram district, shade dried the collected samples, dissolved in ethanol and condensed with Rotary evaporator, the *S. asperum* ethanolic extract EESA used for all activities.

Cell culture and Treatment of cells

The 3T3 L1 pre adipocytes were cell cultures cultured DMEM - Dulbecco's Modified Eagle's Medium contains glucose (25 mM/L). Around 80-90% confluent cells were treated with H₂O₂ (100 μ M) for 2 hrs in serum free medium contain high glucose and then passaged and allowed to cultivate the cells for 70% confluent and again route, after one week the cells were again pulsed with H₂O₂ (100 μ M) 2 hrs and then route, this treatment was continued for fourteen days.

Cell Viability Assay

Chemotherapy-induced was determined by MTT Cell Proliferation Assay (Roch) as per manufacturer's instructions. Momentarily, 1×10^4 cells / well were plated in smooth underneath 96 well plates in a concluding volume (100 μ l), and then allowed the cells to attach for overnight until cell attachment and then EESA agents were added in complete culture medium at various concentrations. Culture plates were incubated for 48 hours. After treatments, in each well MTT 10 μ L (5 μ g/mL) was added. After 4 hours incubation (37°C) supplementary, medium was removed and in well dimethyl sulfoxide (150 μ L) was added to re-suspend MTT metabolic creation. The dissolved Formosan absorbance was considered at 490 nm (A490) using the microplate spectrophotometer.

Extraction of protein and analysis of western blot

The treated cells were clean with cold phosphate buffer saline phosphate buffer saline thrice and frayed hooked on Radio-Immunoprecipitation Assay (RIPA) (100 μ L) Tris HCl buffer (pH 7.6; 25 mM), Sodium chloride NaCl (150 mM), 1,2-bis(o-aminophenoxy)ethane-N,N,N',N'-tetraacetic acid (EGTA) (1 mM), SDS (1%), triton-X 100 (1%), Sodium deoxycholate (1%), and leupeptin (1 μ g/mL), sonicated and centrifuged (12,000 g; 15 mins) then chowder was collected, followed by quantified that protein via Nano-drop. Protein aliquots (50 μ g) were added with the gel loading buffer (5X) and it was alienated in SDS-PAGE (10%), finally western blot was performed. Blots investigated with detailed antibodies of the protein of concern p53, p21, p16, TNF- α , Adiponectin and IL6 (Iqbal and Ahmad, 2017; Manish et al., 2017).

Single cell gel electrophoresis - Comet assay

Single stranded breaks of DNA can be accessed through comet assay, approximately 10^4 cells briefly were washed with cold phosphate buffer saline and shabby and balanced in agarose (200 μ L; 0.5%) little melting after that the in microscopic slide cells layered percolated with agarose (1%). The slides were then immersed and incubated in Lysis buffer (5M - NaCl₂, 100 mM - EDTA, Tris - 10 mM with pH 10, DMSO - 10% and Trion X-100 - 1%) for 1 hour in 4°C. Slides

were transferred into electrophoresis tank for DNA, it contain electrophoresis buffer (EDTA - 1 mM, NaOH - 300 mM; pH - 13) there it was 20 mins incubated after that electrophoresis carried out at 200 mV in 20 mints end of sprint slides were drenched in Neutralizing buffer for 15 mins (Tris 400 mM; pH 7.5) and discolored with 20 µg/ml Ethidium Bromide and analyzed under fluorescent microscopy the amount damage of DNA was intended by Comet Imager (2.1) the DNA damage amount was represented in DNA damage % (Ghosh and Roth, 2014).

Reactive Oxygen Species (ROS) measurement

Intracellular ROS was calculated by Fluorogenic Probe Dichlorodihydrofluorescein diacetate (DCFDA) method. After treated cells incubated with DCF-DA (15 µM; 45 mints), cells were momentarily centrifuged down to eliminate the dye and resuspended in N-(2-Hydroxyethyl)piperazine-N'-(2-ethanesulfonic acid) (HEPES) and measured using Spectrofluorometer for excitation at 485 nm and emission 530 nm (Qilong and Ming-Hui, 2019).

DNA isolation and gene analysis

The DNA was isolated from the treated cells through digestion with proteinase-K and extraction with phenol/chloroform and was quantified using Nano Drop, uptake of 2-Deoxyglucose; after the serum undernourishment, cells washed three times with Krebs-Ringerphosphate (KRH) buffer (10 mM phosphate buffer with pH 7.4, NaCl 136 mM, KCl 4.7 mM, MgSO₄; 1.25 mM) and encouraged with insulin (2 nM) for fifteen mints. After that the cells were over again washed three times with KRH buffer and its incubated with 2-[³H] deoxy glucose for 45 mins (0.5 µCi/mL; 0.1 mM,) after that the cells were washed with cold PBS and SDS (0.1%) was added and reserved for suddenly incubation at extent hotness and then it was understand by liquid scintillation counter (Alberts et al., 2003). The amplification by Polymerase Chain Reaction (PCR) was carried out by ABI-7000 (Applied Biosystems) with suitable cycle conditions, the RNA expression level of indomitable through 2-DDCt and normalized using -GAPDH, the primer sequence of a detailed gene.

Statistical Analysis

The one-way analysis of variance (ANOVA) was used to estimate the implication of the dissimilarity of means of data from assorted experiments by using SPSS arithmetical software package (version: 16). The principles are offered as mean±SD and P < 0.05 is measured as noteworthy (Vinothiniet al., 2017).

Results and Discussions

The 3T3-L1 pre-adipocyte cells treated through the different samples; EESA, hydrogen peroxide (H₂O₂), H₂O₂+EESA, and standard Rosiglitazone were used for different biological studies; oxidative stress test, Reactive Oxygen Species (ROS), COMET assay, glucose uptake, expression of mRNA through RT-PCR method and expression of protein were analyzed.

Cancer cell death (cytotoxicity) of EESA on 3T3-L1 pre- adipocyte

Cytotoxicity EESA effect of 3T3-L1 pre-adipocyte 3T3-L1 cells were treated with an assortment of concentrations (5 to 100 $\mu\text{g/mL}$) of EESA for 48 h. EESA behavior didn't influence the pre-adipocyte practicability up to 50 $\mu\text{g/mL}$. However, supplementary increments (75 and 100 $\mu\text{g/mL}$) exhibit slightly cytotoxic belongings on the 3T3-L1 cells as compared with organized. Consequently, protected quantity ranges of EESA were from 5-50 $\mu\text{g/mL}$ for the additional experimentations (Figure 1).

Ratio of p21/ β -actin

Beta actin were tested with the 3T3-L1 pre-adipocyte cells treated through the different samples; H_2O_2 , EESA, H_2O_2 with EESA and standard Rosiglitazone were used for p21/ β -actin, hydrogen peroxide very toxic to cells, it damage more cells (1.36), highly toxic to the cells. Then EESA treated alone it showed (0.97), standard Rosiglitazone observed (0.60), H_2O_2 +EESA treated cells showed very good potential results showed (0.44), compare with others also standard it showed excellent result (Figure 2).

Ratio of p53/ β -actin

The Ratio of p53/ β -actin is more or less similar to the Ratio of p21/ β -actin; the 3T3-L1 pre-adipocyte cells treated with different samples; H_2O_2 damage more cells it showed (1.3), then EESA treated alone it showed (0.95), Rosiglitazone standard obtained (0.61), H_2O_2 treated cells treat with EESA showed very good potential results, it showed (0.60), compare with others also standard it showed outstanding results, H_2O_2 +EESA combinations reduced the toxic to the cells (Figure 3).

Ratio of p16/ β -actin

The 3T3-L1 pre-adipocyte cells treated with different samples; Dissimilar samples; H_2O_2 , EESA, H_2O_2 +EESA and Rosiglitazone standard were used p16 ratio were tested; H_2O_2 always extremely toxic to treated cells, it damage more cells (0.93), greatly toxic to the cells. Then EESA treated alone it showed (0.36), alone also it showed good result, the standard Rosiglitazone noticed (0.33), H_2O_2 +EESA treated cells observed that awfully superior results showed (0.39), compare with others EESA alone showed outstanding results (Figure 4).

Ratio of Adiponectin/ β -actin

Adiponectin method of analysis looked for increasing good protein content in the cells, H_2O_2 , H_2O_2 +EESA, and Rosiglitazone standard were subjected to the adiponecatin analysis; of 3T3-L1 pre-adipocyte cells treated through the different samples; H_2O_2 highly damaged protein content (0.48) in the cells, EESA alone showed wonderful result it obtained (1.00), H_2O_2 +EESA showed highest protein increment in the cells it observed that (1.16), standard showed (0.96), overall tests showed that very good results from H_2O_2 +EESA showed excellent for increasing the protein content (Figure 5).

Ratio of TNF- α / β -actin

Tumor necrosis factor (TNF) plays an significant responsibility in immune regulating responses, its reducing toxic level in cells, the 3T3-L1 pre-adipocyte cells treated through the different samples; the H₂O₂ treated cells highly toxic it showed (1.59), EESA alone treated observed (0.60), H₂O₂+EESA showed (0.68), standard showed (0.74), overall tests showed that EESA alone treated cells showed very good activity, successfully stimulate the downstream pathways to induce apoptosis. The proteins efficiently guarantee the soft progress of the pre-immunization for antibody identification, drug development and screening processes (Figure 6).

Interleukin 6 (IL6)

The IL-6 is an interleukin that acts as together an anti-inflammatory myokine and pro-inflammatory cytokine, reducing the toxic level in cells. The 3T3-L1 pre-adipocyte cells were treated through different samples. The H₂O₂ treated cells extremely toxic it obtained (1.14), alone EESA treated observed (0.70), H₂O₂ treated cells treated with EESA showed (0.42) excellently reducing the toxic content in cells, standard showed that (0.62), overall tests showed that H₂O₂+EESA treated cells showed very result effectively (Figure 7).

Relative fluorescent unit (RFU)

Reactive oxygen species (ROS) are extremely reactive chemicals formed from O₂; examples of ROS include superoxide, peroxides, OH radical, singlet oxygen and α -oxygen, its roles in homeostasis and signaling in cells. The reduction of molecular O₂ produces superoxide ($\bullet$ O₂⁻), which is the forerunner to the majority of other ROS. RFU tested with different samples; H₂O₂ showed less cell death was observed (375.5), EESA alone, H₂O₂+EESA treated cells and standards showed more or less similar results (222; 211.5; 210) in 3T3-L1 pre-adipocyte cells (Figure 8).

Single-cell gel electrophoresis (COMET) assay

The comet assay is a single cell gel electrophoresis, easy system for measuring deoxyribonucleic acid (DNA) beachfront breaks in eukaryotic cells, an uncomplicated and sensitive fashion for the discovery of damage the DNA at the range of the individual cells. Observed through luminescence microscopy; the concentration of the comet tail qualified to the head reflects the number breaks of DNA. H₂O₂ treated cells showed less DNA damage (14.7%), EESA alone showed more DNA damage compare to hydrogen peroxide, it obtained that (8.9%). The H₂O₂+EESA and standard samples treated cells showed that potential DNA damage (4.95; 4.55%) in 3T3-L1 pre-adipocyte cells (Figure 9).

Glucose Uptake Assay

The glucose uptake assay is a plate- grounded, homogeneous bioluminescent system for determine glucose uptake in cells, grounded on the discovery of 2-deoxyglucose-6-phosphate (2DG6P), H₂O₂ treated cells showed glucose update

(43.33), EESA alone showed that (57.9), H₂O₂ EESA treated cells attained (68.45), the standard showed that (75.5), overall results showed that veritably good glucose uptake was noticed on H₂O₂ EESA treated cells. Glucose is simple sugar molecules contain with transported transversely the cell's membrane and attentive by living being phosphorylated. In adipocyte cells, glucose uptake measuring can cover the glucose transporters over expression or recognize glucose transporter impediments. Through fat and strength cells, changes in GLUT4 translocation upon insulin stimulation container be present experimental through the measuring glucose uptake (Figure 10).

Relative mRNA expression of p53

Mutation of p53 is a common point of cancer; p53 is expressed as lower isoforms, some of which inhibit wild- type p53, p53 as the bookmaker is of the significant experimental significance of the tumours. H₂O₂ treated cells showed (0.28), EESA alone showed that (0.14), H₂O₂+EESA treated cells obtained (0.07), standard showed that (0.21), overall results showed that very good p53 mRNA expression was noticed on H₂O₂+EESA treated cells (Figure 11).

The mRNA variants (p53 isoform mRNAs), including the one garbling the canonical p53 tumour suppressor protein. We've developed scientific tools to specifically descry and quantify p53 isoform expression at the mRNA position by nested RT-PCR (rear recap-polymerase chain response), p53 gene, as a variety of cancer protein antigen, also as a cancer gene, and eventually as a tumour suppressor gene. Farther elaborative exploration and experimental trials accomplished that the mutation of the p53 gene might affect in tumorigenesis or cell metamorphosis. The mutant protein of p53 can be measured as a uveal carcinoma protagonist. In addition, the wild- type p53 gene is a variety of tumour suppressor gene; hence, mutation of the p53 gene will encourage tumorigenesis (Maniwa et al., 2001).

Relative mRNA expression of p21

The p21 Cip1 was primary insulated as one of the cyclin needy kinase (Cdk) interrelate proteins convinced through wild- type p53 gene merchandise, and it appears to occupy yourself an necessary nonsupervisory part in the control of cell proliferation as a powerful, tight- list asset of the cyclin-Cdk complex that blocks the G1/ S transition of the cell cycle. The H₂O₂ treated cells showed (3.9), alone EESA tested showed that (2.8), then H₂O₂+EESA treated cells obtained (1.8), standard showed that (2), overall results showed that very good p21 mRNA expression was noticed on H₂O₂+EESA treated cells (Figure 12).

The p21 protein is decoded through WAF1/ CIP1 (part 1 of wild- type p53/ protein 1 unthinking to a cyclin-dependent kinase (CDK)), a tumour gene suppressor situated on chromosome 2.6 p21 (Atsushi et al., 2016) [16]. This protein is recognized to be connected to the cell cycle; p21 inspiration is a ordinary medium inhibition of enlargement in dissimilar physiological conditions, p21 is noticed in geriatric courses or genes concerned in conditions connected with ageing (Alzheimer's complaint, amyloidosis) (Baguley, 2006); reversible and unrecoverable forms of expansion inhibition payable to harm and concluding

isolation to postmitotic cells are enthused brusquely and fleetingly (Shirakawa et al., 2000).

Relative mRNA expression of Adiponectin

The adiponectin is a hormone of cytokine firstly plant to be buried fully through white adipose towel; still, current pieces of substantiation propose that adiponectin is also fashioned in brown adipose towel and cadaverous influence. The H_2O_2 treated cells showed very less expression it obtained (0.75), EESA alone tested mRNA expression showed that (2.2), then H_2O_2 +EESA treated cells expression obtained (3.25), standard showed that the mRNA expression (2.05), overall results showed that very good mRNA expression of adiponectin was noticed on H_2O_2 +EESA treated cells (Figure 13).

Adiponectin also called GBP28, apM1, Acrp30, adipoQ is a 30-kDa adipocytokine hormone completely concealed from the adipose tissue in numerous mammalian species (Hu et al., 1996), consists of an N-terminal collagenous sphere and C-terminal globular field (Pajvani et al., 2003), thoughtful glucose utilization effects, lipid synthesis, insulin sensitivity, and power homeostasis in numerous mammalian species.

Relative mRNA expression of TNF- α

The probable of tumour necrosis factor (TNF) as a therapeutic intention was browbeaten and has been well characterized in an assortment of diseases, the H_2O_2 treated cells showed in TNF- α (3.65), alone EESA TNF- α tested showed that (2.4), then mRNA expression in H_2O_2 +EESA treated cells TNF- α obtained (1.95), standard showed that (1.7), overall results showed that very good p21 mRNA expression of TNF- α was noticed on H_2O_2 +EESA treated cells (Figure 14).

The TNFSF members are articulated extensively and cooperate main roles in resistant responses, cell homeostasis, inflammation, and tissue repair (Sedger and McDermott, 2014) [21]. In calculation, they also donate to disease pathogenesis, and consequently, are also referred to as twofold edged swords (Foster et al., 2004) [22]. The majority of the TNFSF members are type II transmembrane proteins, whereas several can be concealed proteins with activity in biological (Bodmer et al., 2002), formerly, macrophages were reported as a foundation of TNF (Carswell et al., 1975).

Relative mRNA expression of IL6

The interleukin-6 (IL-6) is a cytokine concerned in several processes of immunological, but it is also connected to implement and probably power position. During the implement, muscle IL-6 plasma IL-6 and mRNA levels are increased, in performance a position in the energy mobilization as free fatty acids (FFA) in comeback. The hydrogen peroxide treated cells showed in mRNA expression of il6 very it obtained (3.4), EESA alone tested mRNA expression of il6 showed that (2.7), then H_2O_2 +EESA treated cells expression obtained (2), standard shows that the mRNA expression of il6 (2.15), generally results obtained that very good mRNA expression of IL6 was noticed on H_2O_2 +EESA treated cells.

The discharge of biologically vigorous interleukin 6 (IL-6) regularly occurs in human glioblastomas, preponderance of malignant brain tumours (Van Meir et al, 1994) [25]. In the ferociousness of glial tumours and IL-6 expression degree at RNA echelon (Rolhion et al, 2001) [26]. As IL-6 has possessions of a development reason for tumour cells (Goswami et al, 1998) [27] and can obstruct apoptosis induced throughout a variety of chemotherapeutic compounds or ROS (Miwa et al, 2003).

Relative mRNA expression of p16

The reverse transcription PCR (RT-PCR) in quantitative was used to quantify expressions gene (relative mRNA levels) of p16 and the exchange transcript p16b in oesophageal and gastric tumours. The expression of p16 gene was untraceable in 13 of 25 oesophageal squamous cell carcinomas. The H₂O₂ treated cells showed mRNA expression of p16 (3.85), alone EESA tested showed that p16 expressions (2.6), then H₂O₂+EESA treated cells obtained in p16 expression (2), standard showed that 1.75), overall results showed that very good p16 mRNA expression was noticed on H₂O₂+EESA treated cells (Figure 16).

Three separate types of p16 differences have been linked in tumour cells; succession changes in genomic DNA similar as small elisions or position mutations (Pollock et al., 1997 [29] homozygous omission (HD) of DNA long parts that include p16 (Stone et al., 1995) [30]; and transcriptional repression of p16 through methylation of the exon1 and protagonist region (Merlo et al., 1995) [31], the restoration of p16 expression in cancer cells beget spectacular growth repression and p16 knockout mouse develop robotic tumours at an untimely age (Serrano et al., 1993).

Diabetes mellitus is a group of metabolic diseases characterized through a elevated blood sugar position larger than a prolonged period. Type-2 diabetes mellitus is nearly associated with adipogenesis and its factors. In this study, we demonstrated that TNF- α has a major part in 3T3-L1 pre-adipocyte gene expression and picky induction of pre-adipocyte genes (Ruan et al., 2002) [33]. Isolation of multipotential 3T3-L1 adipocytes is one of the the majority widespread *in vitro* models used in adipocytes biology, it's involved in enlargement capture, counting agents similar as insulin (Shona Morrison and Sean, 2015).

Served as a major cell line for studying mechanisms of adipocytes differentiation adipocytes gene expression, triglyceride synthesis, insulin and β -adrenergic signal transductions, insulin dependent glucose uptake for white adipose tissue (Fraher et al., 2015) [34]. P53 help fend inedible the commencement and sequence of growth by suggest cell rotation arrest, senescence or programmed cell death in cells. So, marine sources are utilized with progress to treat this metabolic disorder. Phytochemicals of marine Seaweeds were formed to possess various biochemical properties, diabetes mellitus is a metabolic process of occurs when abnormal reaction body does not deliver or appropriately use insulin, antidiabetic activity was observed in *S. asperum* (Sivakumar et al. 2011).

Conclusion

In the current study, Rosiglitazone management stimulates the p53,p21, TNF- α , adiponectin and GLUT-4 gene appearance in the adipocyte cells, we establish approximately comparable enormity responses in EESA treatments. It supports the p53, p21, TNF- α , GLUT-4 expression and adiponectin. Further, the EESA augment glucose consumption in adipocytes in the course of up-regulation of p53, p21, TNF- α , adiponectin and GLUT-4 expression. The GLUT-4 is concerned in glucose transport into adipocyte cells. These data recommended that EESA increased consumption of glucose in the adipocytes through activation of p53,p21, TNF- α , adiponectin and GLUT-4 gene expression.

Type-2 diabetes mellitus is intimately associated with adipocyte, inadequate adipocyte demarcation causes fat declaration in the liver and muscle which guide to insulin confrontation and type-2 diabetes. We identified that *S. asperum* effectively activate the p53,p21, TNF- α , adiponectin and GLUT-4 adipocyte differentiation, glucose utilization and is accountable for the encouraging adipogenesis regulation. Consequently, the current conclusion recommended that the EESA efficiently modulates the accretion of lipids and discrimination in 3T3-L1 pre-adipocyte cells throughout GLUT-4 mediated signaling pathways.

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Author contributions

AS, AP and MR conducted the *in vitro* experiments, RV, GC and GS designed the study and interpreted the data; all authors revised it significantly and finally approved the description to be submitted.

Disclosure statement

The authors report no conflict of interest.

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Figure 1. Cytotoxic effects of EESA on 3T3-L1 cells. 3T3-L1 cells were treated with different concentrations (5, 10, 15, 20, 25, 50, 75 and 100 $\mu\text{g}/\text{ml}$) of EESA for 48 h. The concentration at 75 and 100 $\mu\text{g}/\text{ml}$ exhibited slight cytotoxic effects on the 3T3-L1 cells

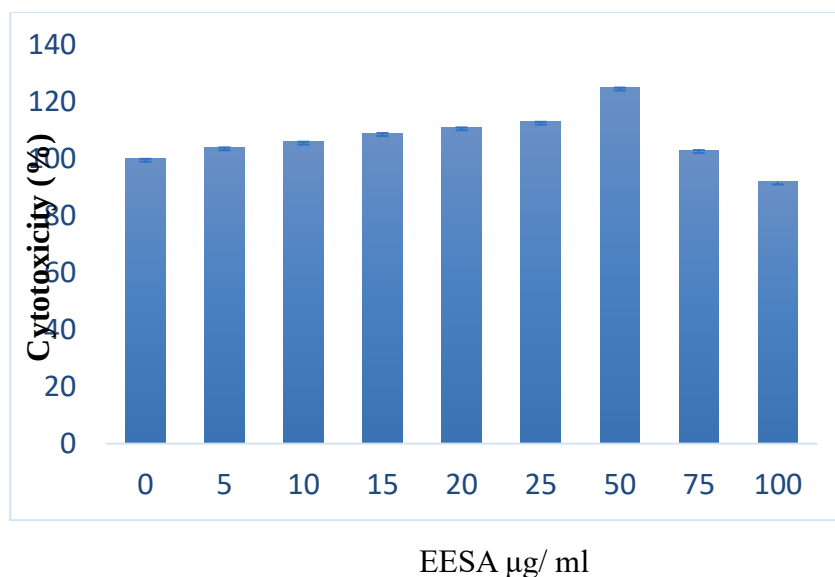


Figure 2. Protein expression patterns of p21

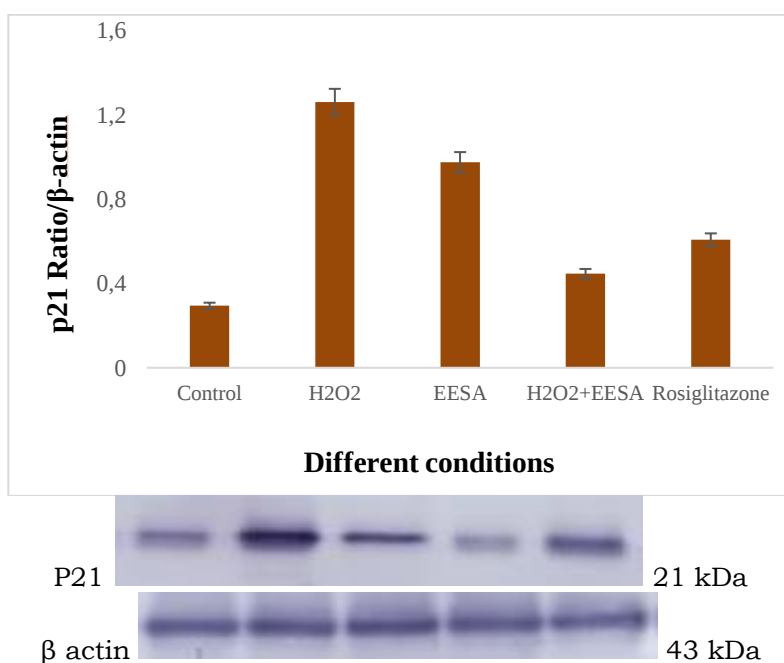


Figure 3. Protein expression patterns of p53

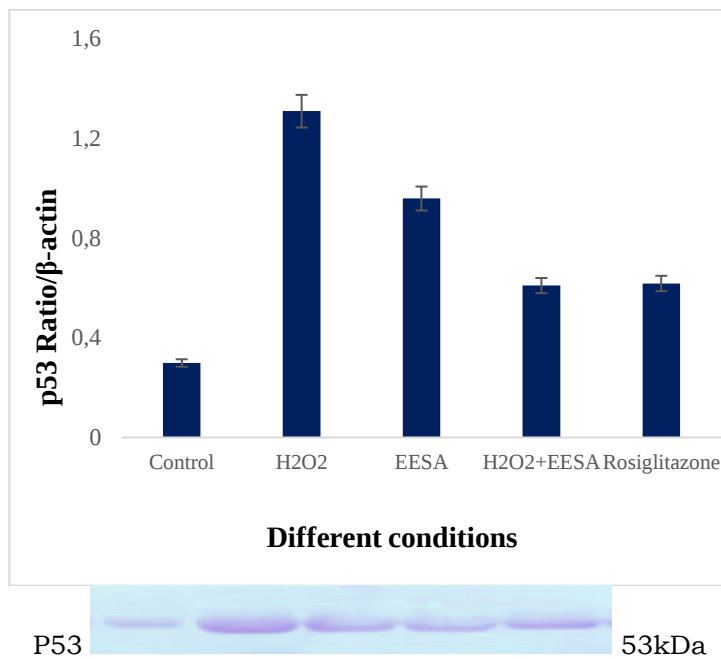


Figure 4. Protein expression patterns of p16

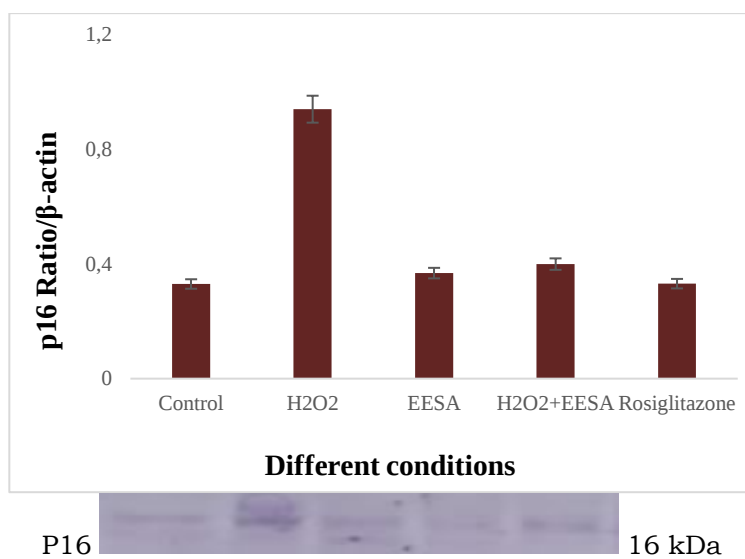


Figure 5. Protein expression patterns of Adiponectin

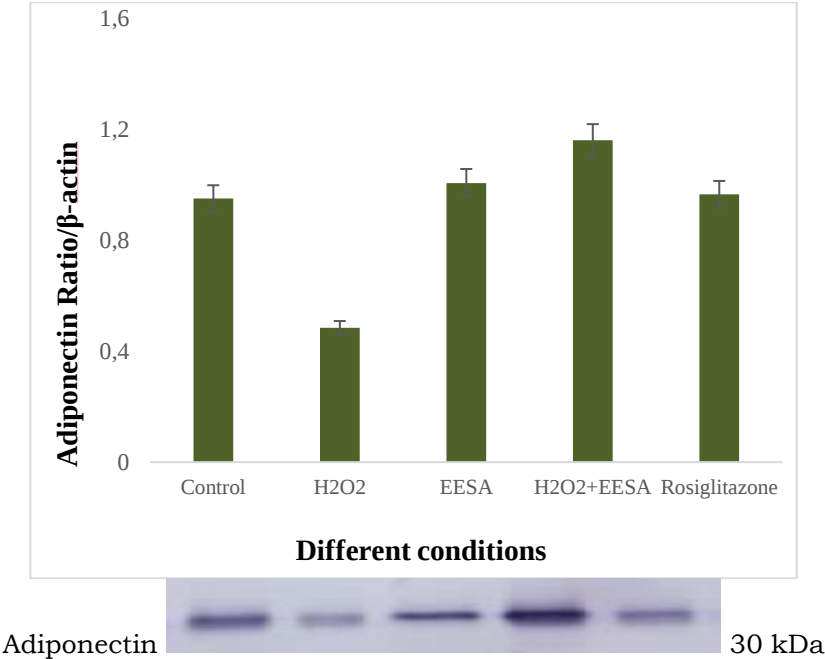


Figure 6. Protein expression patterns of TNF- α

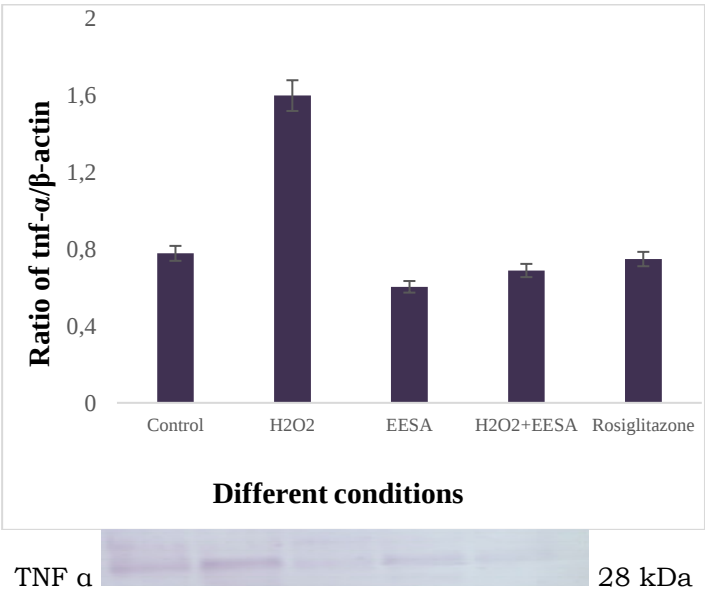


Figure 7. Protein expression patterns of IL6

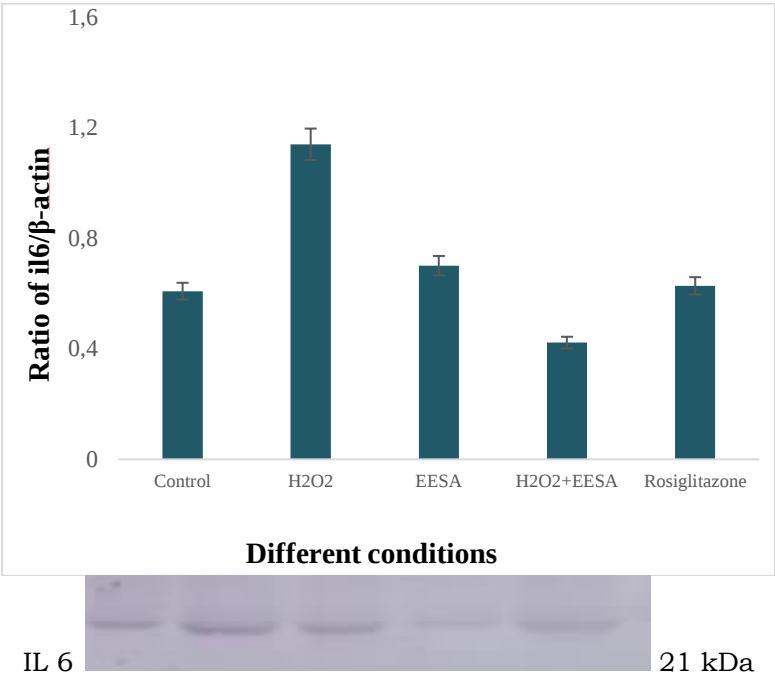


Figure 8. Cumulative data on ROS generation (relative fluorescence unit, RFU)

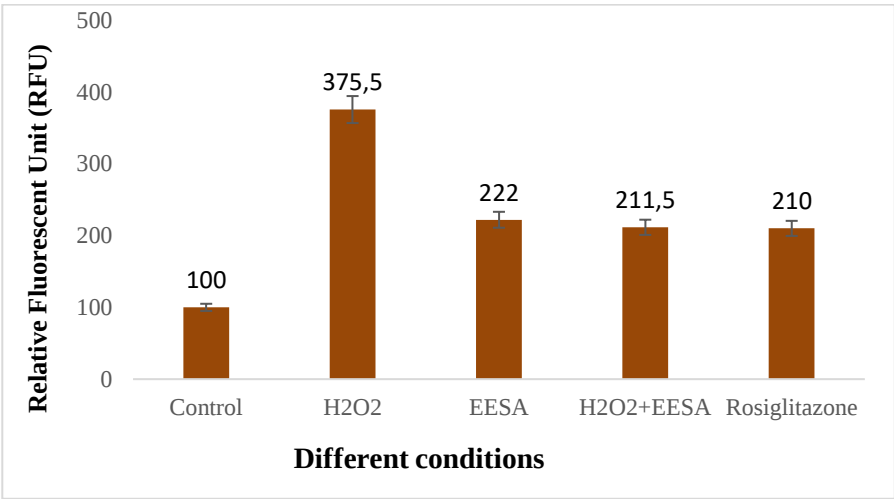


Figure 9. Percentage DNA damage in 3T3-L1 adipocytes under different experimental conditions

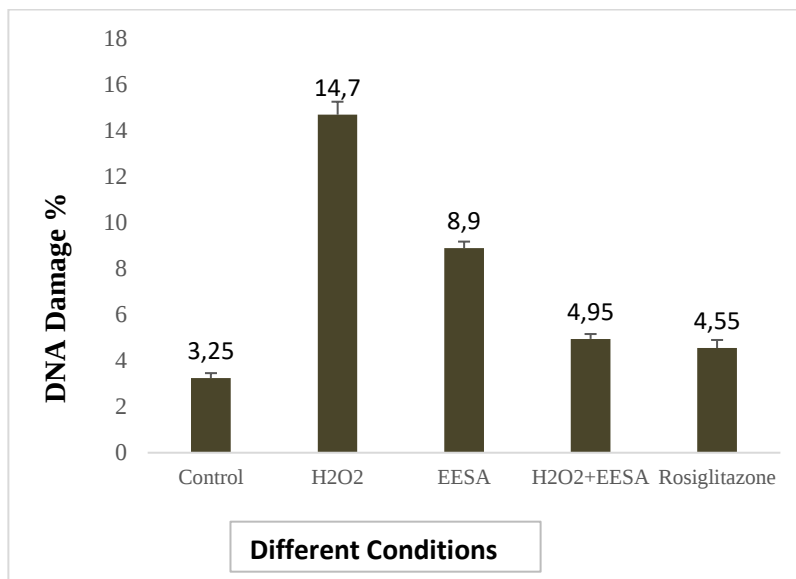


Figure 10. Insulin-stimulated glucose uptake (mean $\pm$ SE) from 3T3-L1 adipocytes under different experimental conditions

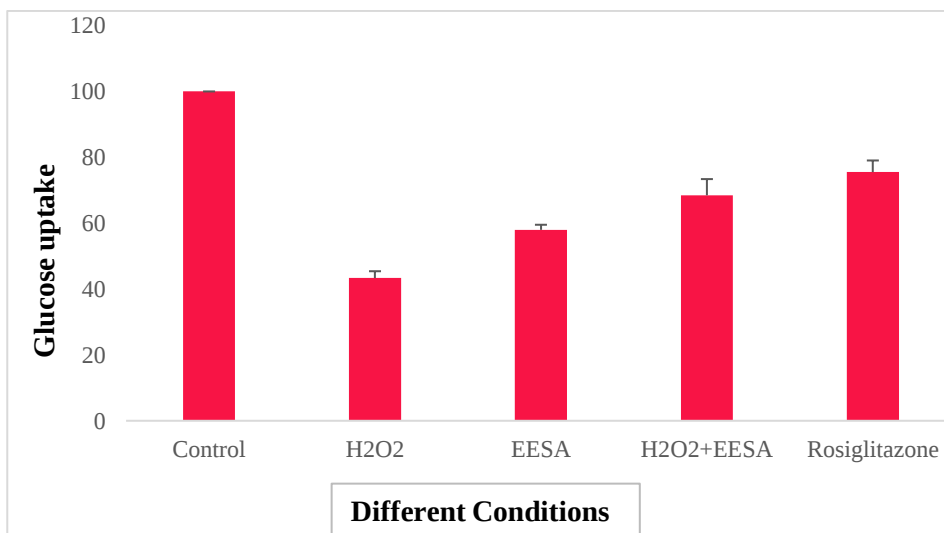


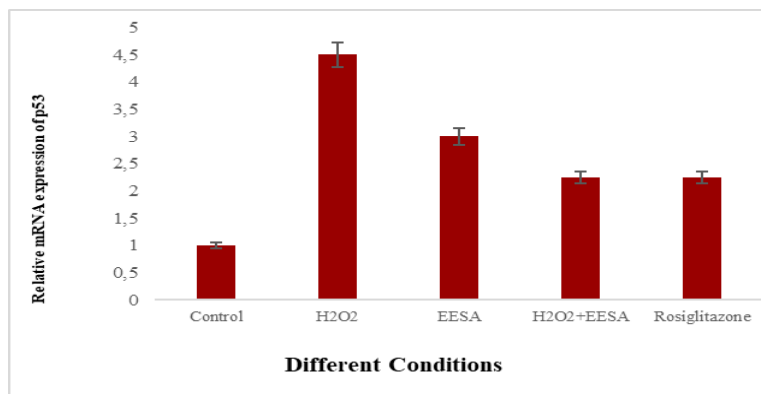
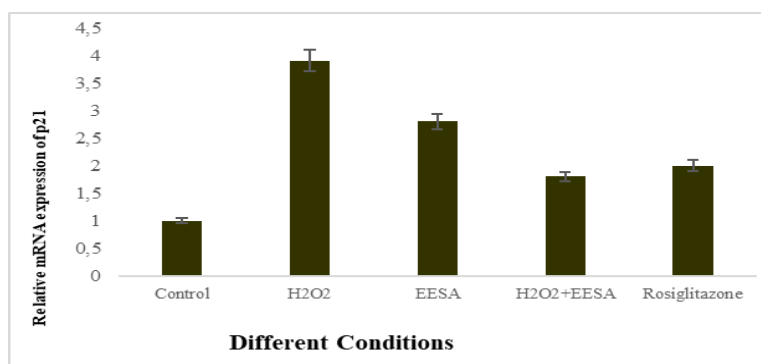
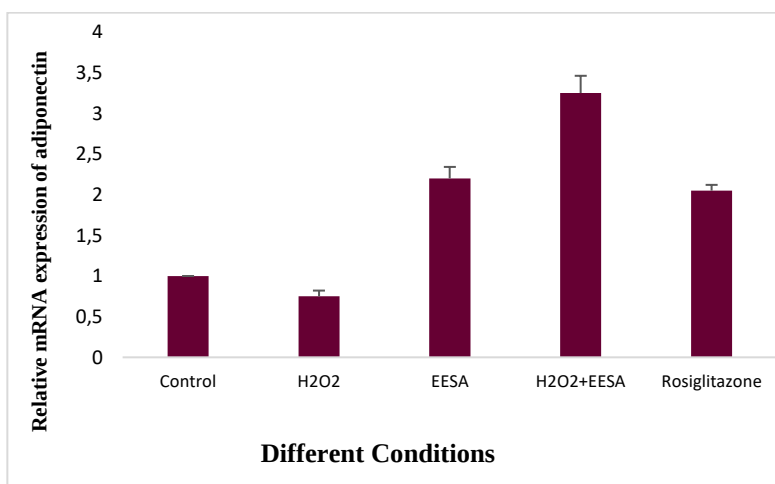
Figure 11. Real-time PCR gene expression patterns (mean $\pm$ SE) of p53Figure 12. Real-time PCR gene expression patterns (mean $\pm$ SE) of p21Figure 13. Real-time PCR gene expression patterns (mean $\pm$ SE) of Adiponectin

Figure 14. Real-time PCR gene expression patterns (mean $\pm$ SE) of TNF- α

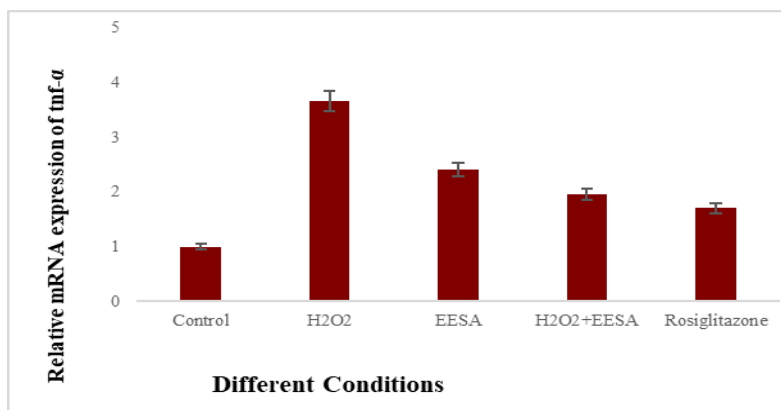


Figure 15. Real-time PCR gene expression patterns (mean $\pm$ SE) of IL6

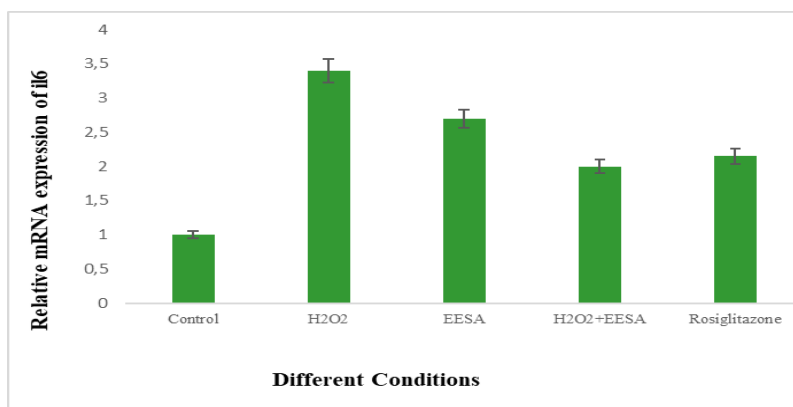


Figure 16. Real-time PCR gene expression patterns (mean $\pm$ SE) of p16

