IJPSR (2017), Volume 8, Issue 11 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 23 March, 2017; received in revised form, 24 May, 2017; accepted, 27 May, 2017; published 01 November, 2017

ANTIDIABETIC ACTIVITY OF METHANOLIC LEAVES EXTRACTS OF TRANSFORMED SOYBEAN IN 3T3-L_1 PRE-ADIPOCYTES CELL LINE

C. Janani and B. D. Ranjitha Kumari*

Department of Plant Science, Bharathidasan University, Tiruchirappalli - 620024, Tamil Nadu, India.

Keywords:

Adipogenesis, Transgenics, Southern blot, Lipid Metabolism

Correspondence to Author:
B. D. Ranjitha Kumari

Professor,
Department of Plant Science,
Bharathidasan University,
Tiruchirappalli - 620024, Tamil Nadu, India.

E-mail: ranjithakumari2004@yahoo.co.in

ABSTRACT: The present study was initiated to quantify the Isoflavones content that accumulated in soybean transgenics with RnPPARγ gene by using high performance liquid chromatography. The effects of methanolic leaves extract of transformed soybean in 3T3-L_1 adipocyte cell line. The RnPPARγ transgenic soybean variety JS335 was developed and transgenics were confirmed by PCR and Southern blot techniques. The quantification of isoflavones compounds by HPLC showed that methanolic leaves extracts of transformed soybean contained Genistein (1.67µg/g extract), Daidzein (2.76µg/g extract), Rutin (32.06µg/g extract) and Quercetin (48.40µg/g extract) and methanolic leaves extracts of non-transformed soybean contained Genistein (1.23µg/g extract), Daidzein (2.33µg/g extract), Rutin (26.16µg/g extract) and Quercetin (43.03µg/g extract) by comparing the chromatogram of the reference standards. In the present study revealed that secondary metabolite content was higher in the transformed plant than non-transformed plants. After treatment of transformed methanolic leaves, extracts on 3T3-L_1 mature adipocytes significantly ameliorated lipid droplet accumulation and droplet reduction in adipocytes might be the result of lipid metabolism by lipolysis.

INTRODUCTION: The obesity and overweight with physical inactivity are evaluated to cause diabetes [1]. Fat deposition in excess is the strongest risk factor for type 2 diabetes and it is considered in the characteristic features of physical activity and diet in human body. Almost 1.9 billion of adults as overweight were observed among these 600 million were obese [2] but the risk for other diseases such as T2DM, cardiovascular ailments and hypertension also increasing for them [3]. Diabesity, characterized by adipose tissue expansion by increased adipogenesis *i.e.* increase in adipocytes number.

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.8(11).4732-44

Article can be accessed online on: www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.8 (11).4732-44

To store excess energy and fat in layer of subcutaneous has lower capacity and free fatty acids are release into liver. The overflow of free fatty acid into peripheral tissue and abdominal fat which leads to loss the insulin sensitivity and causes peripheral insulin resistance and also associated with type 2 diabetes [4-6].

The cytoskeletal proteins that are convert the fibroblastic cells into adipocytes. The adipocyte differentiation and maturation called adipogenesis and the function is metabolism of fat [7-9]. The process involves several events where multiple transcriptional factors are involved and their expressions are stage specific. The phosphorylation of C/EBP-α and C/EBP-β which activate expression of PPAR-γ and C/EBP-δ and C/EBP-β are observed after adipogenesis [10, 11]. The proliferation of adipocytes activate by the

expression of C/EBP-α which is to initiate the adipocyte differentiation into mature adipocytes [12, 13]. In adipocytes, transcriptional factor C/EBP-α and PPAR-γ levels are elevated. The increased level of the development of fat and creates adipose tissue obesity which mediates insulin resistance [14]. The PPAR-γ plays the major regulator for adipogenesis, which expressed specifically in adipose tissue [15-17]. It was found that adiponectin is directly affected by obesity and the correlation between adiponectin and Body Mass Index [18]. Adiponectin activate pathway of AMPK and PPARs which regulate adipocyte maturation marker, fatty acid oxidation and gluconeogenesis[5, 18-21]. Dietary polyphenols are the subject of enhancing scientific interest due to their possible beneficial effects on human health [22]. The soy flour and bread are containing polyphenolics and isoflavone to inhibit lipogenesis [23] and the ethanolic extracts of soybean had potential for anti-adipogenesis in 3T3-L_1 cell line [24]. Numerous studies have reported for insulin sensitivity that activation of both PPARγ and PPARα to prevent the diabetes by improving homeostasis of lipid mechanisms due to the presence of isoflavones[25-33]. 3T3-L_1 cell line serves as best reliable and characterized *in vitro* model for adipogenesis [34, 35].

Soy isoflavones regulate metabolism of lipid in diet-induced obesity rats [36]. The oxidized soy oil rich in omega-6 fatty acids exposure and interaction demonstrated between triacylglyceride (TAG) accumulations within 3T3-L_1 cells were reported [36]. So the present study was aimed that whether the RnPPARγ gene in transformed soybean leaves can enhance the level of isoflavonoids such as genestein, daidzein, quercetin and rutin which are responsible for PPARγ expression. The methanolic leaves extracts of both transformed and non-transformed soybean were prepared for the quantification through HPLC and also analyzed some biochemical and antioxidant activities. The effects of methanolic leaves extracts of transformed and non-transformed soybean on accumulation of lipid were studied through *in vitro* method.

MATERIALS AND METHODS:

Extraction of Isoflavones from Soybean:

Preparation of Sample: The leaves of both transformed which harbouring PPARγ gene and non-transformed soybean were collected and dried under shade. Leaves (10g) were extracted using 50 ml of methanol in an orbital shaker (LabTech RC2100) at room temperature for 1 hour. The extracted leaves were centrifuged at 10,000 rpm for 10 min (Eppendorf 5830) then the solution was filtered using a micro filter [37] with some modifications. The filtrate was evaporated using a vacuum evaporator at 80 °C and crude methanolic leaves extracts were used for further analysis.

High-Performance Liquid Chromatography (HPLC): The isoflavones used as standard in purified form such as Quercetin, Rutin, Daizein and Genistein were purchased from Sigma-Aldrich, Bangalore. About 20µL of each sample was used for HPLC analysis. The analysis was performed on a C18 column (0.4 mm ID, 5.0 µm particle size, Japan) in a HPLC system (Waters 2690) with a photodiode array detector (Waters 996, USA). The wavelength was at 254 nm for Genistein and Daidzein and at 370 nm for Quercetin and Rutin and binary gradient solvent system was employed. The mobile phases with HPLC grade methanol (0.025%) and acidified Milli-Q water (0.025%) in the ratio of 1:1 were used. The initial gradient was 20% solvent and after 40 min reaching 100% then returning to 20% at 45 min and maintaining the same condition up to 60 min. The flow rate (1 ml/min) of the sample was carried out with a temperature of 25 °C. The contents of Isoflavones were separated within 60 minutes and the compounds were identified by comparing the UV spectra of standard and retention times.

Formula: Concentration of Sample = (Area of Sample / Mean of Standard Area) × (Standard Weight / Dilution of Standard) × (Sample Dilution / Sample Weight).

Biochemical and Antioxidant Activities:

Total Protein Content: The Total Protein Content of both transformed and non-transformed methanolic leaves extracts were determined by the following the method of Bradford [38].

α-Amylase Activity: The activity of α-Amylase was determined by following the method of Bernfeld [39]. A reaction mixture of 1% soluble starch for 0.5ml prepared using acetate buffer (0.2 M) and 0.5ml of enzyme solution was added and then incubated at 50 °C for 10 min. After incubation, the reaction was terminated by adding

1.0ml of DNS solution (1g of DNS dissolved in 20 ml of 2 M NaOH, with 30g of sodium potassium tartrate and water to make it 100ml). A reaction mixture was kept in water bath at boiling temperature for 15 min and after cooling make up to 20ml of water. Absorbance was measured at 540 nm (Shimadzu Co., Kyoto, Japan). The activity was defined as the amount of releasing reducing sugar in 1 minute under the controlled conditions.

Antioxidant Activities:

1, 12-picryl--diphenyl-hydrazyl (DPPH) Radical Scavenging Activity: DPPH, a stable radical with a characteristic strong absorbance at 517 nm in spectroscopy of deep violet colour is used to evaluate the scavenging activity of free radical for the sample. The best known natural and synthetic antioxidant standard such as an Ascorbic acid was used as positive control for comparison.

The free radical scavenging activity of transformed and non-transformed methanolic leaves extract of soybean was measured by 2, 2-diphenyl-1-picryl-hydrazil (DPPH) calorimetric method of Shirwaikar *et al.*, [40]. The DPPH solution (0.1 mM) was prepared using ethanol; 1ml of DPPH solution was added to 3ml of soybean extracts solution in water at different concentrations (20, 40, 60, 80 and 100 µg/ml). After 30 minutes of incubation, the absorbance was measured at 517 nm (Shimadzu Co., Kyoto, Japan). The absorbance of the reaction mixture lower which indicates free radical scavenging activity is higher.

Superoxide Radical Scavenging Activity: The superoxide radical is generated by added sodium hydroxide to dimethyl sulfoxide by following the method of Elizabeth and Rao [41]. The generated superoxide remains stable in solution, which reduces nitroblue tetrazolium (NBT) into formazan dye and incubated at room temperature then it can be measured at 560 nm. To the reaction mixture of 1ml of alkaline DMSO (1ml DMSO containing 5 mM NaOH in 0.1ml water) added 0.3ml of various concentration (20, 40, 60, 80 and 100µg/ml) of methanolic leaves extracts of both transformed and non-transformed soybean.

The reaction was initiated by addition of 0.1ml of NBT (1mg/ml). The absorbance was measured at 560 nm (Shimadzu Co., Kyoto, Japan). The same procedure was repeated for the standard of ascorbic acid. For the above mentioned calorimetric activities was calculated using the standard formula: An IC_{50} value of the extract (50% of inhibition of DPPH and Superoxide radicals).

Cytotoxicity Assay: Chemicals and Reagents: Acridine orange was obtained from Sigma, USA. MTT (3-[4, 5-dimethylthiazol-2-yl]- 2, 5- diphenyl tetrazolium bromide) from Invitrogen USA. All other fine chemicals were obtained from Sigma–Aldrich, St. Louis.

MTT Assay: The cytotoxicity of transformed and non-transformed methanolic leaves extracts of soybean on the viability of the 3T3-L_1 cells was measured by the MTT assay. The 3T3-L_1 cells were growing onto a 96-well plate for 24 hr.

Then 3T3-L_1 cells were treated with different concentrations (0 to 100µg/ml) of both transformed and non-transformed methanolic leaves extracts of soybean for 48 hr. At the end of the incubation period, 20µl of MTT (5mg/ml) was added to each well and then incubated at 37 °C for 4 hr [42]. The medium was then discarded. A total of 200µl DMSO was added, the optical density values were measured at 492 nm and the percentage (%) cell viability relative to control cells was calculated.

Oil Red O Staining: The 3T3-L_1 cells were treated with different concentrations (0 to 100µg/ml) of both transformed and non-transformed methanolic leaves extracts of soybean after 8 days of differentiation, the intracellular lipid accumulation in the 3T3-L_1 cells was visualized by Oil Red O staining. Cells were washed with PBS, fixed with 10% neutral buffered formalin for 15 min. Cells were then stained with Oil Red O solution (a mixture of three parts of 0.5% (w/v) Oil Red O in isopropanol and two parts of water) for 20 min followed by washing twice with water. Cells were kept in water and photographed. The Oil Red O was then dissolved in 0.3ml of isopropanol per well and the absorbance was read at 495 nm [42].

Statistical Analysis: The results were analyzed statistically with three replicates and expressed as Mean ± Standard Error. Values are analyzed by Duncan's Multiple Range Test (One-Way ANOVA using SPSS 16 version) and $P \leq 0.05$ as considered significant value.

RESULTS: HPLC: The yield of methanolic leaves extracts of non-transformed (26.45%) and transformed (24.1%). The polyphenolic profiles (isoflavones) of soybean are shown in **Table 1**.

TABLE 1: QUANTIFICATION OF ISOFLAVONES OF TRANSFORMED AND NON-TRANSFORMED METHANOLIC LEAVES EXTRACTS THROUGH HPLC ANALYSIS

Name of the Samples	Isoflavones (µg/g)				
	Genistein	Daidzein	Rutin	Quercetin	Total Content
Non-transformed	1.23 ± 0.30[b]	2.33 ± 0.30[b]	26.16 ± 0.32[b]	43.03 ± 0.14[b]	73.13 ± 0.29[b]
Transformed	1.67 ± 0.40[a]	2.76 ± 0.90[a]	32.06 ± 0.23[a]	48.40 ± 0.12[a]	85.16 ± 0.32[a]

Retention time for the methanol extract of transformed soybean leaves at 19.206, 8.446, 13.269 and 18.781 min corresponded well with the standard chromatogram peaks of Quercetin, Rutin, Genistein and Daidzein and at 19.806, 8.967, 13.497 and 18.254 min respectively (**Fig. 1a-d**).

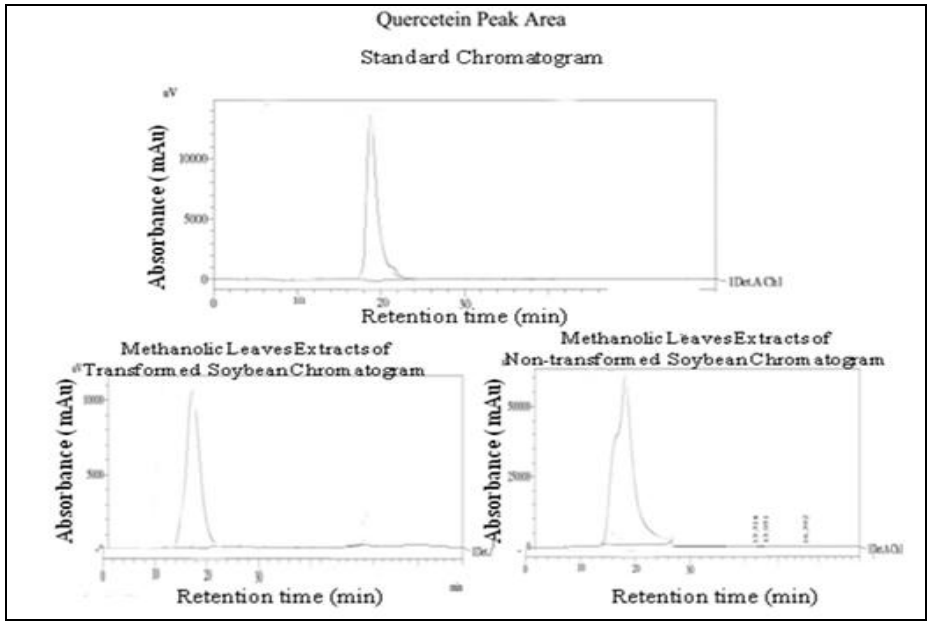


FIG. 1A: HPLC CHROMATOGRAM OF METHANOLIC LEAVES EXTRACTS OF TRANSFORMED AND NON-TRANSFORMED SOYBEAN WITH QUERCETIN AT 370 nm

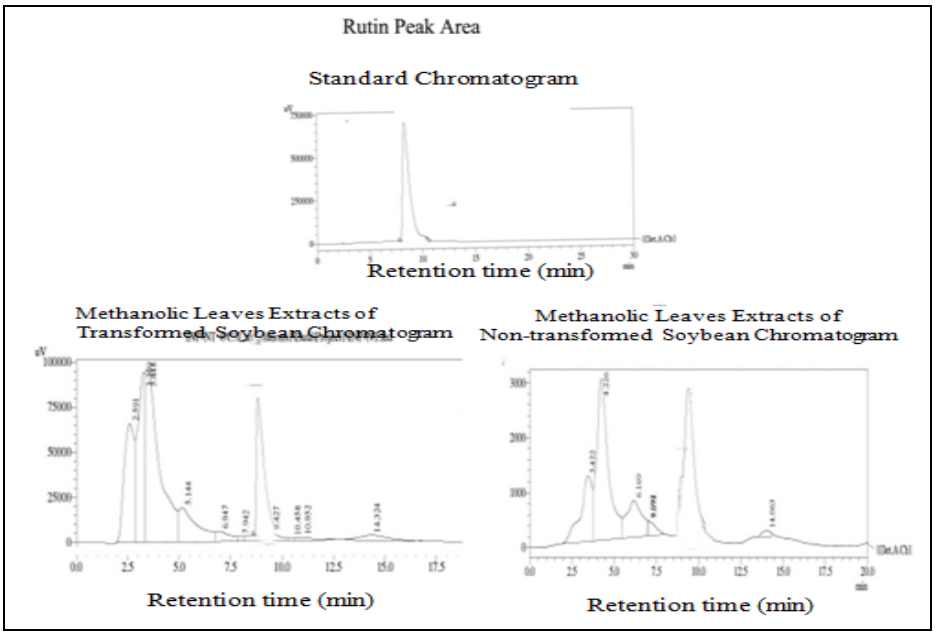


FIG. 1B: HPLC CHROMATOGRAM OF METHANOLIC LEAVES EXTRACTS OF TRANSFORMED AND NON-TRANSFORMED SOYBEAN WITH RUTIN AT 370 nm

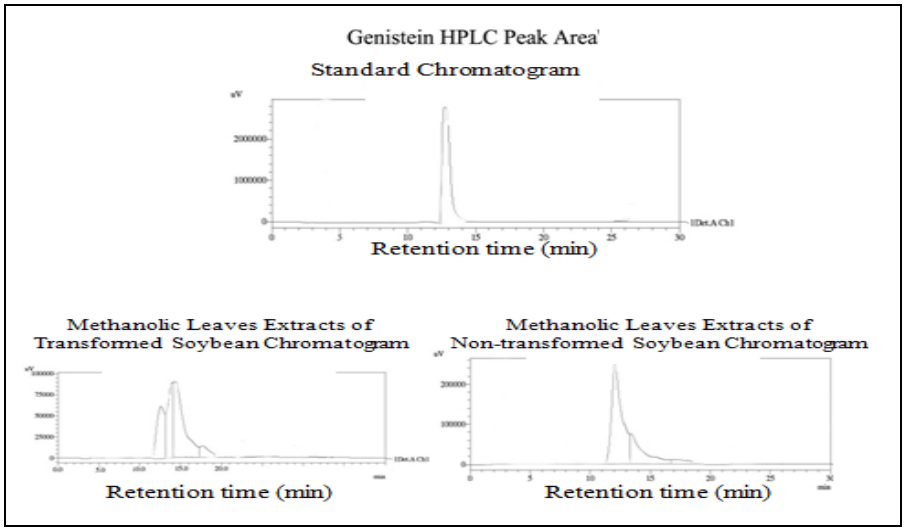


FIG. 1C: HPLC CHROMATOGRAM OF METHANOLIC LEAVES EXTRACTS OF TRANSFORMED AND NON-TRANSFORMED SOYBEAN WITH GENISTEIN AT 254 nm

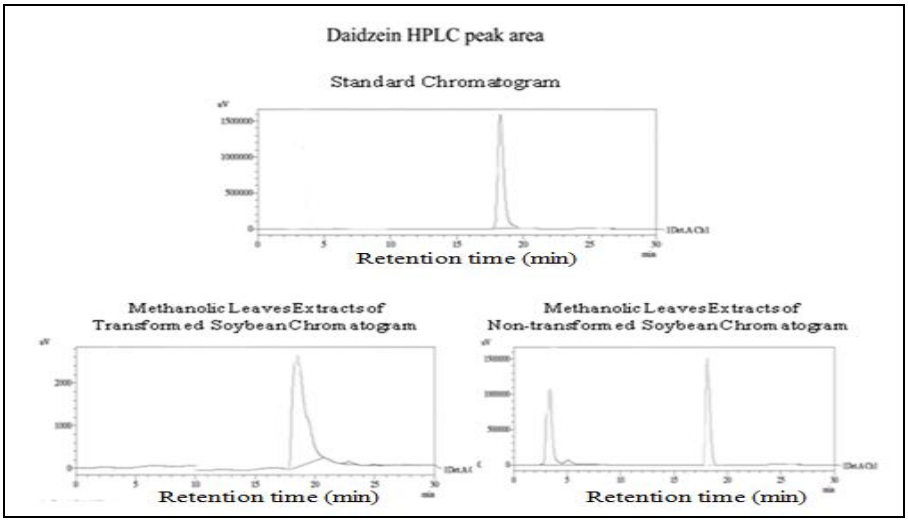


FIG. 1D: HPLC CHROMATOGRAM OF METHANOLIC LEAVES EXTRACTS OF TRANSFORMED AND NON-TRANSFORMED SOYBEAN WITH DAIDZEIN AT 254 nm

The quantification of isoflavones compounds by HPLC showed that methanolic leaves extracts of transformed soybean contained Genistein (1.67µg/g extract), Daidzein (2.76µg/g extract), Rutin (32.06µg/g extract) and Quercetin (48.40µg/g extract) and methanolic leaves extracts of non-transformed soybean contained Genistein (1.23µg/g extract), Daidzein (2.33µg/g extract), Rutin (26.16µg/g extract) and Quercetin (43.03µg/g extract) by comparing the chromatogram of the reference standards. In the present study revealed that secondary metabolite content was higher in the transformed plant than non-transformed plants.

Biochemical Activities: Total protein content was increased in transformed (5.58mg/g) than non-transformed (5.03mg/g) methanolic leaves extracts of soybean (**Fig. 2**).

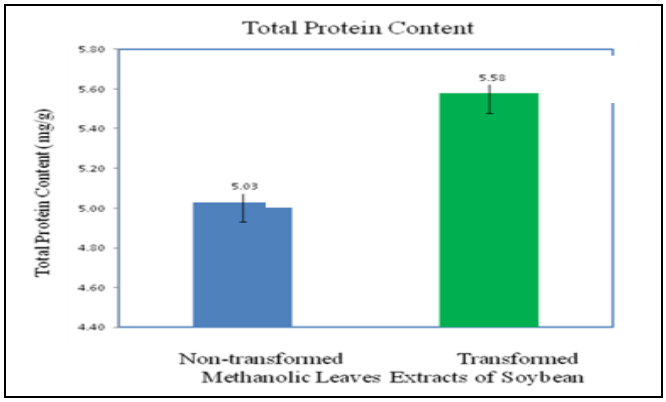


FIG. 2: TOTAL PROTEIN CONTENT OF BOTH TRANSFORMED AND NON-TRANSFORMED METHANOLIC LEAVES EXTRACTS OF SOYBEAN ± SE

The α-amylase activity was decreased in non-transformed (110.54 ± 0.2) and slightly increased

in transformed (116.89 ± 0.4) methanolic leaves extracts of soybean (**Fig. 3**).

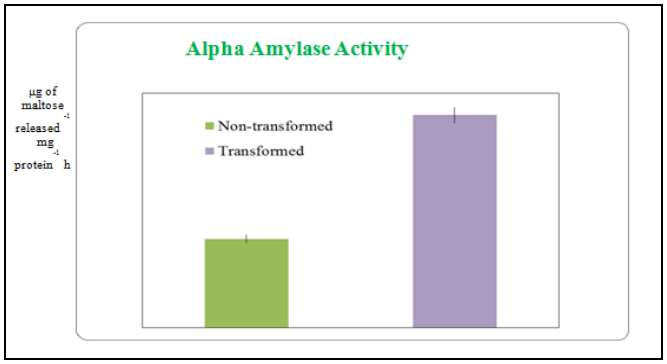


FIG. 3: *IN VITRO* ALPHA AMYLASE ACTIVITY OF TRANSFORMED AND NON - TRANSFORMED METHANOLIC LEAVES EXTRACTS OF SOYBEAN ± SE

Antioxidant Activity: In the present study, transformed and non-transformed methanolic leaves extracts was able to interact intensively with DPPH and reduced the stable violet DPPH radical to the yellow DPPH-H, reaching their 50% reductive plateau ranging between 2.09 and 69.50 µg/ml for extracts and standard respectively (**Fig. 4**). The reference antioxidant ascorbic acid recorded the highest scavenging efficiency toward DPPH radicals (2.09µg/ml) followed by transformed (44.76µg/ml) and non-transformed (69.50µg/ml).

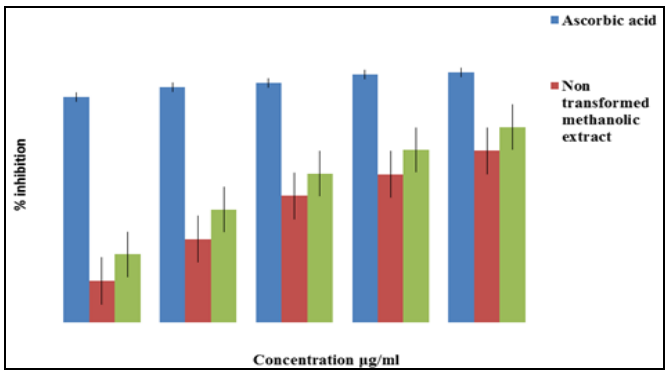


FIG. 4: DPPH RADICAL SCAVENGING ACTIVITY OF METHANOLIC LEAF EXTRACTS OF TRANSFORMED AND NON-TRANSFORMED SOYBEAN ± SE

Superoxide radical scavenging activity of transformed and non-transformed methanolic leaves extracts were assessed by alkaline DMSO method. The plant extracts moderately inhibit the superoxide radical generation. The values were represented in **Fig. 5**. The reference antioxidant ascorbic acid recorded the highest scavenging efficiency toward superoxide radicals (21.09 µg/ml) followed by transformed (25.90µg/ml) and non-transformed (33.91µg/ml).

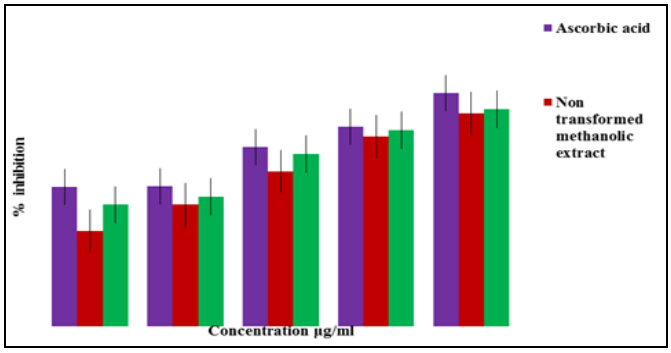


FIG. 5: SUPEROXIDE DISMUTASE SCAVENGING ACTIVITY OF METHANOLIC LEAF EXTRACTS OF TRANSFORMED AND NON-TRANSFORMED SOYBEAN ± SE

Adipocytes are the major target cells of PPARγ-agonists *in vitro* and *in vivo*. PPARγ is well demonstrated which has a major role in inhibiting differentiation of adipose tissue.

Effect of Methanolic Leaves Extracts of Transformed and Non-transformed Soybean on 3T3-L_1 Cell Viability: The effect of transformed and non-transformed methanolic leaves extracts of soybean on 3T3-L_1 cells viability was determined by the MTT assay. The results indicated that 48 hr exposure of cells with transformed and non-transformed methanolic leaves extracts of soybean caused a concentration-dependent reduction of pre-adipocyte proliferation and viability (**Fig. 6a** and **b**).

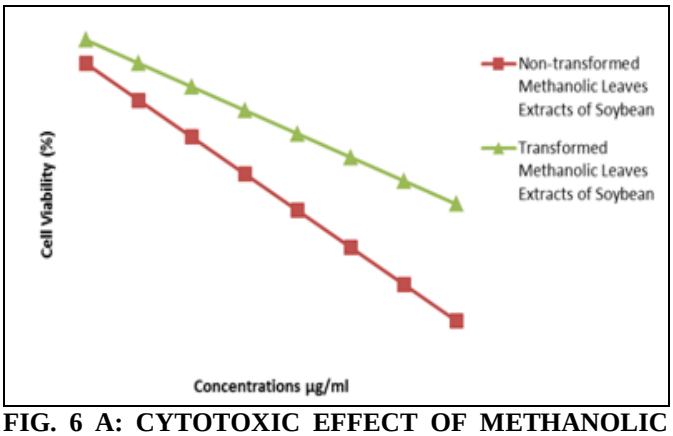


FIG. 6 A: CYTOTOXIC EFFECT OF METHANOLIC LEAVES EXTRACTS OF TRANSFORMED AND NON-TRANSFORMED SOYBEAN BY MTT ASSAY ± SE

The effect was found to suppress the growth of the 3T3-L_1 pre-adipocytes for transformed methanolic leaves extracts of soybean with 47.33 %.

A. CONTROL

B. NON-TRANSFORMED METHANOLIC LEAVES EXTRACTS OF SOYBEAN (100 µg/ml)

C. TRANSFORMED METHANOLIC LEAVES EXTRACTS OF SOYBEAN (100 µg/ml)

FIG. 6B: CYTOTOXIC EFFECT OF METHANOLIC LEAVES EXTRACTS OF TRANSFORMED AND NON-TRANSFORMED SOYBEAN BY MTT ASSAY

Methanolic Leaves Extracts of Transformed and Non-transformed Soybean inhibits lipid accumulation in 3T3-L_1 adipocytes: To investigate the effect of transformed and non-transformed methanolic leaves extracts of soybean on adipogenesis, intracellular lipid accumulation was determined (**Fig. 7a** and **b**). Confluent 3T3-L_1 cells were differentiated into mature adipocytes with a quantity of oil droplets in cells. Microscopic observation showed that 3T3-L_1 cells treated with methanolic leaves extracts maintained the fibroblastic morphology and a few fat droplets were observed after differentiation. Quantification of the extracted Oil Red O stain showed that the lipid content was also significantly reduced (**Fig. 7 a**).

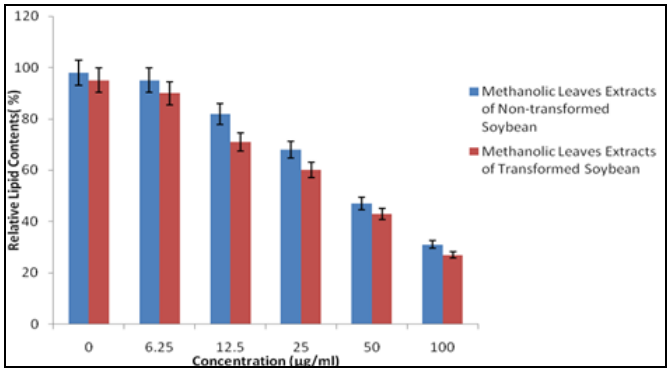
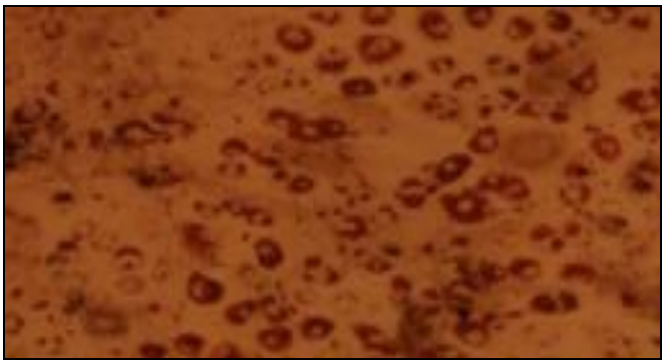
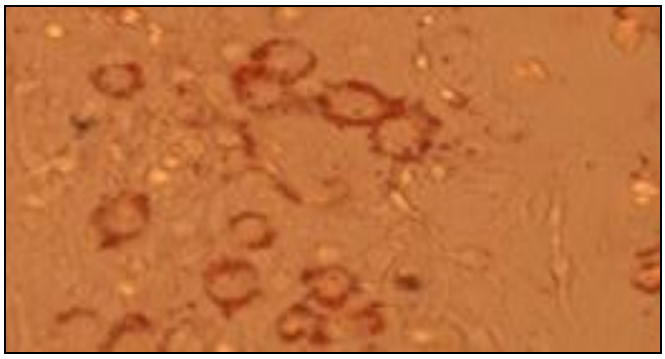


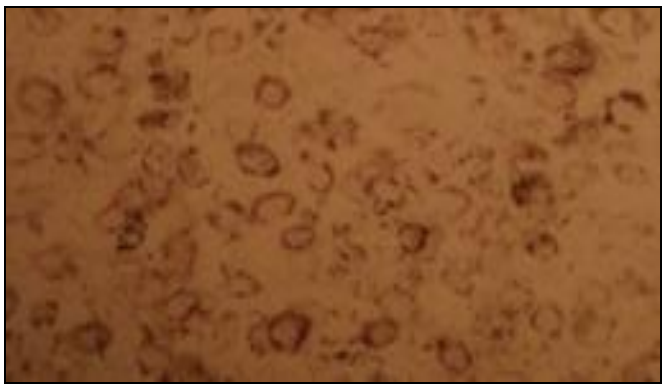
FIG. 7A: OIL RED O STAINING FOR LIPID ACCUMULATION OF TRANSFORMED AND NON-TRANSFORMED METHANOLIC LEAVES EXTRACT OF SOYBEAN FOR ANTIDIABETIC ACTIVITY IN 3T3-L_1 PRE-ADIPOCYTE CELL LINE ± SE



A. CONTROL



B. NON-TRANSFORMED METHANOLIC LEAVES EXTRACTS OF SOYBEAN (100 µg/ml)



C. TRANSFORMED METHANOLIC LEAVES EXTRACTS OF SOYBEAN (100 µg/ml)

Methanolic leaves extracts of transformed soybean (100µg/ml) could significantly reduce oil droplets (27%) compared with the negative adipogenesis control (**Fig. 7b**). Oil Red O staining indicated that transformed soybean could strongly suppress the differentiation of 3T3-L_1 pre-adipocytes.

DISCUSSION: Isoflavones are used for their health beneficial roles such as protection against hormone dependent osteoporosis and cancers in human and animal systems [43, 44]. There have been efforts to engineer their biosynthesis in non-leguminous plants, but very limited success could be achieved [45-51]. So the present study conducted that the RnPPARγ gene incorporated transformed and non-transformed methanolic leaves extracts of soybean with were enhanced the level of isoflavones. The well established beneficial effects of soy isoflavones on insulin sensitivity and lipid metabolism have been recently attributed to the activation of PPARγ and PPARα respectively [25, 52, 53]. However, little is known about the possibility that isoflavones activate PPARβ/ δ, which play a critical role in the regulation of metabolic homeostasis and also in cardiac lipid metabolism [54, 55]. Our results indicated that level of soy isoflavones may be enhanced due to expression of PPARγ.

In this study the level of isoflavones such as Quercetin, Rutin, Genistein and Daidzein were quantified (**Table 1**). Quercetin also reduced maltose induced postprandial hyperglycemia in type 2 diabetics by inhibiting intestinal α-glucosidase activity [56], inhibition of insulin-dependent activation of a phosphoinositol-3 kinase (PI-3K) [57], increase adiponectin levels [58] and decrease the intestinal maltose activity [59]. The PPAR regulated gene, carnitine palmitoyl-transferase I (CPT-I) that is regulated with fatty acid oxidation was identified in a genetic screen searching for soy isoflavones regulated mRNAs [60]. Recently evaluation of the PPAR induced acyl-CoA oxidase (ACO) gene demonstrated in a set of OZRs that ACO mRNA was significantly increased in rats fed diets containing either casein with rosiglitazone or isoflavone compared to levels of casein alone in rats [61].

The quercetin improves nutritional parameters and ameliorates the metabolic alterations were noticed this may enhanced by methanolic leaves extracts of soybean treatment. Moreover, quercetin normalized glucose levels and minimized the extracts related toxic effects on liver and kidney functions. These effects are associated with the powerful antioxidant properties of quercetin [62]. Anthocyanins of soybean have antioxidant effect and can be useful for the treatment of diabetes, cardiovascular disorders and cancers [63]. Recently genistein has been reported to act as a ligand of PPARγ [64]. Accordingly this study quantified the levels of isoflavones in that amount of quercetin was higher in transformed methanolic leaves extracts of soybean compared with non-transformed.

Sakamoto *et al.,* [65], suggest that daidzein regulates pro-inflammatory gene expression by activating PPAR-α and -γ and inhibiting the JNK pathway in adipocyte and macrophage co-cultures. Accordingly this study revealed that the levels of daidzein were enhanced in transformed leaves of soybean with RnPPARγ. If daidzein was LBD-independent, it might be alternatively due to post-translational modifications of PPARγ that affect its activity. Indeed, they have found that daidzein induces a translocation from the cytosol to the nucleus by using western blot techniques. Although the nuclear receptor super family was named after their nuclear localization, evidence supports that they also present an extra nuclear localization.

Depending upon ligand stimulation appears to exert important roles for nuclear receptors due to changes in localization, although little information is known about the mechanism that provokes cytosolic fraction or nucleo cytoplasmic shuttling of PPARγ [66, 67]. PPARγ activates ligand binding to control gene expression at the nucleus [68]; in addition, ligand binding also appears to enable translocation of PPARγ from the cytosol into the nucleus [69, 70] and associated with an enhancement of its nuclear activity. Hsu and Yen [71] found that rutin exhibits anti adipogenic activity mediated by the inhibition of the expression of C/EBPα and PPARγ2.

In the digestive system, Alpha-amylase is a key enzyme and catalyzes the hydrolysis of starch, which is a principal source of glucose in the diet [72]. In the small intestine, the activity of α -amylase correlates with postprandial glucose levels, the control of important factor in postprandial hyperglycemia, which is linked to the type 2 DM.

Our results are in agreed with previous studies and demonstrated that phenolic phytochemicals derived from plant have lower α-amylase inhibitory activity and stronger inhibition potential against α-glucosidase [73-75] and such natural inhibitors of enzyme would offer an attractive therapeutic approach for the treatment of postprandial hyperglycemia [74]. Therefore, these results indicate that soybean methanolic extract enriched with Genistein, Daidzein, Rutin and Quercetin have the potential to contribute the management of type 2 diabetes, because of their potent inhibition against of α -amylase.

For the past two decades, an explosive interest in the role of oxygen free radicals, more generally known as "reactive oxygen species" (ROS) and of "reactive nitrogen species" (RNS) in clinical medicine and experimental [76]. In biological systems, ROS or RNS are known to play a dual role since they can be either harmful or beneficial to living systems [77]. ROS can be important mediators of damage to cell structures, including nucleic acids, proteins and membranes of lipids termed as oxidative stress [78]. Reactive oxygen species and reactive nitrogen species such as hydroxyl radical, superoxide anions and nitric oxide inactivate enzymes and damage intracellular components causing injury through binding and lipid peroxidation.

The harmful effects of ROS are balanced by the antioxidants which hinder the processes of oxidative and thereby prevent oxidative stress [79]. Despite the presence of the antioxidant defense system to counter act oxidative damage from ROS, during the life cycle oxidative damage accumulates and damage to DNA, to proteins and to lipids due to radical has been proposed to play a role in the development of diseases such as, arteriosclerosis, cancer, arthritis, neurodegenerative disorders and other conditions [80].

To measure the efficiency of natural antioxidants either as plant extracts or as pure compounds developed by great number of *in vitro* methods. *In vitro* methods can be divided into two major groups: a) Hydrogen atom transfer reactions like Total radical trapping antioxidant potential (TRAP), Oxygen Radical Absorbance Capacity (ORAC) and β carotene bleaching; b) Electron transfer reactions like Ferric reducing antioxidant power (FRAP), Superoxide anion radical scavenging assay, α- diphenyl-β-picrylhydrazyl radical scavenging assay (DPPH), Trolox equivalent antioxidant capacity (TEAC), Hydroxyl radical scavenging assay, total phenol assay and Nitric oxide radical scavenging assay [80]. These methods are popular due to their high speed and sensitivity. However, it is essential to evaluate antioxidant capacity of plant materials because of the complex nature of the phytochemicals [81].

Non enzymatic antioxidant activity is measured by DPPH radical scavenging activity. An antioxidant scavenges the DPPH free radical by donating hydrogen, the purple colour of the DPPH solution becomes light yellow. The decrease in the absorbance was measured of radical scavenging activity. IC_{50} value corresponds with the higher antioxidant power was lower. DPPH radical scavenging activity of soybean methanolic leaves extracts were shown in the **Fig. 4**.

In the present study, DPPH and Superoxide radical scavenging assays were used to evaluate the antioxidant capacity of both transformed and non-transformed methanolic leaves extracts. DPPH assay is based on a hydrogen donor is an antioxidant. The activity of an antioxidant was measured by spectrophotometrically determining its absorbance at 515 nm to scavenge DPPH. Free radical with a dark purple colour becomes colourless when it reacts with antioxidants to form non-radicals. The results expressed that the transformed methanolic extract of soybean contain more amount of hydrogen donor molecules such as Genistein, Daidzein, Rutin and Quercetin which may reduce the production of radicals and the decolorization in the DPPH assay.

Our results further support the view that strong scavenging properties of soybean extracts on superoxide anion possibly render them as promising antioxidants. The results suggested that all the extracts are capable of scavenging free radicals, so it is able to prevent the initiation of free radical-mediated chain reactions by stabilizing reactive species before they can participate in deleterious reactions. In the present, the superior antioxidant activity of transformed leaves methanolic extract due to the present of higher content of Isoflavones.

Recent data proved that ROS react with thiol protein to produce sulphur oxidations which attenuates signal of insulin receptor and inhibits cellular uptake of TG from the blood stream [82]. Antioxidant activities could be inversely correlated with leptin and insulin resistance. Administration of quercetin enhanced SOD and GSH-Px activities in monosodium glutamate-treated rats and only doses of 75mg/kg quercetin were able to reduce both insulin and glucose levels in the rats. Quercetin acts as an effective antioxidant either by promoting antioxidant activities or by scavenging ROS after diffusion into the lipid bilayer membranes [83].

The differentiation of new fat cells termed as adipogenesis, considered a vigorous process and also intensive research field [84]. Numerous reports of adipogenesis have been performed using the 3T3-L1 cell line [85-88]. The changes in lipid accumulation and morphology of the cells were clearly observed on 4th day after the addition of differentiation media. The complete adipocyte differentiation could be achieved between 8 to 10 days [89]. The viability of control, quercetin or isorhamnetin treated and non-differentiated cells was higher than 90%; 3T3-L_1 cells were maintained for 10 days for further observations in terms of lipid accumulation, morphology and biomarker measurements [90].

Yang *et al.,* [90] investigations stated that concentration of 25µM decreased lipid accumulation around 15.9 ± 2.5% and concentration of 12.5µM was not significantly different from control. Furthermore, combinations of quercetin with resveratrol down-regulated the lipid accumulation by 68.7 ± 0.7% was found. Yang *et al.,* [90] also reported that quercetin concentrations from 0.1 to 20µM have potential in demonstrating dependent inhibition of adipocyte differentiation. Our results suggest that transformed methanolic leaves extracts of soybean might be effective in inhibiting lipid accumulation may be due to the presence of PPARγ gene. In this study, we demonstrated that incubation with both transformed and non-transformed methanolic leaves extracts reduced the lipid accumulation in a dose-dependent manner (**Fig. 7a** and **b**). This antidiabetic effect of soybean with PPARγ gene was achieved at different concentrations that did not affect cell viability according to the MTT assay. Several reports were suggested that the phytochemicals are potential anti-obesity agents and understanding the activities of these compounds during adipogenesis is essential to the development of new treatments for obesity [91]. Due to lipid accumulation, the differentiation of preadipocytes into adipocytes is associated with an increased number of oil red o-positive cells.

PPARγ is one of the isotypes of PPAR, which are ligand-activated transcription factors. They play a crucial role in the regulation of lipid and glucose metabolism in the condition of obesity [92]. It involves in the regulation of adipogenic process by binding to PPAR response elements in the promoter region of several target genes such as FABP4, lipoprotein lipase, glucose transporter-4, adiponectin and leptin. C/EBPα protein involves in the adipose tissue development by interacting with PPAR in their respective transcriptional targets [93]. Cdk4 kinase activity induced in response to signals of extracellular such as growth factors and signal translation from extracellular into cell cycle activation. Transcription factor E2F1 is the effector of the cdk/cyclin pathway, which stimulates the transition between pre-adipocyte proliferation and adipocyte differentiation through activation the PPARγ expression. Furthermore, Cdk4 is an upstream regulator of the E2F1/RB pathway and participates in adipocyte differentiation [94].

Fang *et al.,* [95] reported that in the mature 3T3-L_1 cell system, kaempferol and quercetin significantly improved insulin-stimulated glucose uptake, similar to a PPARγ agonist, such as rosiglitazone. Further experiments with transfected cells demonstrated in the PPARγ reporter gene assay that kaempferol and quercetin served as weak partial agonists. When combination of PPARγ agonist, rosiglitazone to 3T3-L_1 pre-adipocytes, they could inhibit 3T3-L_1 differentiation in a dose-dependent manner. A recent study that combined G, quercetin, and R and synergistically inhibited preadipocyte differentiation [96]. The competitive ligand binding assay confirmed that kaempferol and quercetin compete at the same binding site of PPARγ with rosiglitazone. The HPLC results proved that methanolic transformed soybean leaves extracts had quercetin was quantified with high content than the non-transformed that this may trigger the lipid accumulation.

CONCLUSION: The present study demonstrated that methanolic leaves extracts of transformed soybean had beneficial effects on α-amylase activity. After treatment of transformed methanolic leaves, extracts on 3T3-L_1 mature adipocytes significantly ameliorated lipid droplet accumulation and droplet reduction in adipocytes might be the result of lipid metabolism by lipolysis.

ACKNOWLEDGEMENT: Nil.

DECLARATION OF INTEREST: Nil.

REFERENCES:

1. WHO: Global status report on non-communicable diseases 2014.
2. WHO: Obesity and overweight 2016.
3. Shah A, Mehta N and Reilly MP. Adipose inflammation, insulin resistance, and cardiovascular disease. J Parenter Enteral Nutr. 2008; 32: 638-644.
4. Klein S, Allison DB, Heymsfield SB, Kelley DE, Leibel RL, Nonas C and Kahn R: Waist circumference and cardio-metabolic risk: a consensus statement from shaping America's health: Association for Weight Management and Obesity Prevention; NAASO, the Obesity Society; the American Society for Nutrition and the American Diabetes Association. Obesity 2007; 15: 1061-1067.
5. Maury E and Brichard S: Adipokine dysregulation, adipose tissue inflammation and metabolic syndrome. Mol Cell Endocrinol 2010; 314: 1-16.
6. Stephens JM: The fat controller: adipocyte development. PLoS Biol 2012; 10: e1001436.
7. Gesta S, Tseng YH and Kahn CR: Developmental origin of fat: tracking obesity to its source. Cell 2007; 31: 242-256.
8. Vestergaard P: Bone metabolism in type 2 diabetes and role of thiazolidinediones. Opin Endocrinol Diabetes Obes 2009; 16: 125-131.
9. Coelho M, Oliveira T and Fernandes R: Biochemistry of adipose tissue: an endocrine organ. Arch Med Sci 2013; 9(2): 191-200.
10. Ramji D and Foka P: CCAAT/enhancer-binding proteins: structure, function and regulation. Biochem J. 2002; 365: 561-575.
11. Tang QQ, Gronborg M, Huang H, Kim JW, Otto TC, Pandey A and Lane MD: Sequential phosphorylation of CCAAT enhancer-binding protein β by MAPK and glycogen synthase kinase 3β is required for adipogenesis. Proc Natl Acad Sci. 2005; 102: 9766-9771.
12. Rosen ED, Hsu CH, Wang X, Sakai S, Freeman MW, Gonzalez FJ and Spiegelman BM: C/EBPα induces adipogenesis through PPARγ: a unified pathway. Gen Dev 2002; 16: 22-26.
13. Huang H and Tindall DJ: Dynamic FoxO transcription factors. J Cell Sci 2007; 120: 2479-2487.
14. Xu H, Barnes GT, Yang Q, Tan G, Yang D, Chou CJ, Sole J, Nichols A, Ross JS and Tartaglia LA: Chronic inflammation in fat plays a crucial role in the development of obesity-related insulin resistance. J Clin Invest 2003; 112: 1821-1830.
15. Zhu Y, Qi C, Korenberg JR, Chen XN, Noya D, Rao MS and Reddy JK: Structural organization of mouse peroxisome proliferator-activated receptor gamma (mPPAR gamma) gene: alternative promoter use and different splicing yield two mPPAR gamma isoforms. Proc Natl Acad Sci 1995; 92: 7921-7925.
16. Kajimura S, Seale P, Tomaru T, Erdjument-Bromage H, Cooper MP, Ruas JL, Chin S, Tempst P, Lazar MA and Spiegelman BM: Regulation of the brown and white fat gene programs through a PRDM16/CtBP transcriptional complex. Genes and development 2008; 22: 1397-1409.
17. Ishibashi J, Firtina Z, Rajakumari S, Wood KH, Conroe HM, Steger DJ and Seale P: An Evi1-C/EBPβ complex controls peroxisome proliferator-activated receptor γ2 gene expression to initiate white fat cell differentiation. Mol Cell Biol 2012; 32(12): 2289-22.
18. Arita Y, Kihara S, Ouchi N, Takahashi M, Maeda K, Miyagawa JI, Hotta K, Shimomura I, Nakamura T and Miyaoka K: Paradoxical decrease of an adipose-specific protein, adiponectin, in obesity. Biochem. Biophys. Res. Commun. 1999; 257: 79-83.
19. Kadowaki T, Yamauchi T, Kubota N, Hara K, Ueki K and Tobe K: Adiponectin and adiponectin receptors in insulin resistance, diabetes, and the metabolic syndrome. J Clin Invest 2006; 116: 1784-1792.
20. Capeau J: The story of adiponectin and its receptors AdipoR1 and R2: to follow. J Hepatol 2007; 47: 736-738.
21. Yamauchi T, Nio Y, Maki T, Kobayashi M, Takazawa T, Iwabu M, Okada-Iwabu M, Kawamoto S, Kubota N and Kubota T: Targeted disruption of AdipoR1 and AdipoR2 causes abrogation of adiponectin binding and metabolic actions. Nat Med 2007; 13: 332-339.
22. He X and Sun LM: Dietary intake of flavonoid subclasses and risk of colorectal cancer: Evidence from population studies. Oncotarge 2016; 7: 26617–26627.
23. Shin DJ, Choi I, Yokoyama WH, Kim MJ and Kim Y: Decreased fat accumulation in 3T3-L_1 pre-adipocytes treated with extracts of heat-processed soy flour and breads. Intern J Food Sci and Technol. 2014; 49: 759–767.
24. Hidayat M, Prahastuti S, Fauziah N, Maesaroh M, Balqis B and Widowati W: Modulation of adipogenesis-related gene expression by ethanol extracts of Detam 1 soybean and Jati belanda leaf in 3T3-L_1 cells. Bangladesh J Pharmacol. 2016; 11(3): 697-702.
25. Mezei O, Banz WJ, Steger RW, Peluso MR, Winters TA and Shay N: Soy isoflavones exert antidiabetic and hypolipidemic effects through the PPAR pathways in obese Zucker rats and murine RAW264.7 cells. J. Nutrit. 2003; 133(5): 1238–1243.
26. Bitto A, Altavilla D and Bonaiut A: Effects of aglycone Genistein in a rat experimental model of postmenopausal metabolic syndrome. J. Endocrinol. 2009; 200(3): 367–376.
27. Dang ZC: Dose-dependent effects of soy phyto-oestrogen genistein on adipocytes: mechanisms of action: other review. Obesity Rev. 2009; 10(3): 342–349.
28. Kwon DY, Jang JS, Lee JE, Kim YS, Shin DH and Park S: The isoflavonoid aglycone-rich fractions of Chung kook jang, fermented unsalted soybeans, enhance insulin signaling and peroxisome proliferator activated receptor-*γ* activity *in vitro*. Bio Fact 2006; 26(4): 245–258.
29. Lopez LN, Tovar AR and Gonzalez-Granillo M: Pancreatic insulin secretion in rats fed a soy protein high fat diet depends on the interaction between the amino acid pattern and isoflavones. J Biol Chem 2007; 282(28): 20657–20666.
30. Ronis MJ, Chen Y, Badeaux J and Badger TM: Dietary soy protein isolate attenuates metabolic syndrome in rats via effects on PPAR, LXR and SREBP signaling. J. Nutri. 2009; 139(8): 1431–1438.
31. Shen P, Liu MH, Ng TY, Chan YH and Yong EL: Differential effects of isoflavones, from *Astragalus membranaceus* and *Pueraria thomsonii*, on the activation of PPAR*α*, PPAR*γ* and adipocyte differentiation *in vitro*. J Nutrit 2006; 136(4): 899–905.
32. Wagner JD, Zhang L and Shadoan MK: Effects of soy protein and isoflavones on insulin resistance and adiponectin in male monkeys, Metabol. 2008; 57(1): S24–S31.
33. Kim S, Shin HJ, Kim SY, Kim JH, Lee YS, Kim DH and Lee MO: Genistein enhances expression of genes involved in fatty acid catabolism through activation of PPARα. Molecul and cell endocrinol 2004; 220(1): 51-58.
34. Ruiz-Ojeda FJ, Ruperez AI, Gomez-Llorente C, Gil A and Aguilera CM: Cell Models and Their Application for Studying

Adipogenic Differentiation in Relation to Obesity: A Review. Int. J. Mol. Sci 2016; 17: 1040.
35. Hsieh CC, Chou MJ and Wang CH: Lunasin attenuates obesity-related inflammation in RAW 264.7 cells and 3T3-L_1 adipocytes by inhibiting inflammatory cytokine production. PLoS ONE. 2017; 12(2): e0171969.
36. Huang C, Pang D, Luo Q, Chen X, Gao Q, Shi L, Liu W, Zou Y, Li L and Chen Z: Soy Isoflavones Regulate Lipid Metabolism through an AKT/mTORC1 Pathway in Diet-Induced Obesity (DIO) Male Rats. Molecul. 2016; 21(5): 586.
37. Behnaz M, Davood EA and Atena A: Diurnal change in rutin content in *Capparis spinosa* growing wild in Tafresh/Iran. Europ J Experimen Biol. 2013; 3(3): 30-34.
38. Bradford MM: A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analyt. Biochem. 1976; 72: 248–254.
39. Bernfeld P: Amylase α and β, In Methods in Enzymology (Colowick SP, Kaplan NO, ed.), Academic Press Inc. 1955; 1: 149-158.
40. Shirwaikar A, Shirwaikar A, Rajendran K and Punitha ISR: *In vitro* antioxidant studies on the benzyl tetra tsoquinoline alkaloid berberine. Biol Pharm Bull 2006; 29: 1906-1910.
41. Elizabeth K and Rao MNA: Oxygen radical scavenging activity of curcumin. Int J Pharm. 1990; 58: 2370240.
42. Li KK, Liu CL, Shiu HT: Cocoa tea (*Camellia ptilophylla*) water extract inhibits adipocyte differentiation in mouse 3T3-L_1 preadipocytes. Scientific Rep. 2016; 6: 20172.
43. Peterson G and Barnes S: Genistein inhibition of the growth of human breast cancer cells: independence from estrogen receptors and the multi-drug resistance gene. Biochem Biophys Res Commun 1991; 179: 661–667.
44. Occhiuto F, Pasquale RD, Guglielmo G, Palumbo DR, Zangla G, Samperi S, Renzo A and Circosta C: Effects of phytoestrogenic isoflavones from red clover (*Trifolium pratense* L.) on experimental osteoporosis. Phytother Res 2007; 21: 130–134.
45. Jung W, Yu O, Lau SMC, O'Keefe DP, Odell J, Fader G and McGonigle B: Identification and expression of isoflavone synthase, the key enzyme for biosynthesis of isoflavones in legumes. Nat Biotech 2000; 18: 208–212.
46. Yu O, Jung W, Shi J, Croes RA, Fader GM, McGonigle B and Odell JT: Production of the isoflavones genistein and daidzein in non-legume dicot and monocot tissues. Plant Physiol 2000; 124: 781–794.
47. Liu CJ, Blount JW, Steele CJ and Dixon RA: Bottlenecks for metabolic engineering of isoflavone glycoconjugates in Arabidopsis. Proc Natl Acad Sci. 2002; 99: 14578–14583.
48. Tian L and Dixon RA: Engineering isoflavone metabolism with an artificial bifunctional enzyme. Planta 2006; 224: 496–507.
49. Liu R, Hu Y, Li J and Lin Z: Production of soybean isoflavone genistein in non-legume plants via genetically modified secondary metabolism pathway. Metab Eng. 2007; 9: 1–7.
50. Shih CH, Chen Y, Wang M, Chu IK and Lo C: Accumulation of isoflavone genistin in transgenic tomato plants overexpressing a soybean isoflavone synthase gene. J. Agric. Food Chem. 2008; 56: 5655–5661.
51. Misra P, Pandey A, Tewari SK, Nath P and Trivedi PK: Characterization of isoflavone synthase gene from *Psoralea corylifolia*: a medicinal plant. Plant Cell Rep. 2010; 29: 747–775.
52. Ricketts ML, Moore DD, Banz WJ, Mezei O and Shay NF: A molecular mechanism of action of the soy isoflavones includes activation of promiscuous nuclear receptors. A review. J Nutr Biochem 2005; 16: 321-330.
53. Davis J, Higginbotham A, O'Connor T, Moustaid-Moussa N, Tebbe A and Kim YC: Soy protein and isoflavones influence adiposity and development of metabolic syndrome in the obese male ZDF rat. Ann Nutr Metab 2007; 51: 42-52.
54. Gilde AJ, Van der Lee KA, Willemsen PH, Chinetti G, Van der Leij FR and Van der Vusse GJ: Peroxisome proliferator-activated receptor (PPAR) alpha and PPAR beta/delta, but not PPAR gamma, modulate the expression of genes involved in cardiac lipid metabolism. Circ Res 2003; 92: 518-524.
55. Sheng L, Ye P, Liu YX, Han CG and Zhang ZY: Peroxisome proliferator-activated receptor beta/delta activation improves angiotensin II-induced cardiac hypertrophy *in vitro*. Clin Exp Hypertens 2008; 30: 109-119.
56. Hussain SA, Ahmed ZA, Mahwi TO and Aziz TA: Quercetin Dampens Postprandial Hyperglycemia in Type 2 Diabetic Patients Challenged with Carbohydrates Load. J Diabetes Res 2012; 1(3): 32-35.
57. Steward LK, Wang Z, Ribnicky D, Soileau JL, Cefalu WT and Gettys TW: Failure of dietary quercetin to alter the temporal progression of insulin resistance among tissues of C57BL/6J mice during the development of diet-induced obesity. Diabetologia. 2009; 52: 514-523.
58. Wein N, Avril A and Bartoli M: Efficient bypass of mutations in dysferlin deficient patient cells by antisense-induced exon skipping. Hum Mutat 2010; 31: 136–142.
59. Kim YJ, Kim YA and Yokozawa T: Pycnogenol modulates apoptosis by suppressing oxidative stress and inflammation in high glucose-treated renal tubular cells. Food Chem Toxicol 2011; 49: 2196–2201.
60. Iqbal MJ, Yaegashi S, Ahsan R, Lightfoot DA and Banz WJ: Differentially abundant mRNAs in rat liver in response to diets containing soy protein isolate. Physiol Genomics. 2002; 11: 219–26.
61. Banz WJ, Iqbal MJ, Ahsan, Winters TA and Lightfoot DA: The effects of high- and low isoflavones soy protein diets on gene expression in lean and obese rats. FASEB J 2001; 15: A632.
62. Seiva FRF, Chuffa LGA, Braga CP, Amorim JPA and Fernandes AAH: Quercetin ameliorates glucose and lipid metabolism and improves antioxidant status in postnatally monosodium glutamate - induced metabolic alterations. Food Chem Toxicol 2012; 50: 3556 – 3561.
63. A Critical Review on Polyphenols and Health Benefits of Black Soybeans. Ganesan K, Xu B: Nutrients 2017; 9: 455.
64. Dang ZC, Audinot V, Papapoulos SE, Boutin JA and Lowik CW: Peroxisome proliferator-activated receptor gamma (PPAR gamma) as a molecular target for the soy phytoestrogen genistein. J Biol Chem 2003; 278: 962–967.
65. Sakamoto Y, Kanatsu J, Toh M, Naka A, Kondo K and Iida K: The Dietary Isoflavone Daidzein Reduces Expression of Pro-Inflammatory Genes through PPARα/γ and JNK Pathways in Adipocyte and Macrophage Co-Cultures. PLoS One 2016; 11(2): e0149676.
66. Kelly D, Campbell JI, King TP, Grant G, Jansson EA, Coutts AG, Pettersson S and Conway S: Commensal anaerobic gut bacteria attenuate inflammation by regulating nuclear-cytoplasmic shuttling of PPAR-gamma and RelA. Nat. Immunol 2004; 5: 104–112.
67. Burgermeister E and Seger R: MAPK kinases as nucleo-cytoplasmic shuttles for PPAR gamma. Cell Cycle 2006; 6: 1539–1548.
68. Tontonoz P and Spiegelman BM: Fat and beyond: the diverse biology of PPAR gamma. Annu Rev Biochem 2008; 77: 289–315.
69. Shibuya A, Wada K, Nakajima A, Saeki M, Katayama K, Mayumi T, Kadowaki T, Niwa H and Kamisaki Y: Nitration of PPAR gamma inhibits ligand dependent translocation into the nucleus in a macrophage-like cell line, RAW 264. FEBS Lett 2002; 525: 43–47.
70. Xu and Xing: A new dromaeosaurid (Dinosauria: Theropoda) from the Upper Cretaceous Wulansuhai Formation of Inner Mongolia, China." Zootaxa 2010; 24(3): 1-9.
71. Hsu CL and Yen GC: Effects of flavonoids and phenolic acids on the inhibition of adipogenesis in 3T3-L_1 adipocytes. J Agric Food Chem 2007; 55: 8404–8410.

72. Tarling CA, Woods K, Zhang R, Brastianos HC, Brayer GD, Ersen RJ and Withers SG: Chem Bio Chem 2008; 9: 433–438.
73. Apostolidis E, Kwon YI and Shetty K: Potential of cranberry-based herbal synergies for diabetes and hypertension management. Asia Pac. J. Clin. Nutr 2006; 15: 433–441.
74. Kwon YI, Apostolidis E and Shetty K: *In vitro* studies of eggplant (*Solanum melongena*) phenolics as inhibitors of key enzymes relevant for type 2 diabetes and hypertension. Bioresour. Technol 2008; 99: 2981–2988.
75. Apostolidis E and Lee C: *In vitro* potential of *Ascophyllum nodosum* phenolic antioxidant-mediated –α- glucosidase and β-amylase inhibition. J. Food Sci 2010; 75: H97–H102.
76. Halliwell B and Gutteridge JMC: Free radicals in biology and medicine. 4th Edition, Oxford UK 2007.
77. Valko M, Izakovic M, Mazur M, Rhodes CJ and Tesler J: Role of oxygen radicals in DNA damage and cancer incidence. Mol Cell Biochem 2004; 266: 37-56.
78. Poli G, Leonarduzzi G, Biasi F and Chiarpotro E: Oxidative stress and cell signaling. Curr Med Chem 2004; 11: 1163-1182.
79. Rajkapoor B, Burkan ZE and Senthilkumar R: Oxidants and Human diseases: Role of antioxidant medicinal plants- A Review. Pharmacol 2010; 1: 1117-1131.
80. Huang DJ, Chen HJ, Lin CD and Lin YH: Antioxidant and antiproliferative activities of water spinach (*Ipomoea aquatica* Forsk) constituents. Bot Bull Acad Sin 2005; 46: 99-106.
81. Salazar R, Pozos ME, Cordero P, Perez J, Salinas MC and Waksman N: Determination of the antioxidant activity of plants from Northeast Mexico. Pharm Biol 2008; 46: 166-170.
82. Chen YE, Fu M, Zhang J, Zhu X, Lin Y, Akinbami MA and Song Q: Peroxisome proliferator-activated receptors and the cardiovascular system. Vitam Horm 2003; 66: 157-188.
83. Vieira EK, Bona S, Di Naso FC, Porawski M, Tieppo J and Marroni NP: Quercetin treatment ameliorates systemic oxidative stress in cirrhotic rats. ISRN Gastroenterol 2011; 1–6.
84. Ailhaud G and Hauner H: Development of white adipose tissue. In: Bray AGB (ed) Handbook of obesity: Etiology and pathophysiology. Marcel Dekker, New York 2004.
85. Moon HS, Chung CS, Lee HG, Kim TG, Choi YJ and Cho CS: Inhibitory effect of epigallocatechin-3-gallate on lipid accumulation of 3T3-L_1 cells. Obesity 2007; 15(11): 2571–2582.
86. Trayhurn P: Adipocyte biology. Obes Rev 2007; 8(1): 41–44.
87. Park T and Kim Y: Phytochemicals as potential agents for prevention and treatment of obesity and metabolic diseases. In: Atta-ur-Rahman, Choudhary MI (eds) Anti-obesity drug discovery and development. Bentham Sci 2011; 150–185.
88. Monteros LG, Ramon-Gallegos E, Torres-Torres N and Mora-Escobedo R: Effect of germinated soybean protein hydrolysates on adipogenesis and adipolysis in 3T3-L_1 cells. Plant Food Hum Nutr 2011; 66(4): 355-62.
89. Zebisch K, Voigt V, Wabitsch M and Brandsch M: Protocol for effective differentiation of 3T3-L_1 cells to adipocytes. Anal Biochem 2012; 425(1): 88-90.
90. Yang L, Li XF, Gao L, Zhang YO and Cai GP: Suppressive Affects of Quercetin 3 O (6 ″Feruloyl) β D Galactopyranoside on Adipogenesis in 3T3-L1 Preadipocytes Through Down regulation of PPARγ and C/EBPα Expression. Phyto Res 2012; 26(3): 438-444.
91. Sharma AM, Bramlage P and Kirch W: Antihypertensive effect of irbesartan and predictors of response in obesity-associated hypertension: a prospective, open-label study. Clin Drug Investig 2005; 25: 765-776.
92. Kadowaki T, Hara K, Kubota N, Tobe K, Terauchi Y, Yamauchi T, Eto K, Kadowaki H, Noda M, Hagura R and Akanuma Y: The role of PPARγ in high-fat diet-induced obesity and insulin resistance. J Diabetes Complications 2002; 16(1): 41-45.
93. Moseti D, Regassa A and Kim WK: Molecular regulation of adipogenesis and potential anti-adipogenic bioactive molecules. International J Mol Sci 2016; 17(1): 124.
94. Abella A, Dubus P, Malumbres M, Rane SG, Kiyokawa H, Sicard A, Vignon F, Langin D, Barbacid M and Fajas L: Cdk4 promotes adipogenesis through PPARγ activation. Cell metabol 2005; 2(4d): 239-249.
95. Fang XK, Gao J and Zhu DN: Kaempferol and quercetin isolated from *Euonymus alatus* improve glucose uptake of 3T3-L_1 cells without adipogenesis activity. Life Sci 2008; 82(11): 615-622.
96. Ahmed B, Liu S and Si H: Antiadipogenic Effects and Mechanisms of Combinations of Genistein, Epigallocatechin-3-Gallate, and/or Resveratrol in Preadipocytes. J Med Food 2016; 1–9.

How to cite this article:
Janani C and Kumari BDR: Antidiabetic activity of methanolic leaves extracts of transformed soybean in 3T3-L_1 pre-adipocytes cell line. Int J Pharm Sci Res 2017; 8(11): 4732-44.doi: 10.13040/IJPSR.0975-8232.8(11).4732-44.