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Research Article

Evaluation Of *Solanum Melongena* Linn. Fruit Extract On Myonectin Expression- An In Vitro Approach

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ABSTRACT

Myonectin is a significant endogenous protein which was discovered in 2011 and many research works were done on this protein. It has tremendous physiological effects in human body. Myonectin secreted by skeletal muscle and it regulate many metabolic processes in human body, and its low concentrations may play a role in the pathophysiology of Metabolic syndrome, by favouring the accumulation of abdominal fat and the consequent development of IR. Myonectin is also known as C1q/TNF-related protein isoform 15 (CTRP15), whose gene is located on locus2q37.3. This myokine was discovered very recently by Seldin and coworkers. According to the authors, myonectin expression is stimulated by two main factors: exercise and nutrients. Fasting circulating myonectin levels were shown to be low and to increase 2 hours after the intake of glucose or lipid Ethnopharmacological evidence shows that Solanum melongena Linn. Fruits possesses various pharmacological effects including anti-oxidant, anti-diabetic, antihypertensive, anti – hyperlipidaemic, and anti-obesity etc. Myonectin expression was quantified by RT-qPCR method. Total phenol content in solanum melongena extract was estimated by folin-ciocalteu method and total flavonoid content was estimated by aluminium chloride colorimetric method. Purpose of this study was to evaluated the effect of solanum melongena Linn. fruits extract effect on myonectin expression by using RAW 267.4 cell line. The invitro result showed that Solanum melongena Linn. Fruits extract modulate the myonectin gene expression in vitro and this result helps the future researchers. In future this positive effect of Solanum melongena Linn. Fruits shall test for clinical efficacy for the treatment of myonectin deficiencies.

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INTRODUCTION

Myokines are cytokines or peptides synthesised and released by myocytes in muscle tissue in response to muscular contractions and are released by the skeletal muscles during the physical activity1.Myokines are implicated in the autocrine regulation of metabolism in muscles as well as in the paracrine or endocrine regulation of other tissues and organs 2. Myostatin was first identified as a myokine in 1997, secretome-based analysis of human myocyte culture medium has revealed over 600 myokines till date3. Myonectin is a novel myokine that is mostly expressed in skeletal muscle tissues. It is also referred to as C1q (complement component 1q), tumour necrosis factor-related protein 15 (CTRP15), and Acute exercise and nutrition, like glucose and fatty acids, increase myonectin expression as the primary regulators4,5,6. which encourages adipocytes to absorb fatty acids and hepatocytes 6, Myonectin expression is predominant in slow-twitch muscles, compared to that in fast-twitch muscles5. Its domain structure is comparable to that of adiponectin, a well-known adipocytokine4. Myonectin may play a role in glucose, lipid, and energy metabolism disorders, including obesity and diabetes, according to a number of studies 4,6,7, 8,9. Studies reveals that myokines provide protection against charges in metabolism and metabolic syndrome risk factors. Irisin, apelin, and interleukin-6 (IL-6) enhance lipid metabolism and glycemic control in this manner 10,11,12. Similarly, it has been suggested that myonectin (erythroferrone or CTRP15) is a myokine that positively affects lipid control by lowering amounts of total FFA in circulation, most likely through enhancing fatty acid transport in hepatocytes and adipocytes in vitro13. Also, myonectin acts as a protective factor against skeletal muscle dysfunction and it also described as nutrient responsive regulator of liver autophagy. Myonectin attenuates the inflammatory response in macrophages. It reduces the expression of pro inflammatory cytokines such as TNF-α, IL-6, and MCP-1 in macrophages stimulated with lipopolysaccharide14. Myonectin acts as a biomarker of various disorders like diabetes, liver steatosis, PCOD etc. Progressive resistant training, high intensity interval training and aerobic exercise are the endogenous factors which upregulating the expression of myonectin. Dietary vitamin B12, curcumin, culinary herbs and spices, olive oil and other oils are the exogenous factors stimulating myonectin expression14. This study investigates the role of solanum melongena Linn. fruits effects on myonectin expression in RAW 267.4 cell line. Eggplant is an annual or short-lived perennial plant that is very sensitive to cold temperatures. It grows fastest when the temperature ranges between 70 and 85 degrees. It produces an edible shiny glossy fruit. The plant may grow 2 to 4 feet tall and is multi-branched. The leaves and stems have star-shaped hairs, and the small violet flowers are also star-shaped. Eggplant or Aubergine is a member of the Solanaceae or nightshade family which also includes tomatoes, potatoes, and peppers. It is grown primarily as a food crop. The eggplant grows best in full sun. It prefers moist, well-drained, fertile, sandy, and loamy soils with a pH range of 5.5 to 6.8. It is propagated by seeds and germination occurs in 8 -12 days. The fruits may be harvested in about 105-133 days. The plant's flowers attract bumblebees. The leaves and stems are covered with star-shaped hairs and sometimes prickles. The flowers are solitary, star-shaped, and usually violet in colour. The fruit is a large fleshy smooth berry. The fruit colour varies from white, green, or purple to black depending on the cultivar. The fruit has many pale brown kidney-shaped seeds. The medicinal properties of the plant are derived from its chemical constituents. The plant’s antioxidant property is due to the flavonoids. The

terpenes (steroids) make it useful for bronchitis. Analgesic property is because of the alkaloids. The plant also shows antipyretic activity, Anti-asthmatic activity, Antiplatelet and Calcium channel blocking activities, CNS Depressant Activity etc. Plenty of the research reveal that the plant has antioxidant, hepato protective and hypolipidemic effects. The current research examines the impact of Solanum melongena Linn fruits effect on myonectin by using RAW 267.4 cell line.

MATERIALS AND METHODS

MATERIALS:

Solanum melongena Linn. fruits, Alcohol

1. **COLLECTION OF Solanum melongena LINN. FRUITS**

Solanum melongena Linn. Fruits of about 4kg were collected from Kottayam, Kerala, India on January 2024 in the morning and it was identified and authetified by Dr. Rojimon P. Thomas, HOD, Department of Botany, CMS College Kottayam. (Herbarium number 2879). it was shade dried for 1 week. After drying it was coarsely powdered.

2. **ETHANOLIC EXTRACTION AND PHYTOCHEMICAL ANALYSIS OF Solanum melongena Linn. FRUITS**

490g of dried and coarsely powdered Solanum melongena Linn. Fruits were extracted using maceration by ethanol (96%) as a solvent. Evaporation done with the help of Rotary evaporator.

Percentage yield = [weight of the dried extract / weight of the fruits] *100

= [41.47/331] * 100

= 12.5 % w/w

A **B**

Figure 1. A) Maceration 1.B) Rotary evaporator

3. **PHYTOCHEMICAL SCREENING**

Preliminary phytochemical analysis

Medicinal plants have been used in the treatment of various diseases as they possess potential pharmacological activities including antineoplastic, antimicrobial, antioxidant, anti-inflammatory, analgesic, anti-diabetic, anti-hypertensive, antidiarrheal and other activities. Phytoconstituents individually or in combination, determine the therapeutic value of a medicinal plant. Alkaloids, flavonoids, phenolics, tannins, saponins, steroids, glycosides, terpenes, etc. are some of the important phytochemicals with diverse biological activities. The pharmacological activity of a plant can be predicted by the identification of the phytochemicals. The extract obtained from natural sources has definite specific chemical constituents to which their biological or pharmacological activity is attributed. The ethanol extract of Physalis minima L. fruits was subjected to preliminary phytochemical analysis to assess the presence of various phytoconstituents15.

4. **ESTIMATION OF TOTAL PHENOLIC CONTENT**

Method: Folin-Ciocalteu method

The total phenolic content was determined using a Folin–Ciocalteu reagent according to the procedure reported by Singleton and Rossi in 196516. Many available methods of quantification of total phenolic content in food products or

biological samples are based on the reaction of phenolic compounds with a colorimetric reagent, which allows measurement in the visible portion of the spectrum. The Folin–Ciocalteu (F–C) assay is such a method and has been proposed as a standardized method for use in the routine quality control and measurement of the antioxidant capacity of food products and dietary supplements. The F–C assay relies on the transfer of electrons in an alkaline medium from phenolic compounds to phosphomolybdic/phosphotungstic acid complexes to form blue complexes that are determined spectroscopically at approximately 765 nm. The exact chemical nature of the F–C reaction is unknown; it is believed that sequences of reversible one- or two-electron reduction reactions lead to blue species17.

Phenolic content was estimated by the equation;

Total phenol content, C = x(V/M)

x-obtained from the equation for straight line of the standard graph of gallic acid

y = mx+c

y - mean absorbance of test at 765 nm

m – slope

c - y-intercept

V - volume of extract taken

M - amount of extract in the taken volume

5. ESTIMATION OF TOTAL FLAVONOIDS

Method: Aluminium Chloride Colorimetric Method

Flavonoids consist of a large group of polyphenolic compounds having a benzo-γ-pyrone structure and are ubiquitously present in plants. They are synthesized by phenylpropanoid pathway. Total flavonoid contents can be determined in the sample extracts by reaction with sodium nitrite, followed by the development of pink-coloured flavonoid-aluminium complex formation using Aluminium Chloride in alkaline conditions which can be monitored spectrophotometrically at a wavelength of 510 nm18.

Flavonoid content was estimated by the equation;

Total flavonoid content, C = x(V/M)

x-obtained from the equation for straight line of the standard graph of quercetin

y = mx + c

y - mean absorbance of test at 510 nm

m – slope

c - y-intercept

V - volume of extract taken

M - amount of extract in the taken volume.

6. IN-VITRO STUDY

6.a. EFFECT OF SOLANUM MELONGENA LINN. FRUITS ON RAW 267.4 CELLS BY REAL TIME PCR ANALYSIS

1. Isolation of total RNA (trizol) method.
2. cDNA synthesis
3. Gene expression analysis by RT-Qpcr
4. Agarose gel electrophoresis

ISOLATION OF TOTAL RNA (TRIzol METHOD)

Total RNA was isolated using the total RNA isolation kit. Addition of TRIzol solution causes the disruption of cells and the release of RNA. Chloroform extraction following centrifugation, exclusively in the aqueous phase whereas proteins are in the interphase and organic phase. On mixing with isopropanol, RNA gets precipitated as a white pellet on the side and the bottom of the tube. 1ml of TRIzol reagent was added to culture well plate and incubated for 5 minutes. The contents were then transferred to a fresh sterile Eppendorf tube. 200µL of chloroform was at room temperature, followed by centrifugation at 14000rpm for 15 minutes at 40 C. The aqueous layer was collected and 500µL of 100% isopropanol was added. It was incubated for 10 minutes at room temperature and then centrifuged at 14000rpm for 15 minutes at 40 C. The supernatant was discarded and pellet thus obtained was washed with 200µL of 75% ethanol (Merck). It was then centrifuged at 14000rpm for

5 minutes at 40 C in a cooling centrifuge (Remi CM12). The RNA pellet was dried and suspended in TE buffer20,21.

cDNA SYNTHESIS

Total RNA was extracted using Trizol (Invitrogen, USA). The purity and the concentration of total RNA was determined. Template complementary DNA was synthesized using the cDNA preparation kit (G BIOSCIENCES, Product code- 786-5019s, 786-5020, master premix for first-strand cDNA synthesis). About 5µL of RT Easy mix, 0.5µL of oligo dT, and 2µL of RNA template (0.5µg of total RNA) were added to an RNAse free tube. Then the total reaction volume was made up to 10µL with the addition of sterile distilled water. The solution was mixed by pipetting gently up and down. The thermal cycler (Eppendorf Master Cycler) was programmed to undergo cDNA synthesis. The following cycling conditions were employed, 20minutes at 42ºC and 5 minutes at 85ºC.

Table 1: cDNA synthesis

Step	Temperature ºC	Time (minutes)	Number of cycles
cDNA synthesis	42	20	1
Inactivation	85	5	1

GENE EXPRESSION ANALYSIS BY RT-qPCR

Real-Time qRT-PCR analysis was carried out using SYBR Green Master Mix (G BIOSCIENCES, Product code-786-5062) using Light cycler 96 (Roche). All reactions were performed in triplicates and data were analysed according to ΔΔCt method (using Light Cycler 96 SW 1.1 Software).

Table 2: Gene expression analysis by RT-qPCR

Steps	Time required	Temperature
Initial activation step	2 minutes	95ºC
3 step cycling		
Denaturation	10 seconds	95ºC
Annealing	1 minutes	58ºC
Extension	1 min/kb	72ºC
Number of cycles	40 cycles	
End of PCR cycling	Indefinite	4ºC

Table 3: The primer sequences

OLIGO NAME	FORWARD		REVERSE	
	SEQUENCE (5’ ->3’)	Tm	SEQUENCE (5’ ->3’)	Tm
GAPDH	AGGTCGGTGTGAACGGATTTG	65.8	GGGGTCGTTGATGGCAACA	65.9
Myonectin	TGCTTGGATGCTGTTCGTCAA	65.8	CAGATGGGATAAAGGGGCCTG	65.5

AGAROSE GEL ELECTROPHORESIS

Agarose gel electrophoresis is a method for separating and visualizing DNA fragments. The fragments are separated by charge and size and move through agarose gel matrix, when subjected to an electric field. The electric field is generated by applying potential across an electrolyte solution (buffer). When boiled in an aqueous buffer, agar dissolve and upon cooling solidifies to a gel. 1.5% agarose gel was prepared in 1x TE buffer and melted in hot water bath at 90◦C.Then the melted agarose was cooled down to 450C. 6µl of 10 mg/mL of ethidium bromide was added and poured in to a gel casting apparatus with the gel comb. After setting, the comb was removed from the gel. The electrophoresis buffer was poured in the gel tank and the platform with the gel was placed in it so as to immerse the gel. The gel was loaded with the samples and run at 50 V for 30 minutes. The stained gel was visualized using a gel

documentation system (ChemiDoc imaging system,Biorad).

RESULTS

a. EXTRACTION

The process of phytochemical processing that leads to the identification of bioactive components in plant materials includes a crucial step called extraction, choosing an appropriate extraction method is crucial for the standardization of herbal products. The quality and purity of the medicine are determined by the crude drug's extraction yield. Many factors influence extraction yields from biomass, and some of these factors can even influence one another in interdependent ways (174).490g of dried and coarsely powdered Solanum melongena Linn. Fruits were extracted using maceration by ethanol (96%) as a solvent. Evaporation done with the help of Rotary evaporator.

Extract yield of Solanum melongena Linn. Fruits prepared by maceration with ethanol is determined by = [weight of the dried extract / weight of the fruits] *100

= [41.47/331] * 100

= 12.5 % w/w

The extraction yield was used as an indicator of the effects of the extraction conditions. The percentage yield obtained after ethanolic extraction of Solanum melongena Linn. fruit by maceration method is 12.5 % w/w.

b. Phytochemical studies

Qualitative phytochemical analysis

Ethanol extract of Solanum melongena Linn. fruit was subjected to qualitative chemical analysis for the identification of various phytoconstituents like alkaloids, glycosides, flavonoids, carbohydrates, sterols, etc.

Table 4: Qualitative phytochemical analysis

Chemical test	Observation	Inference
Test for Carbohydrates:		
Molisch's test	Formation of violet ring at the junction	+
Fehling's test	Formation of red precipitate	++
Benedict's test	Formation of reddish-brown precipitate.	++
Test for Alkaloids:		
Mayer's test	Absence of white/creamy white precipitate	+
Wagner's test	Absence of reddish-brown precipitate	+
Dragendroff's test	Absence of reddish orange precipitate	++
Test for phenolics		
Ferric chloride test	Formation of blue black or violet	++
Lead acetate test	Formation of bulky white precipitate	++
Test for flavonoids:		
Aqueous NaOH test	Formation of yellow orange Colour	++
H_2SO_4 test	Formation of orange colour	++
Shinoda test	Formation of orange red	++
Test for Glycosides:		
Legal's test	Formation of pink to red colour	+
Test for saponins:		
Foam test	Presence of foam formation	+ +
Test for proteins:		
Biuret test	Formation of pink colour in the ethanolic layer	++
Ninhydrin test	Formation of purple colour	+
Millon's test	Formation of purple colour	+
Xanthoproteic test	Absence of yellow colour	-

Test for terpenoids: Isoprenoid test	Reddish brown colouration	+

(++) - Indicate active constituents in high amount
(+) - Indicate active constituents in low amount
(−) - Indicate the absence of active constituents

The preliminary phytochemical screening showed the presence of carbohydrates, glycosides, phenolics, flavonoids, protein, terpenoids and steroids, etc. The extract of Physalis minima L. fruits showed an abundance of phenol and flavonoids during the preliminary phytochemical screening.

c. Quantitative phytochemical analysis

Estimation of total phenolic content

Table 5: Standard graph values of gallic acid

Concentration (µg/mL)	Mean absorbance (750nm)
25	0.232 ± 0.07
50	0.451 ± 0.01
75	0.67 ± 0.01
100	0.88 ± 0.06
125	1.1 ± 0.10
150	1.368 ± 0.12

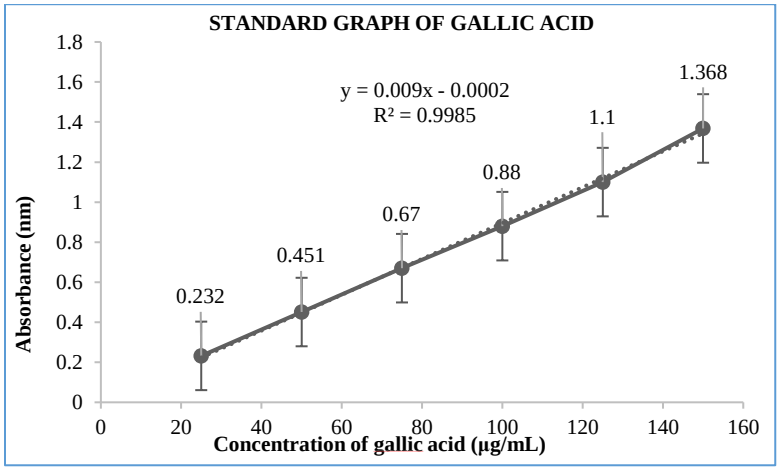

Figure 3: Standard graph of quercetin

Calculation

Mean absorbance of solanum melongena Linn fruit extract at 515nm = 0.521nm

Concentration from graph (µg/ml) = 102.01 (µg/ml)

From the calibration curve of quercetin phenolic content of ethanolic extract were found to be 102.01 (µg/ml)

e. IN-VITRO STUDY

REAL TIME PCR ANALYSIS

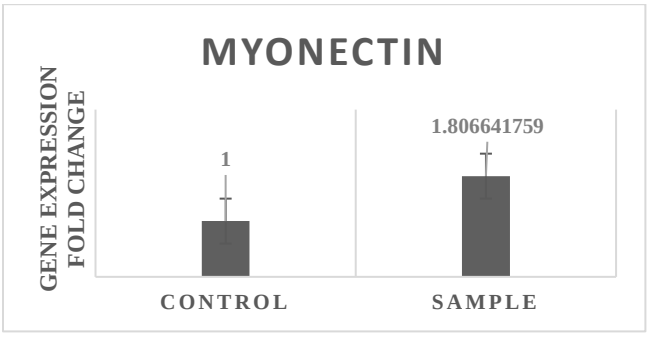

Figure 4: Graphical representation of expression fold change of Myonectin

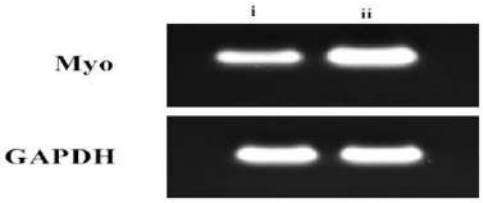

Figure 5: PCR gel image

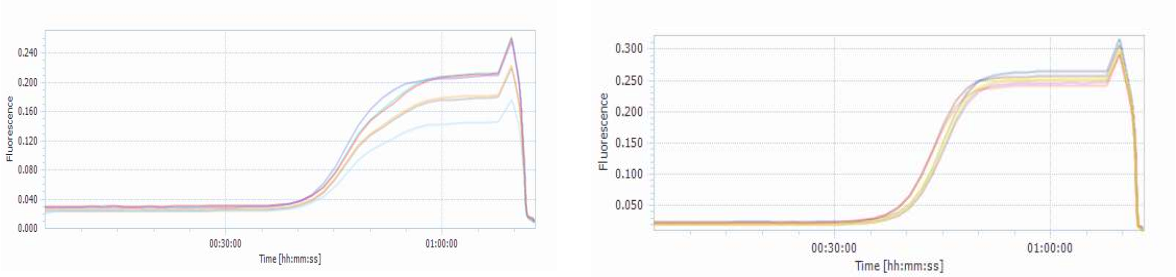

Figure 6 A) Fluorescence curves of GAPDH B) Fluorescence curve of Myonectin

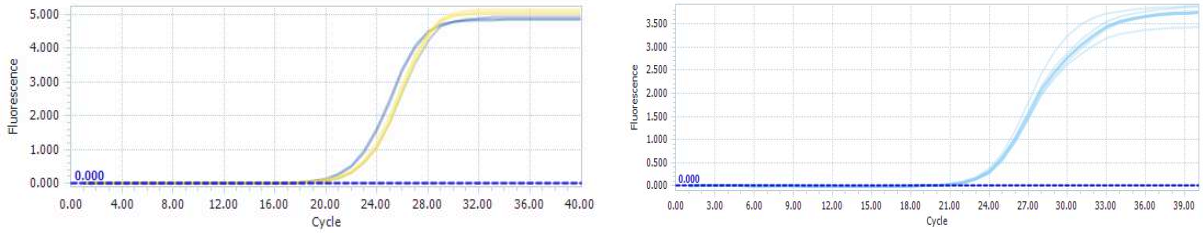

Figure 7 A) Amplification curve of GAPDH B) Amplification curve Myonectin

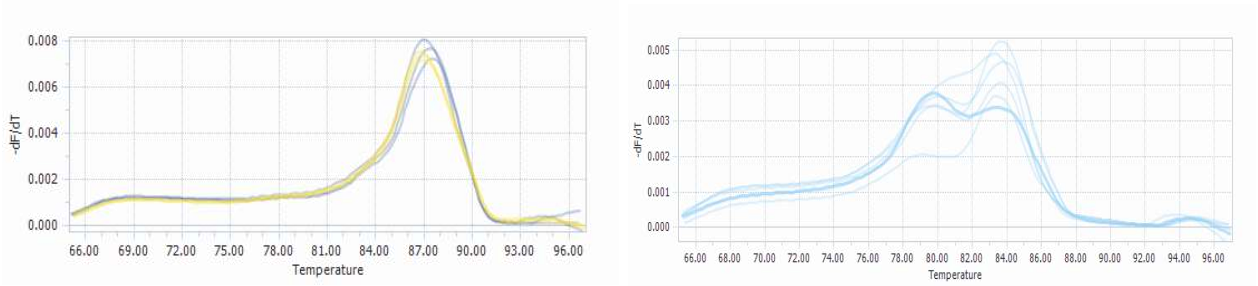

Figure 8 A) Melting curve of GAPDH B) Melting curve of myonectin

DISCUSSION

The aim of this work was to evaluate the influence of ethanolic extract of solanum melongena Linn fruit on myonectin expression in RAW 267.4 cell line. The in vitro study revealed that the ethanolic extract of solanum melongena Linn fruit has an effect on myonectin expression. After ethanolic extraction, the phytochemical screening of solanum melongena Linn. fruits extract showed the presence of carbohydrates, glycoside, flavonoids, terpenoids, protein and steroid. Total flavonoid content was found to be 102.01 µg/ml and total phenolic content was found to be 111 µg/ml. Total flavonoid content was estimated by Aluminium Chloride Colorimetric Method. Flavonoids are a class of natural products that exhibit several pharmacological properties and affects various physiological and biochemical functions in the body22. A standard graph of quercetin was plotted and the total flavonoid content was determined. From the calibration curve of gallic acid phenolic content of ethanolic extract were found to be 102.01 (µg/ml). Total phenolic content was estimated by Aluminium Chloride Colorimetric Method. Phytochemical evaluation revealed that the plant extract has phenolic content, which are predominantly secondary metabolites of plants. The standard graph was plotted using various concentrations of gallic acid and total phenolic content in the extract was determined. From the calibration curve of quercetin phenolic content of ethanolic extract were found to be 102.01 (µg/ml). Myonectin is an important mediator in inter-organ cross-talk and its secretion by skeletal muscle increases with the higher availability of glucose and fatty acids in the insulin-resistant and T2D state as a compensatory mechanism to improve glucose tolerance and increase fatty acid oxidation23,24. Recent study evidences shows that myonectin as a nutrient-responsive regulator of liver autophagy. The skeletal muscle-derived myonectin induced by food intake or the availability of nutrients (e.g. glucose and free fatty acids), conveys a hormonal signal to inhibit autophagy in hepatocytes; this is evidence of a novel skeletal muscle-liver axis in modulating tissue homeostasis25. Circulating myonectin functions as a myokine linking skeletal muscle to lipid metabolism in liver and adipose tissue, providing insights into tissue cross-talk that underlies the integrated control of whole-body metabolism13. In our study, in vitro gene expression analysis by RT PCR study on RAW 267.4 cell line shows treatment with Solanum melongena Linn. Fruits produced notable increase in the expression of myonectin gene. In housekeeping gene, the gene expression fold change was found to be 1 and for solanum melongena treated groups the gene expression fold change was found to be 1.80. This result shows that the ethanolic extract of solanum melongena Linn. Fruits modulate the myonectin gene expression in vitro. This result highlights the interesting link between solanum melongena and myokine expression.

CONCLUSION

Our study reveals that Solanum melongena Linn. Fruits extract modulate the myonectin gene expression in vitro and this result helps the future researchers. Furthermore, given the close link between the extract and myonectin and in future this positive effect of Solanum melongena Linn. Fruits shall test to assess the clinical efficacy for the treatment of myonectin deficiencies.

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