Journal of Medicinal Plants and By-products (2023) 1: 93-108
https://doi.org/10.22092/jmpb.2022.356394.1415

Original Article

Plants Today Drugs Tomorrow: *Cordia grandicalyx* A Possible Future Anti-Hypoglycaemic?

Alinah Mildred Chauke[1*], Leshweni Jeremiah Shai[1] and Motatelo Alfred Mogale[2]

[1]*Department of Biomedical Sciences, Tshwane University of Technology, Private Bag X680, Pretoria, South Africa*
[2]*Department of Biochemistry, Sefako Makgatho Health Science University, Medunsa, South Africa*

Article History

Received: 18 October 2021
Accepted in revised form: 13 April 2022

Keywords
Medicinal Plants
Diabetes Mellitus
Insulin Signals
Cordia grandicalyx

ABSTRACT

Lowering blood glucose levels by enhancing glucose uptake and GLUT4 translocation is an important strategy in glucose homeostasis in insulin-sensitive tissues. Similarly, traditional medicinal plants are used by several traditional healers, and plants are a possible avenue for the discovery and development of antidiabetic drugs. The study aimed to investigate the effects of *Cordia grandicalyx* Oberm. bark, fruit, and leaf extracts through glucose uptake activity by preadipocytes, liver and skeletal muscle cells, relative to their capability on increasing GLUT4 translocation. In addition, the protein expression and phosphorylation of diabetes-related proteins were performed. The *C. grandicalyx* extracts increased glucose uptake activity by enhancing GLUT4 translocation. Moreover, it was established that the co-usage of insulin with plant extracts increased the glucose uptake activity in comparison to insulin. The extracts upregulated total insulin receptor substrate expression and increased the phosphorylation of Akt levels. This data, therefore, suggests that *C. grandicalyx* enhances glucose uptake by modulating insulin signalling, potentially through GLUT4 translocation and upregulation of diabetes-related proteins, possibly mimicking the PI3-K/Akt pathway. This, therefore, suggests that *C. grandicalyx* is a possible candidate for the management of diabetes.

INTRODUCTION

For centuries diabetes has been recognised as a disorder of glucose intolerance and the cause has been directly linked to insufficient insulin production or insulin resistance [1]. The disposal of glucose in the blood system is through insulin-mediated glucose transport [2]. Insulin is activated following increased levels of glucose in the bloodstream, and increases glucose uptake in tissues, thereby increasing functional glucose transport into the plasma membrane [3, 4]. This is achieved through the movement of glucose from the vesicles into tissues, with adipose tissue and skeletal muscle as the primary target cells involved [5,6].

Insulin is also involved in several metabolic actions, such as; glycogen storage in the liver and skeletal muscle tissues, stimulation of lipogenesis and inhibition of lipolysis, and inhibition of gluconeogenesis in the liver [7]. The insulin signaling pathway is initiated when insulin receptors (IR), found on the cell surface of tissues like; skeletal muscle, liver and adipocytes, are activated [8]. The phosphorylated insulin receptor substrate (IRS) protein is involved in two major signalling pathways involved in glucose uptake [9]; namely the mitogen-activated protein kinase (MAPK), and phosphatidylinositol 3 kinase/Akt (PI3K/Akt) pathway [10]. Under pathophysiological conditions such as obesity, type 2 diabetes mellitus and non-alcoholic fatty liver disease (NAFLD), these processes become dysfunctional thereby impairing insulin action and lipid metabolism [11].

Depletion of GLUT4 in cells may result in insulin resistance [12,13], and disturbance of any of the mechanisms results in disruption of glucose uptake into the cell and ultimately hyperglycemia. In diabetes mellitus, emanating from events that lead to insufficient insulin production and secretion, or insensitivity of target cells to insulin, dysregulation of GLUT4 occurs, resulting in decreased glucose

*Corresponding author: Department of Biomedical Sciences, Tshwane University of Technology, Private Bag X680, Pretoria, South Africa
Email Address: Chaukema@tut.ac.za

uptake and utilization by cells [14]. This insufficient phenomenon is due to different metabolic abnormalities which may affect the function of the pancreas but several other factors have been implicated in the defect in translocation of GLUT4 [15,16].

Diabetes mellitus is treated traditionally using medicinal plants, of which many species used have not been investigated for their potential efficacy and cytotoxic activity. Medicinal plants play an important role in the basic health needs in developing countries and the majority of rural populations still rely on traditional systems for the treatment of different ailments [17,18].

In view of the above statements, medicinal plants have been exposed to extensive research, aiming to provide a comprehensive overview of the physiological and biochemical functioning of a cell [19-21]. Knowledge of medicinal plants and plant-based remedies, their efficacy, dosages, mechanisms of action and toxicity is important and must be a central component in traditional medicine before being employed for treatment. Literature on medicinal plants with hypoglycaemic activity is vast, and it is therefore important to identify more plants with potential antidiabetic activity [22, 23].

This study aimed to investigate the effects of fruit, bark and leaf extracts of *C. grandicalyx* on glucose uptake, GLUT4 translocation in the liver, preadipocytes and skeletal muscle cells and expression of diabetes-related proteins. *C. grandicalyx* is a medicinal plant used by traditional healers of Ga-Mashishimale village, in Phalaborwa, South Africa for the management of diabetes mellitus. The majority of *Cordia* species possess a number of ethnomedicinal values, with the leaves used frequently [24]. Moreover, a number of pharmacological activities reported for the extracts and compound include; anti-inflammatory, antioxidant, antimicrobial and antidiabetic to mention a few [25]. It is therefore hypothesised that *C. grandicalyx* possesses potential antidiabetic activity.

MATERIAL AND METHODS

Collection of Plant Material

Fruits, leaves, bark and roots of *C. grandicalyx* were collected from Ga-*Mashishimale* village in Phalaborwa, South Africa. The plant was selected based on its ethnopharmacological use in the management of diabetes, mode of preparation and administration by traditional healers. Fruits, bark, and leaves were collected in woven bags and left to dry at room temperature under suitable conditions away from sunlight in a dark room. Enough ventilation and favourable conditions were maintained to prevent the growth of moulds and other fungi, which may degrade essential metabolites [26].

Preparation of the Plant Extracts for Screening

The dried samples were ground to fine powder on a Polymix laboratory mill (PX-MFC 90D, Kinematica). The powders were stored in clean sterile bottles at room temperature until the extraction procedure. Powders (5 g) of the different plant samples, were placed in glass beakers and extracted with 20 ml of five different solvents of varying polarities; viz., *n*-hexane (SMM instruments), chloroform (Merck), ethyl acetate (SMM instruments), acetone (SMM instruments) and water decoction (hot and cold). The samples were left overnight at room temperature. The extracts were filtered using a Whatman No. 1 filter paper and the filtrates were transferred into pre-weighed glass beakers. The filtered extracts were left to dry in a stream of cold air to ensure that volatiles was retained. The mass of the extract was determined by subtracting the weight of the beaker from the total weight of the beaker and extract. Dried filtrates were made up to 10 *mg/ml* concentrations in 10% dimethyl sulphoxide (DMSO)/Phosphate buffered salt (PBS).

Cell Culture Method

A complete Dulbecco's Modified eagle medium (DMEM) (GIBCO Cell Culture, Carlsbad, CA, USA) was prepared with 450 mL fresh media, 5 mL of penicillin-streptomycin (10,000 U/mL) and 45 mL of fetal bovine serum (10% FBS). The media solutions were stored at 4 °C until use. Murine C2C12 myoblasts, 3T3-L1 preadipocytes, and liver (H4IIE) cells were used in the study. Cells were cultured in media as prepared above. The culture medium was incubated at 37 °C in a 5% CO_2 humidified incubator. The cells were passaged twice or thrice weekly in order to maintain the density of between 1 x 10^5 and 1 x 10^6 cells/*mL*.

Glucose Uptake Assay

The cells (muscle, liver and preadipocytes), were seeded into 96-well microtitre plates and incubated for four days to allow them to reach confluency. Cells were only treated when fully differentiated. The medium was removed by aspiration and replaced with a medium containing plant extracts, controls and compounds at different concentrations. The cells were incubated for a further 1, 3 and 6 h at 37 °C, followed by the determination of glucose in the medium. Glucose in the medium was determined using the Accent 200 glucose assay kit (KAT Medicals SA). Insulin (100 µM) was used as a positive control, while untreated cells were used as negative controls. The absorbance of this colored solution was measured at 540 nm using a Multiskan GO (ThermoScientific). The results were expressed as percentage glucose uptake (mmol/L) in the medium after incubation in presence of plant extracts using this formula:

$$\%\ GLUT4\ Translocation = 100 - \frac{A_{test}\ x\ 100}{A_{control}}$$

Glucose Transporter Translocation

Cells were treated as per the cell culture protocol above. GLUT4 levels at the cell surface of cells were measured by antibody-coupled flow cytometry. Muscle cells and preadipocytes were treated with plant extracts, 100 µM insulin was used as a positive control, while untreated cells served as negative controls. The cells were incubated for 3 h, after which the growth medium was discarded and cells washed with Tris-buffered saline and fixed with 4% formaldehyde for 15 min. The cells were washed once for 5 min with TBS, blocked for 1 h at room temperature in 5% bovine serum albumin (BSA) in TBS-T to prevent non-specific binding. The cells were washed once for 5 min with TBST and incubated with 1:100000 dilution of primary antibody (rabbit anti-mouse GLUT 4) conjugated with fluoresce in isothiocyanate-conjugated antibody (FITC) for 30 min at room temperature in the dark. The cells were washed three times with TBS-T for 5 min. The amount of FITC on the cell surface was measured using Promega-Glomax multi detection system flow cytometer (Promega Corporation) at excitation wavelength 470 nm. The results were expressed as a percentage GLUT4 translocation to the cell surface after incubation in presence of test drug (plant extracts) using the formula:

$$\%\ GLUT4\ Translocation = 100 - \frac{A_{test}\ x\ 100}{A_{control}}$$

Palmitic Acid and Insulin Resistance

Cell cultures were prepared as above and seeded in 96 well plates. Palmitic acid was prepared as per the method of Pu *et al.* [27], with slight modifications. Briefly, sodium palmitate was dissolved in ethanol to a final working concentration of 300 µM. The cells were treated with palmitic acid for 48 h in order to render them resistant to insulin. They were then treated for 3 h with a combination of insulin and plant extract, plant extract alone or insulin. The glucose in the medium was determined using a glucose oxidase-based assay kit (KAT Medicals, South Africa). The absorbance of this colored solution was measured at 540 nm using a Multiscan GO. The results were expressed as a percentage of the glucose uptake in the medium after incubation in presence of plant extracts or insulin using this formula:

$$\%\ Glucose\ uptake\ activity = 100 - \frac{A_{test}\ x\ 100}{A_{control}}$$

Dot Blot

Muscle and liver cells were treated with plant extracts and 100 μM insulin and incubated at 37 ^{0}C for 3 h. The cells were lysed in 1% Triton X-100 in PBS supplemented with NaF (1 mM) and protease inhibitor cocktail (1:1000 dilution). The mixture was incubated on ice for 30 min, and then the lysate spun for 10 min at 12000 rpm. The supernatant was retained and used for the determination of protein concentration using the Biuret assay.

Two microliters of each protein sample were spotted onto a nitrocellulose membrane and left to air dry. The membrane was blocked in 5% BSA/TBS-T (1 h at room temperature) to prevent non-specific binding. The membrane was then incubated with 1:100000 dilution of primary antibody (rabbit anti-mouse glucose transporter 4), dissolved in BSA/TBS-T for 30 min at room temperature. The membrane was washed three times with TBS-T at 5 *min* per wash. The membrane was then incubated with a secondary antibody (goat anti-rabbit IgG) conjugated to horse radish peroxidase (HRP) for 30 *min* at room temperature. The membrane was washed three times with TBS-T (15 min), then once with TBS (5 min). After antibody binding, the

membrane was incubated with a chemiluminescent substrate (ECL reagent) for 1 min in the dark. The excess substrate was removed from the surface of the membrane and exposed to X-ray film in the dark room using the Chemidoc imaging system (Bio-Rad S.A). The signal from the extracts was compared to that of positive and negative control and concentration was estimated as protein density.

Akt Phosphorylation

Muscle and preadipocytes cells were treated for 3 h with plant extracts or insulin, harvested by centrifugation and then lysed using a lysis buffer containing SDS, dithiothreitol and protease inhibitor cocktail (1:1000 dilution). The amount of phosphorylated Akt was determined using a colorimetric-based assay (ab126433 – Akt (pS473) + total Akt ELISA Kit), according to the manufacturer's instructions (Abcam). Protein samples were all adjusted to the lowest protein concentration (22.5 µg).

Total IRS Expression

Muscle and preadipocytes cells were treated for 3 h with plant extracts or insulin, harvested by centrifugation and then lysed using a lysis buffer containing SDS, dithiothreitol and protease inhibitor cocktail (1:1000 dilution). Analysis was performed using a colorimetric-based Rat IRS1 (Insulin Receptor Substrate 1) ELISA assay Kit according to the manufacturer's instructions (Elabscience). Protein samples were adjusted as per the protocol above.

Statistical Analysis

Samples were analysed using three biological replicates (N = 3), in three independent experiments and data was presented as mean ± standard deviation. A two-way ANOVA was used to determine student t-test and confidence intervals. The difference between groups was considered significant * at $P< 0.05$, ** at $P< 0.01$ and *** at $P< 001$.

RESULTS

Glucose Uptake Activity by Skeletal Muscle (C2C12) Cells

Glucose uptake activity was analysed at 1, 3 and 6 h, to assess the effects of *C. grandicalyx* on glucose utilization by the cells.

After a 1 h treatment, the leaf, bark and fruit extracts showed improved glucose uptake activity. The bark acetone (BA) extract and the BEA, both at 2.5 mg/mL, led to a 41% and 55% increase in glucose uptake, respectively, as compared to untreated control cells (Table1). The majority of the extracts used in this section of the study improved glucose uptake even better than insulin, which managed to improve glucose uptake by 20%. The treatment continued after 3 h incubation, with BHW, BA, and BEA increasing the glucose absorption by cells to about 45%, higher than untreated controls. After 6 h treatment of muscle cells with extracts, the improvement was observed with FH (34%), BA (46%) and BH (36%), as compared to insulin which had 20% activity. Extracts FC, BEA as well as β-amyrin had little or no influence on glucose utilisation by cells.

Glucose Uptake on Preadipocytes (3T3-L1) Cells

The same trend as with muscle cells was observed with the highest concentration recording the highest uptake activity. BHW and BA recorded 47% and 53% at 2.5 mg/ml above untreated control cells, with insulin activity at 20% (Table 2). Minimal uptake activity was observed with leaf extracts except for the LHW which recorded 39% at 1.25 mg/mL.

After a further 3 h treatment, all extracts resulted in increased glucose uptake activity of about 70% for BA and 53% for both LH and LA at 2.5 mg/ml higher than untreated controls. Insulin on the other hand, had minimal glucose absorption activity of less than 10%. Surprisingly, after 6 h treatment of preadipocytes with extracts, improved glucose uptake activity was observed with BHW and LB at 48% and 50% respectively at higher concentration (2.5 mg/ml), even after insulin had dropped to below 10% activity.

Glucose Uptake on Liver (H4IIE) Cells

Glucose uptake activity on liver cells had minimal activity at 1 h treatment as compared with muscle and preadipocytes, with the highest activity recorded for LB which was 42% at 2.5 mg/mL, surpassing that of metformin at 38% (Table 3). The treatment continued after 3 h incubation, with BA recording 42%, BH 38% and FH 36%, higher than untreated controls and metformin (25%).

Following 6 h treatment, most plant extracts displayed minimal uptake activity with exception of FH, LHW and BC which had an activity of 48%, 43% and 44% respectively, higher than the untreated control cells. Metformin had minimal or no activity, same as with other plant extracts, below 10%.

In summary, glucose uptake activity was in a concentration depended manner with glucose absorption increasing with increased concentration of the extracts. Promising uptake activity was recorded with preadipocytes at all three different treatment intervals. In addition, at 6 h, uptake activity of up to 50% was recorded for LB compared to that of insulin (below 5%).

GLUT4 Translocation

The plants selected for this section were those that improved glucose uptake above. GLUT4 is the predominant glucose transporter expressed in preadipocytes and muscle cells. We explored the ability of different plant extracts in stimulating translocation of GLUT4 from the cytoplasm to the cell membrane of muscle cells after 3 h treatment at different concentrations (2.5, 1.25, .625, and .312 mg/ml).

GLUT4 Translocation on Muscle Cells

GLUT4 translocation assay assesses mobilisation of GLUT4 from within the cells to the surface (cell membrane) and is key for optimal glucose uptake and absorption by the cells. The reduction of glucose is primarily dependent on insulin-releasing signals and glucose transporter activation. Our data revealed significant ($P< 0.01$) stimulation of GLUT4 translocation up to 16% and 10% by BCW and LB respectively, with a number of plant extracts faring better ($P< 0.05$) than untreated control cells. It was observed that plant extracts BHW, BCW, and LA had increased GLUT4 translocation, except for the BA, BEA, LB and LHW (Fig. 1). This might be attributed to the fact that they probably use a different mechanism to increase glucose uptake.

GLUT4 Translocation by Preadipocytes

Similarly, preadipocytes GLUT4 translocation was significantly increased ($P< 0.01$) on both BEA, LH at 17% and LEA at 21% (Fig. 2). The rest of the extracts, BA, LA and LC had an activity below 5%, indicating minimal GLUT4 translocation. This was suggesting that the glucose uptake activity observed was probably due to a different mechanism other than GLUT4 translocation.

Insulin Resistance

We used the synergic approach of co-usage of prescribed medication (insulin) with plant extracts that had good uptake activity, by treating cells with combined treatment (both insulin and *C. grandicalyx* extracts) after 48 h exposure to 300 µM palmitic acid [27]. Cells were treated for 3 h, followed by an assessment of glucose uptake by cells. The hypoglycaemic activity of the combination of insulin and the plant extract at a ratio of 1:1 on both palmitate-induced and normal cells was analysed.

Insulin Resistance on Muscle Cells

The data revealed a slight increase in glucose uptake activity for both normal and palmitate-treated cells as compared to the plant alone. It was observed that in cells exposed to sodium palmitate (Figure 3 A and C) insulin levels were suppressed indicating that cells were resistant to insulin.

Furthermore, there was limited glucose uptake activity observed with insulin compared to the plant extracts, however, a combination of plant extract with insulin demonstrated improved uptake activity (Fig. 3 B). LHW extracts, at all concentrations, led to improved glucose uptake activity (in a dose-dependent manner) as compared to its combination with insulin, on insulin-resistant cells. However, at 2.5 mg/mL, a slight increase in glucose uptake activity of 48% for the LHW/insulin combination was observed compared to 36% of the LHW extract. A similar picture was displayed by BHW/insulin combination (Fig. 3 D), which resulted in 57% glucose uptake compared to 28% for BHW extract at 2.5 mg/mL.

R esistance on Preadipocytes

Interestingly, it was noted that a combination of LB/insulin resulted in a dose-dependent improvement of glucose uptake activity on preadipocytes, 53% at 2.5 mg/mL (Fig. 4 a) in comparison to 50% for the BHW extract. The highest extract concentration resulted in the highest glucose uptake by the cells. Similarly, BA/insulin combination had the same effect at 51% compared to the 48% BA extract on its own (Fig. 4 c).

Table 1 Skeletal muscle (C2C12) cells glucose uptake activity on *C. grandicalyx* Oberm. extracts with insulin (100 µM) as a positive control, and results expressed percentage (%) glucose uptake; A (Fruits), B (Bark), C (Leaves). NEG (Negative control), POS (Insulin), BB (Bark boiled), BHW (Bark hot water), BCW (Bark cold water), LB ((Leaf boiled), LHW (Leaf hot water), LCW (Leaf cold water), FB (Fruits boiled), BA (Bark acetone), BEA (Bark ethyl acetate), BC (Bark chloroform), BH (Bark hexane), LA (Leaf acetone), LC (Leaf chloroform), LH (Leaf hexane), LCMP (*β*-amyrin)

Skeleta muscle cells																					
1 Hours glucose uptake activity																					
	NEG	POS	BB	BHW	BCW	BA	BEA	BC	BH	FB	FA	FEA	FC	FH	LB	LHW	LCW	LA	LEA	LC	LH	LCMP
2.5	0	9	31	21	8	55	50	20	35	33	16	3	18	21	26	31	12	41	22	18	26	4
1.25	0	20	30	27	29	40	39	17	19	25	25	20	13	17	27	33	28	39	18	16	21	10
0.625	0	19	24	24	30	35	33	23	18	25	28	28	9	11	27	28	24	25	8	15	11	0
0.312	0	15	21	22	30	26	26	23	13	24	28	17	13	10	28	22	31	29	30	25	33	23
3 Hours glucose uptake activity																					
	NEG	POS	BB	BHW	BCW	BA	BEA	BC	BH	FB	FA	FEA	FC	FH	LB	LHW	LCW	LA	LEA	LC	LH	LCMP
2.5	0	13	25	12	0	48	50	10	16	35	1	-7	11	10	26	19	20	44	24	19	0	0
1.25	0	12	31	27	16	32	33	0	13	40	4	6	5	8	28	38	27	32	13	5	3	9
0.625	0	4	28	21	5	31	23	5	12	35	7	6	3	5	24	36	26	25	10	9	9	12
0.312	0	0	23	10	6	22	14	2	4	22	8	4	0	0	21	7	18	13	0	6	0	8
6 Hours glucose uptake activity																					
	NEG	POS	BB	BHW	BCW	BA	BEA	BC	BH	FB	FA	FEA	FC	FH	LB	LHW	LCW	LA	LEA	LC	LH	LCMP
2.5	0	22	12	0	27	46	0	30	36	15	8	0	0	34	17	5	20	18	7	0	0	0
1.25	0	19	26	0	26	27	0	11	23	21	13	13	0	28	28	14	28	5	0	1	2	1
0.625	0	16	21	3	20	13	0	3	14	14	11	11	0	23	26	12	25	3	1	4	0	2
0.312	0	30	28	19	30	12	0	4	7	21	16	10	0	23	34	22	36	0	0	0	0	0

.

Table 2 Preadipocytes (3T3-L1) cells glucose uptake activity on *C. grandicalyx* Oberm. extracts with insulin (100 µM) as a positive control, and results expressed percentage (%) glucose uptake; A (Fruits), B (Bark), C (Leaves). NEG (Negative control), POS (Insulin), BB (Bark boiled), BHW (Bark hot water), BCW (Bark cold water), LB ((Leaf boiled), LHW (Leaf hot water), LCW (Leaf cold water), FB (Fruits boiled), BA (Bark acetone), BEA (Bark ethyl acetate), BC (Bark chloroform), BH (Bark hexane), LA (Leaf acetone), LC (Leaf chloroform), LH (Leaf hexane), LCMP (β-amyrin).

Preadipocyte cells

1 Hour glucose uptake activity

	NEG	POS	BB	BHW	BCW	BA	BEA	BC	BH	FB	FA	FEA	FC	FH	LB	LHW	LCW	LA	LEA	LC	LH	LCMP
2.5	0	22	12	0	27	46	0	30	36	15	8	0	0	34	17	5	20	18	7	0	0	0
1.25	0	19	26	0	26	27	0	11	23	21	13	13	0	28	28	14	28	5	0	1	2	1
0.625	0	16	21	3	20	13	0	3	14	14	11	11	4	23	26	12	25	3	1	4	0	2
0.312	0	30	28	19	30	12	0	4	7	21	16	10	0	23	34	22	36	0	0	0	0	0

3 Hours glucose uptake activity

	NEG	POS	BB	BHW	BCW	BA	BEA	BC	BH	FB	FA	FEA	FC	FH	LB	LHW	LCW	LA	LEA	LC	LH	LCMP
2.5	0	11	31	17	26	39	29	30	45	1	10	16	67	49	32	23	54	46	49	54	49	0
1.25	0	7	38	38	28	44	31	32	42	4	9	16	70	64	47	29	9	0	20	38	11	0
0.625	0	8	29	43	21	37	26	27	35	9	11	16	70	52	48	54	7	23	20	59	19	0
0.312	0	9	29	45	14	44	26	23	23	22	19	30	18	0	0	9	0	0	0	0	0	0

6 Hours glucose uptake activity

	NEG	POS	BB	BHW	BCW	BA	BEA	BC	BH	FB	FA	FEA	FC	FH	LB	LHW	LCW	LA	LEA	LC	LH	LCMP
2.5	0	4	44	44	36	49	46	38	36	-4	7	5	45	35	25	26	30	18	21	3	6	0
1.25	0	4	38	48	28	50	45	39	43	1	6	11	44	30	16	27	29	20	25	6	6	0
0.625	0	5	27	48	18	44	43	34	45	8	11	13	32	29	12	26	25	20	27	10	11	0
0.312	0	0	12	45	13	36	28	22	36	9	8	11	23	21	5	19	17	15	25	5	5	0

Table 3 Liver cells (H4IIE) glucose uptake activity on *C. grandicalyx* Oberm. extracts with metformin (8.5 mg/ml*)* as a positive control, and results expressed percentage (%) glucose uptake. NEG (Negative control), POS (Metformin), BB (Bark boiled), BHW (Bark hot water), BCW (Bark cold water), LB (Leaf boiled), LHW (Leaf hot water), LCW (Leaf cold water), FB (Fruits boiled), BA (Bark acetone), BEA (Bark ethyl acetate), BC (Bark chloroform), BH (Bark hexane), LA ((Leaf acetone), LC (Leaf chloroform), LH (Leaf hexane), LCMP (*β*-amyrin).

Liver cells																					
1 Hour glucose uptake activity																					
	NEG	POS	BB	BHW	BCH	LB	LHW	LCW	FB	FA	FEA	FC	FH	BA	BEA	BC	BH	LA	LEA	LC	LH	LCMP
2.5	0	39	25	0	29	42	12	19	25	27	11	11	0	24	16	0	0	15	0	0	0	0
1.25	0	31	16	0	0	20	0	0	22	24	12	9	0	33	13	0	8	6	0	9	7	0
0.625	0	21	0	0	0	23	0	0	4	0	0	0	0	20	20	0	0	0	0	0	0	0
0.312	0	19	11	7	8	23	6	22	2	7	3	16	0	1	-2	6	0	0	0	0	7	0
3 Hours glucose uptake activity																					
	NEG	POS	BB	BHW	BCH	LB	LHW	LCW	FB	FA	FEA	FC	FH	BA	BEA	BC	BH	LA	LEA	LC	LH	LCMP
2.5	0	25	17	4	16	33	16	31	0	0	0	13	37	41	6	0	38	0	19	5	0	0
1.25	0	30	21	11	21	31	18	31	9	9	0	0	25	31	12	0	11	0	20	0	0	0
0.625	0	14	0	0	0	12	8	20	0	0	0	12	30	23	9	0	27	9	19	0	13	0
0.312	0	12	18	2	7	25	20	27	18	0	5	8	27	21	12	6	0	0	3	0	0	0
6 Hours glucose uptake activity																					
	NEG	POS	BB	BHW	BCH	LB	LHW	LCW	FB	FA	FEA	FC	FH	BA	BEA	BC	BH	LA	LEA	LC	LH	LCMP
2.5	0	0	27	20	17	27	41	15	35	9	1	4	1	31	36	15	7	34	7	5	0	0
1.25	0	4	34	14	25	28	43	26	47	20	5	0	0	16	0	9	2	18	0	5	0	0
0.625	0	0	28	0	23	19	34	13	34	0	0	0	0	4	0	22	0	0	0	0	0	0
0.312	0	0	10	0	0	4	22	0	24	0	0	20	13	28	0	44	23	24	25	22	31	12

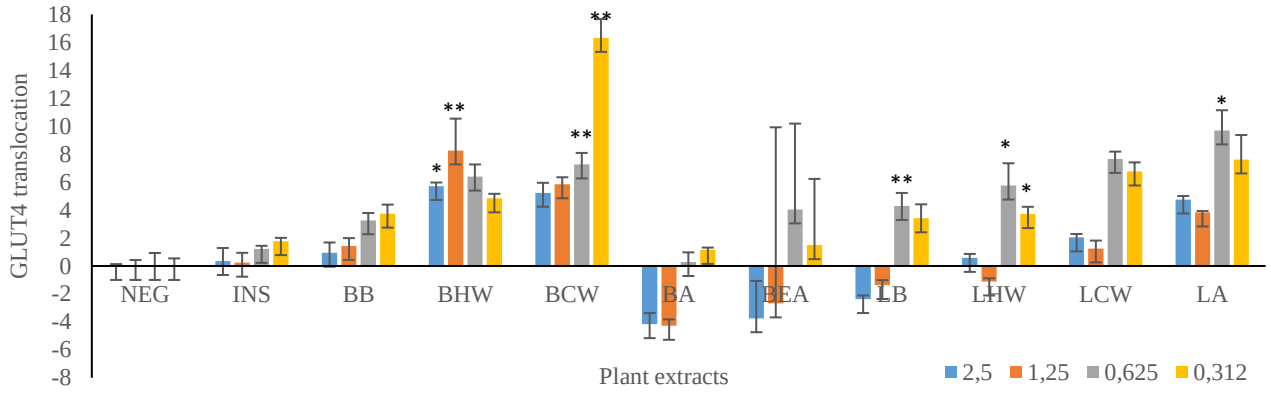

Fig. 1 GLUT4 expression in skeletal muscle (C2C12) cells after 3 h treatment with different concentration of *C. grandicalyx*, insulin (100 µM) as a positive control and results expressed as percentage GLUT4 translocation. Data presented mean ± SD of 3 replicates, p-significant of **P*< 0.05, ***P*< 0.01 and ****P*< 0.001. NEG (Negative control), INS (Insulin), BB (Bark boiled), BHW (Bark hot water), BCW (Bark cold water), BA (Bark acetone), BEA (Bark ethyl acetate), LB ((Leaf boiled), LHW((Leaf hot water), LCW ((Leaf cold water), LA (Leaf acetone) and FB (Fruits boiled).

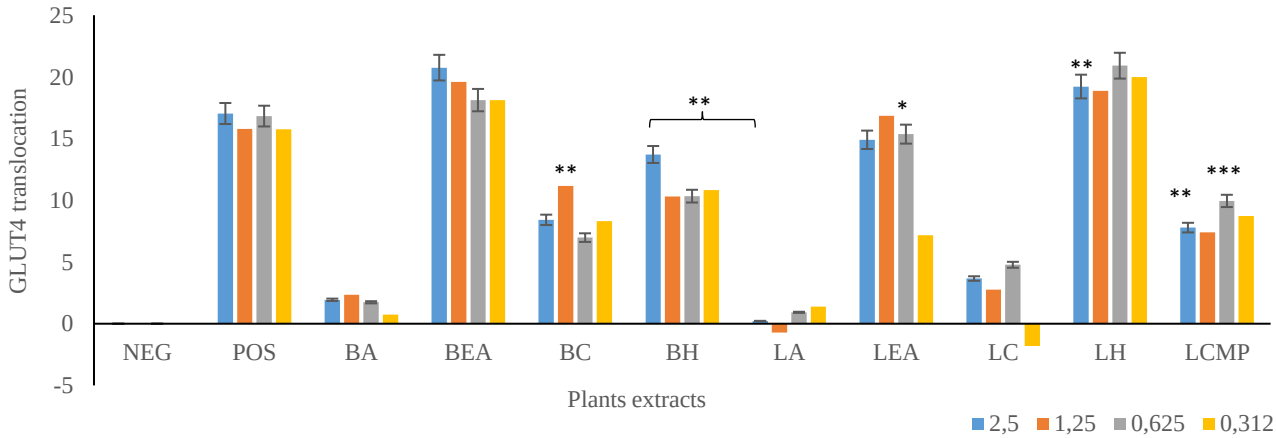

Fig. 2 GLUT4 translocation in preadipocytes (3T3-L1) cells after 3 h treatment with different concentrations of *C. grandicalyx*, insulin as a positive control and results expressed as percentages GLUT4 translocation. Data presented mean ± SD of 3 replicates, p-significant of **P*< 0.05, ***P*< 0.01 and ****P*< 0.001. NEG (Negative control), POS (Insulin), BA (Bark acetone), BEA (Bark ethyl acetate), BC (Bark chloroform), BH (Bark hexane), LA (Leaf acetone), LEA (Leaf ethyl acetate), LC (Leaf chloroform), LH (Leaf hexane), LCMP (β-amyrin).

Protein Expression

We investigated IRS 1/2 and Akt 1/2 expression and phosphorylation on muscle and preadipocytes treated cells. Furthermore, we examined the expression of proteins using dot blot.

Dot Blot

Identification of protein expression resulted in blue-purple spots on positive controls and proteins from plant samples, on muscle and liver cells. The spots indicated the reaction of primer antibody and secondary antibody for GLUT4 transporter. All plant extracts sufficiently expressed GLUT4 primarily with BA and BH extracts (Fig. 5) on muscle cells compared to insulin and untreated cells. However, minimal expression was observed with the liver cells. Expression of proteins was limited, BA and FB had the most expression compared to metformin, with BB, LHW and LCW still faring better compared to untreated cells.

Total IRS Expression

For GLUT 4 to be translocated, insulin receptor substrate (IRS-1 and IRS-2) has to be activated, which then leads to the phosphorylation of protein kinase Akt. Significant activation of insulin receptor substrate 1 and 2 by BA and BB (*P*< 0.05) on muscle cells was observed even though activity was minimal compared to that of insulin (*P*< 0.01) (Fig. 6). In addition, BA and FB had a noticeable increase in total IRS expression with *P*< 0.05 significance in 3T3-L1 preadipocytes treated cells.

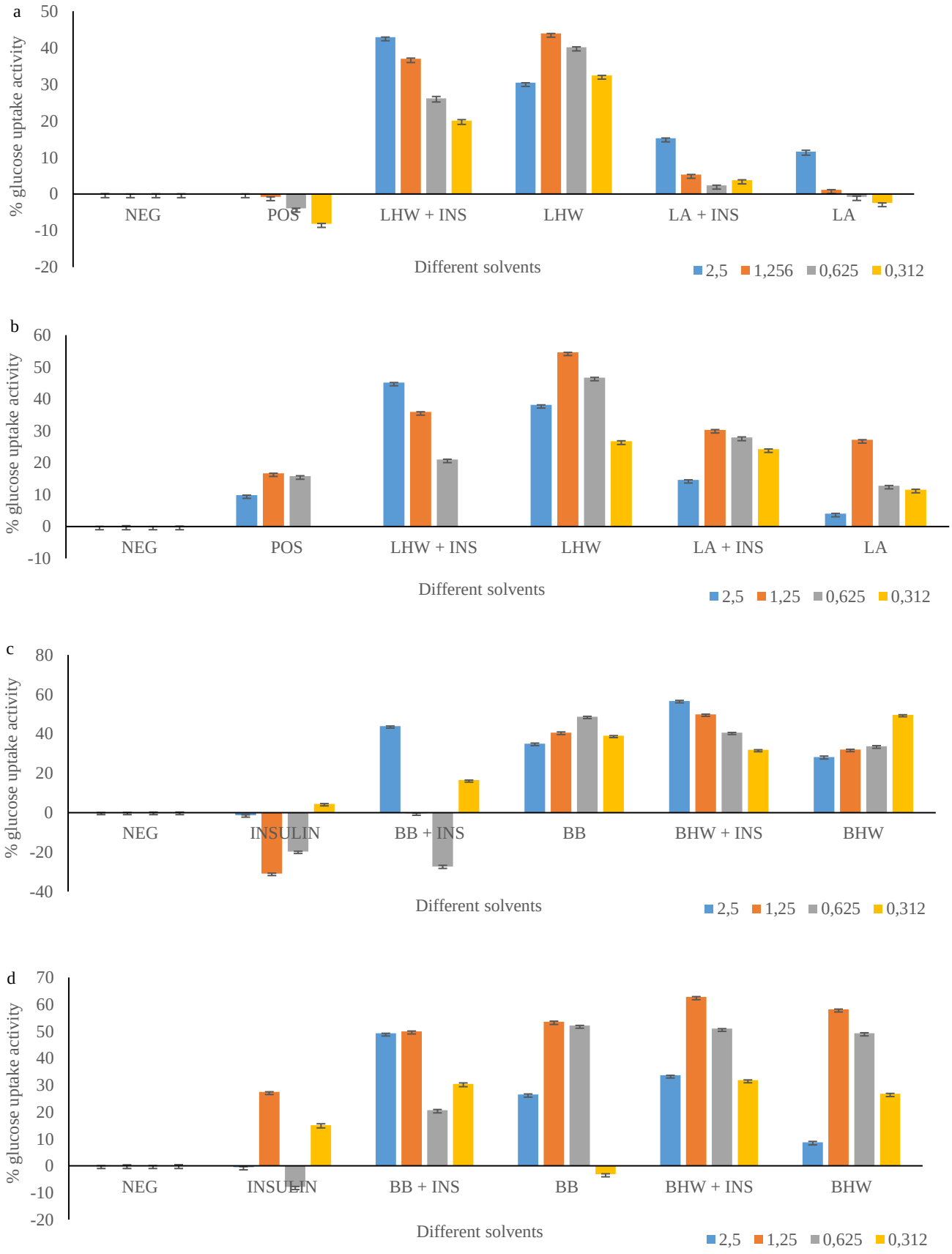

Fig. 3 Hypoglycaemic activity of the different *C. grandicalyx* Oberm. extracts on skeletal muscle (C2C12) cells treated with palmitic acid (300 µM) (a and c) and untreated (b and d) with insulin as a positive control (100 µM) and untreated cells as a negative control. Results expressed as percentage glucose uptake and values are mean ± SD of 3 replicates. NEG (untreated cells); INS (insulin); LA (leaf acetone); BHW (bark hot water); BB (bark boiled); LHW (leaf hot water).

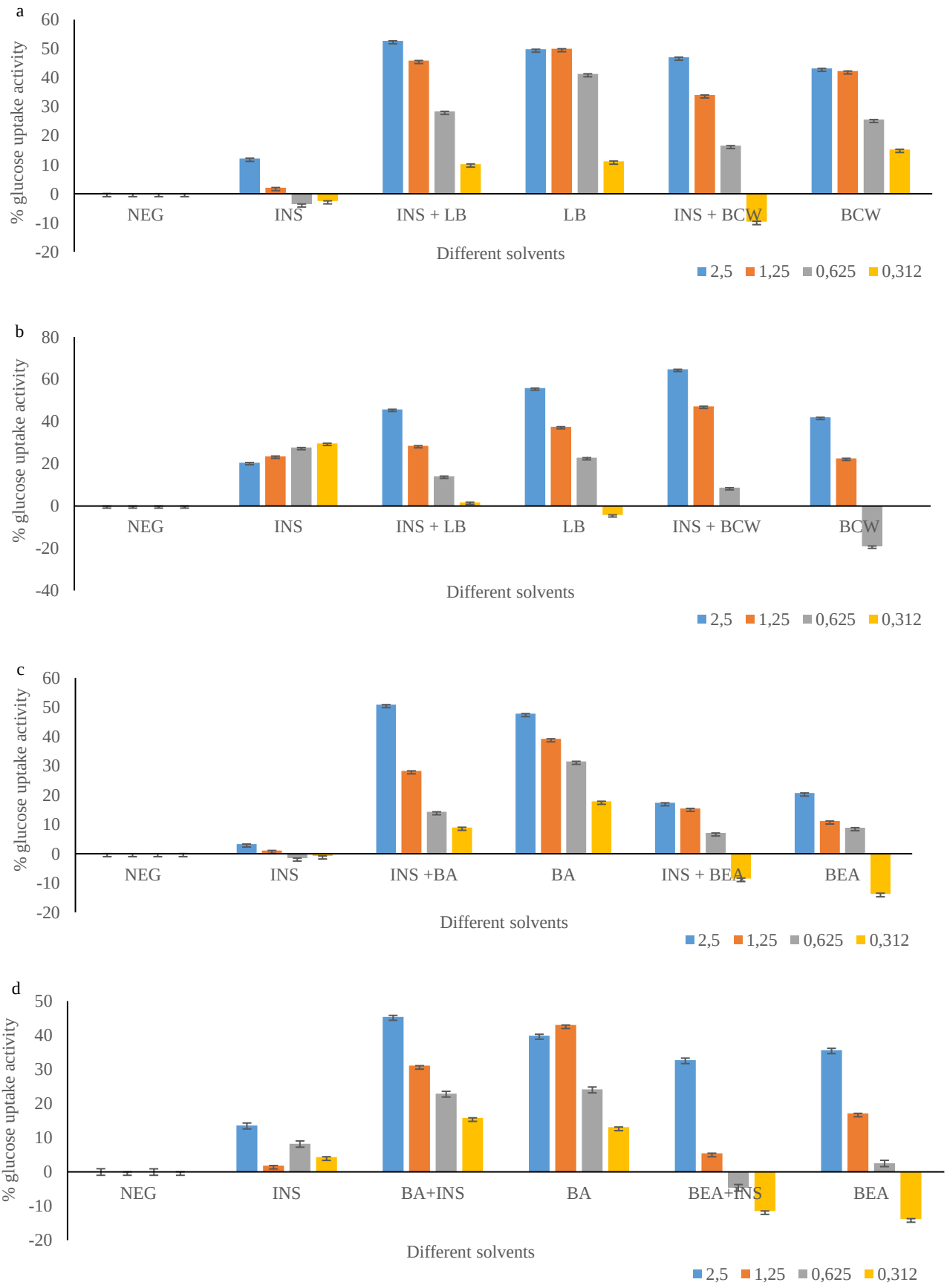

Fig. 4 Hypoglycaemic activit y of the different *C. grandicalyx* Oberm. extracts on preadipocytes (3T3-L1) cells treated with palmitic acid (300 µM) (a and c) and untreated (b and d) with insulin as a positive control (100 µM) and untreated cells as a negative control. Results expressed as percentage glucose uptake and values are mean ± SD of 3 replicates. NEG (untreated cells); INS (insulin); BA (bark acetone); BEA (bark ethyl acetate); LB (leaf boiled); BCW (bark cold water).

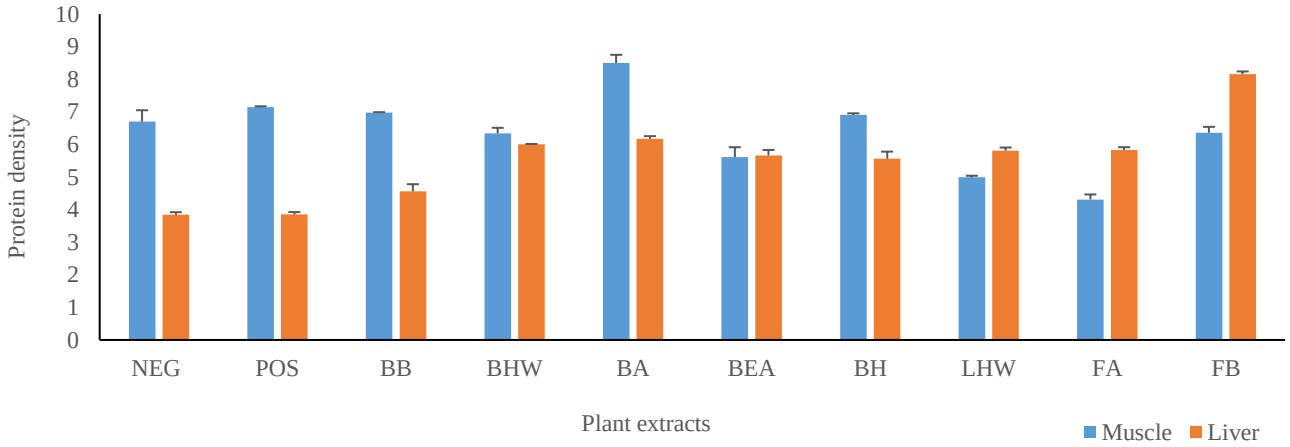

Fig. 5 Dot blot analysis on skeletal muscle (C2C12) and liver (H4IIE) cells exposed to GLUT4 antibodies, blot protein expression calculated as protein density using ImageJ software. POS (Insulin, 100 µM and Metformin 8.5 mg/ml)); NEG (untreated cells); BB (Bark boiled); BHW (Bark hot water); BA (Bark acetone); BEA (Bark ethyl acetate); BH (Bark hexane); FB (Fruits boiled); FEA (Fruit ethyl acetate); FA (Fruit acetone); LH (Leaf hexane); LC (Leaf chloroform), LA (Leaf acetone); LHW (Leaf hot water).

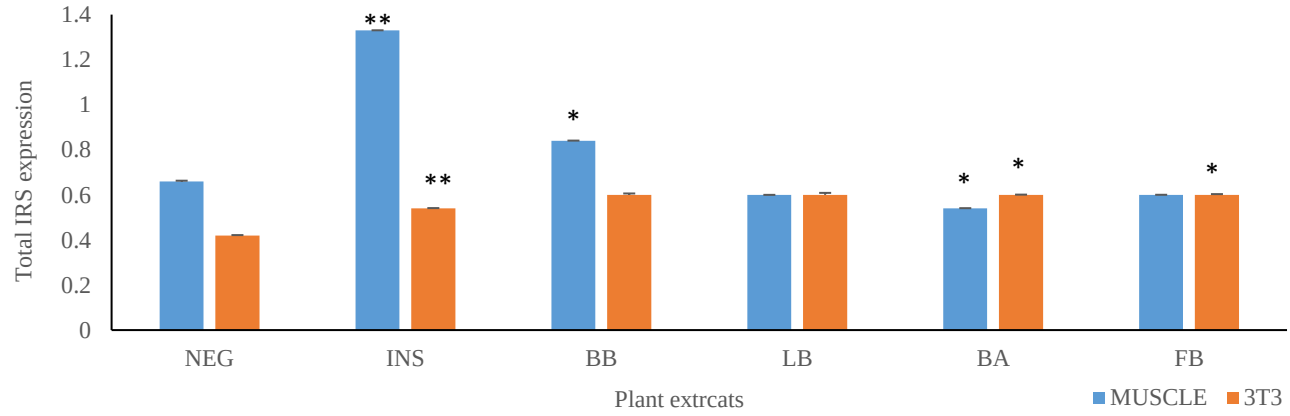

Fig. 6 Total IRS expression on skeletal muscle (C2C12) and preadipocytes (3T3-L1) cells after 24 h treatment with *C. grandicalyx* Oberm. extracts. Significant differences is represented by **P*< 0.05 and ***P*< 0.01 with respect to the control. NEG (Negative control);, INS (Insulin); BB (Bark boiled); LB (Leaf boiled); BA (Bark acetone); FB (Fruits boiled).

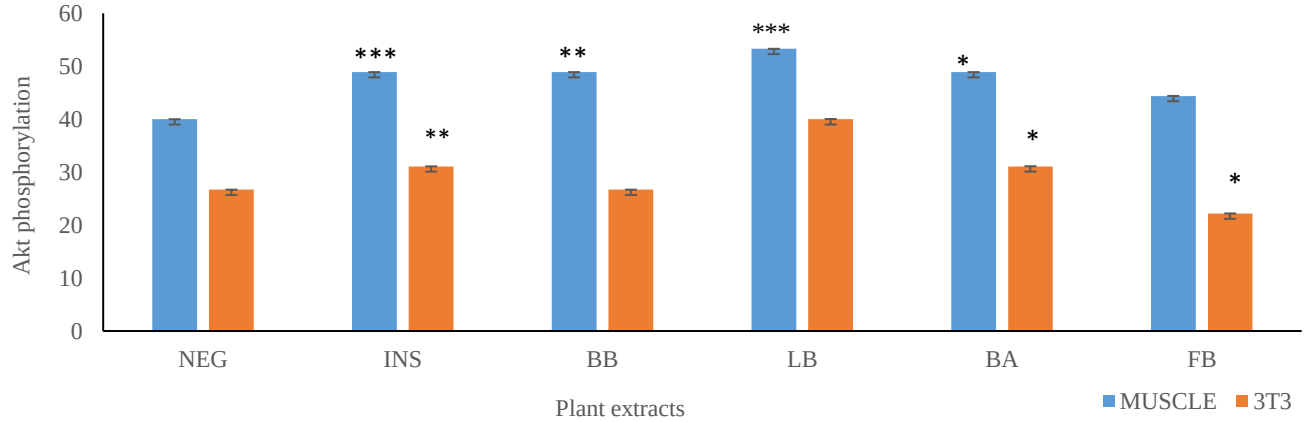

Fig. 7 Expression of the active phosphorylated form of Akt (ser 473) on Skeletal muscle (C2C12) cells after 24 hrs treatment with *C. grandicalyx* Oberm. extracts. Significant differences is represented by **P*< 0.05 and ***P*< 0.01 with respect to the control. NEG (Negative control), INS (Insulin), BB (Bark boiled), LB (Leaf boiled), BA (Bark acetone), FB (Fruits boiled).

Akt Phosphorylation

Total Akt phosphorylation was used to determine the mechanism used by *C grandicalyx* to enhance glucose absorption. After treatment, it was observed that all the selected plant extracts significantly up a ated Akt phosphorylation on muscle cells with BB and LB extracts demonstrating $P< 0.01$ and BA $P< 0.05$ significance respectively (Fig. 7) as compared to the untreated cells. Similarly, BA and FB demonstrated a significant ($P< 0.05$) increase in total Akt phosphorylation on 3T3-L1 cells.

DISCUSSION

Glucose uptake is regulated by several mechanisms, where insulin plays the most prominent role. In diabetes mellitus, because of the absence or insufficient sensitivity to insulin, GLUT4 in the cell membrane is decreased thereby affecting glucose uptake by the cells. An increase in GLUT4 translocation to the cell surface in response to treatment with plant extracts resulted in increased glucose uptake by the cells. There was a significant correlation on glucose uptake activity between adipose, liver and muscle cells; with a number of the plant samples with increased glucose uptake activity, displaying a similar profile to that of GLUT4 translocation.

Some of the extracts with no GLUT4 translocation activity, surprisingly, had very good glucose uptake activity, meaning that they probably employ a different pathway that enhances uptake activity. A study by Kadan *et al*. [28] reported a significant gain in GLUT4 translocation in muscle cells after treatment with eight different medicinal plant extracts, and Anandharajan *et al.* [29] reported upregulation of glucose uptake and transport by *A. marmelos* and *S. cumini*.

Some of the extracts appeared to relieve or reverse insulin resistance. It was observed that in palmitate-induced insulin resistance, insulin barely stimulated any changes in glucose uptake. However, treatment of these cells with insulin and plant extracts combination led to an improvement in glucose uptake by cells. This could suggest that the palmitate-induced insulin resistance is overcome by the plant extracts, using a mechanism that is yet to be unraveled. Khedher *et al*. [30] reported improved insulin sensitivity, reduced inflammation and lipogenesis inhibition on adipocytes by Sage methanol extract, while Yang *et al.* [31] reported potential anti-diabetic activity of guava leaf extract by enhancing insulin resistance through the activation of PI3K/Akt signaling pathway.

Type II diabetes mellitus is a result of insulin resistance, and a plant capable of reversing the effects of insulin resistance is interesting. However, the full mechanism employed by the plant extracts needs to be investigated thoroughly *in vivo*. GLUT4 translocation to the cell surface is beneficial for the management of insulin resistance [32]. Given that most extracts resulted in glucose uptake through GLUT4 translocation, we further investigated the effects of *C. grandicalyx* extracts on the downstream signaling molecules, Akt and IRS proteins. The results showed improved expression of IRS-1 and pronounced levels of phosphorylated Akt in cells treated with selected plant extracts. IRS is a docking protein that has to be phosphorylated in order to be activated, thereby activating PI3K, which is necessary in initiating several insulin signals, such as glucose transport [33]. Expression of these proteins is initiated when insulin binds to cell surface insulin receptors, which in turn is phosphorylated and ultimately causes phosphorylation of IRS and activates Akt, which is associated with insulin signaling [34-35]. Moreover, the increase in GLUT4 translocation was in a PI3K/Akt-dependent manner, suggesting that the pathway employed by the plant extract is probably the PI3K/Akt.

Akt directly phosphorylates AS160 thereby inducing GLUT4 translocation from the storage vesicles to the plasma membrane and increasing glucose uptake in skeletal muscle and preadipocytes [36]. Previous research has found that knockdown of Akt or IRS adaptor proteins reduces insulin-induced glucose uptake, whereas overexpression of Akt increases glucose uptake [37]. Similarly, it was reported that low expression of IRS resulted in insulin resistance [38]. Therefore, dysfunctional PI3K/Akt-mediated glucose transport and glycogen synthesis play an important role in the development of obesity and type 2 diabetes mellitus. Biological functions of cells like phosphorylation of Akt improve mRNA expression of the signal transduction pathway [34]. Based on accumulated research evidence, the PI3K/Akt signaling pathway is required for normal metabolism and has been identified as a therapeutic target for treatments of obesity and type 2 diabetes mellitus [39].

The results are corroborated by Anurag and Ganesh [40], who revealed the presence of significant antidiabetic potential of methanol extract of *C. dichotoma* in alloxan-induced diabetic rats. Similarly, Ezequiel *et al*. [41] reported promising antioxidant and alpha-glucosidase inhibitory activity on *C. boissieri* while Jamkhande *et al.* [42] reported significantly reduced blood glucose levels on *C. verbenacea* extract. In addition, Nazim and Kakoti [43] reported the leaf extracts of *C. dichotoma* as having high drug safety and significant hypoglycaemic and antihyperglycaemic effects on Wistar rats.

After decades of research, the PI3K/Akt pathway is still worth studying due to its multiple functions [44]. Therefore, a plant extract that activates the PI3K/Akt signalling pathway is in need.

CONCLUSION

Stimulation of glucose uptake is mostly attributed to increased translocation and moving of the GLUT4 to the plasma membrane [45]. Therefore, our results support the statement and strongly suggest the potential antidiabetic activity of *C. grandicalyx* plant extracts. In summary, *C. grandicalyx* stimulated glucose uptake in the liver, preadipocytes and muscle cells while at the same time enhancing GLUT4 translocation. Furthermore, it asserts that the induced glucose uptake activity observed is dependent on GLUT4 translocation. In addition, *C. grandicalyx* increased the expression of Akt and IRS proteins. These proteins are key markers of the PI3K signaling pathway. PI3K/Akt signalling pathway promotes; lipid biosynthesis inhibits lipolysis, promotes glucose transport, glycogen synthesis and protein synthesis.

This study extends our knowledge on the use of *C. grandicalyx* and therefore supports its use in indigenous settings for the treatment of diabetes mellitus.

Conflict of Interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

ACKNOWLEDGMENT

The authors would like to acknowledge the Tshwane University of Technology for the study opportunity and NRF for funding. LJS is a recipient of NRF funding under the Competitive Support for Rated and Unrated Researchers.

REFERENCES

1. Arnold S.E., Arvanitakis Z., Macauley R,, Shannon L., Koenig A.M., Wang H., Yan A., Rexford S., Craft S., Gandy S.B., Christoph S., Luke E. Holtzman D.M., Nathan D.M. Brain insulin resistance in type 2 diabetes and Alzheimer disease: concepts and conundrums. Nature Reviews Neurology. 2018;14 (3):168-181.
2. Olson A.L., Humphries K.. Recent advances in understanding glucose transport and glucose disposal. 2020;9:F1000.
3. Kim S.H., Park Mi.Jung. Effects of growth hormone on glucose metabolism and insulin resistance in human. Annals of Pediatric Endocrinology & Metabolism. 2017;22 (3):145-152.
4. Schreiber I., Dörpholz G., Ott C.Eric., Kragesteen B., Schanze N., Lee C.Th., Köhrle J., Mundlos S., Ruschke K., Knaus P. BMPs as new insulin sensitizers: enhanced glucose uptake in mature 3T3-L1 adipocytes via PPARγ and GLUT4 upregulation. Scientific Reports. 2017;7 (1):1-3.
5. Ceddia R.B. Direct metabolic regulation in skeletal muscle and fat tissue by leptin: implications for glucose and fatty acids homeostasis. Inter J Obesity. 2005;29 (10):1175-1183.
6. Kalinkovich A., Gregory L. Sarcopenic obesity or obese sarcopenia: A cross talk between age-associated adipose tissue and skeletal muscle inflammation as a main mechanism of the pathogenesis. Ageing Res Reviews. 2017;35:200-21.
7. Kumar Guru I.P., Chandrakumar A., Lite S.S., Saraswathi CH., Arasu N.T., Al-Dhabi M.V, Arshad N.A., Jesu A.A. Molecular process of glucose uptake and glycogen storage due to hamamelitannin via insulin signalling cascade in glucose metabolism. Molecular Biology Reports. 2020;47:6727–6740.
8. Świderska Ewa., Adam Wróblewski J.S., Szemraj J., Drzewoski J., ŚliwińskaA. Role of PI3K/AKT Pathway in Insulin-Mediated Glucose Uptake. Blood Glucose Levels. 2018;1:1-18.
9. Sayem A.S.M., Arya A., Karimian H., Krishnasamy N., Hasamnis A.A., Hossain Ch.F. Action of Phytochemicals on Insulin Signaling Pathways Accelerating Glucose Transporter (GLUT4) Protein Translocation. Molecules. 2018;23 (2):258.
10. Zlobin A., Bloodworth J.C., Osipo C. Mitogen-activated protein kinase (MAPK) signaling. Predictive Biomarkers in Oncology. Springer, Cham. 2019;213-221.
11. Leite N.C., Salles, G.F., Araujo A.L.E., Villela-Nogueira C.A., Cardoso C.R.L. Prevalence and associated factors of non-alcoholic fatty liver disease in

patients with type-2 diabetes mellitus. Liver Inter. 2009;29(1):113-119.

12. Pillon N.J., Frendo-Cumbo S., Jacobson M.R., Liu Zh., Milligan P.L., Bui H.H., Zierath J.R., Bilan Ph.J., Brozinick J.T., Klip A. Cell-autonomous sphingolipid changes do not underlie fatty acid-induced insulin resistance of GLUT4 translocation or pro-inflammatory signaling in muscle cells. J Lipid Res. 2018;59(7):1148-1163
13. Ueda M., Nishiumi S., Nagayasu H., Fukuda I., Yoshida K, Ashida H. Epigallocatechin gallate promotes GLUT4 translocation in skeletal muscle. Biochemical and Biophysical Res Communications. 2008;377(1):286-290.
14. Vijayakumar A., Aryal P., Wen J., Syed I., Vazirani R.P., Moraes-Vieira P.M., Camporez JP, Gallop M.R., Perry R.J., Peroni O.D., Shulman G.I., Saghatelian A., McGraw T.E., Kahn B.B. Absence of Carbohydrate Response Element Binding Protein in Adipocytes Causes Systemic Insulin Resistance and Impairs Glucose Transport. Cell Rep. 2017;21(4):1021-1035.
15. Araujo Nunes E., Rafacho A. Implications of Palmitoleic Acid (Palmitoleate) On Glucose Homeostasis, Insulin Resistance and Diabetes. Current Drug Targets. 2017;18(6):619-628.
16. Huang X., Liu G., Guo J., Su Z. The PI3K/AKT pathway in obesity and type 2 diabetes. International J Biological Sci. 2018;14(11):1483-1496.
17. Fatemeh J.K., Zahra L., Hossein A.KH. Medicinal plants: Past history and future perspective. J Herbmed Pharmaco. 2018;7 (1):1-7.
18. Süntar I. Importance of ethnopharmacological studies in drug discovery: role of medicinal plants. Phytochemistry Reviews. 2020;19 (5):1199-1209.
19. Shanak S., Saad B., Zaid H. Metabolic and Epigenetic Action Mechanisms of Antidiabetic Medicinal Plants. Evidence-Based Complementary and Alternative Med. 2019;3583067.
20. Unuofin J.O., Lebelo S.L. Antioxidant Effects and Mechanisms of Medicinal Plants and Their Bioactive Compounds for the Prevention and Treatment of Type 2 Diabetes: An Updated Review. Oxidative Med & Cell Longevity. 2020;1356893.
21. Wickramasinghe A.S.D., Kalansuriya P., Attanayake A.P. Herbal Medicines Targeting the Improved β-Cell Functions and β-Cell Regeneration for the Management of Diabetes Mellitus. Evidence-Based Complementary & Alternative Medicine. 2021;2920530.
22. Fatima S., Akhtar M.F., Ashraf K.M., Sharif A., Saleem A., Akhtar B., Peerzada S., Shabbir M., Ali S., Ashraf W. Antioxidant and alpha amylase inhibitory activities of Fumaria officinalis and its antidiabetic potential against alloxan induced diabetes. Cell & Mol Biol. 2019;65(2):50–57.
23. Salmi D., Riou C., Issawi M., Titouche Y., Ambrosini V., Smail-Saadoun N., Abbaci H., Houali K. Antibacterial and antioxidant activities of endophytic fungi and nettle (Urtica dioica L.) leaves as their host. Cell & Mol Biol. 2021;67 (3),204–211.
24. Oza M.J., Kulkarni Y.A. Traditional uses, phytochemistry and pharmacology of the medicinal species of the genus *Cordia* (Boraginaceae). J Pharmacy & Pharmaco. 2017;6(7):755-789.
25. Prajapati S., Kar M., Maurya S., Pandey R., Dhakar R.C. Exploring phytochemicals and pharmacological uses of *Cordia dichotoma* (Indian Cherry): a review. J Drug Delivery and Therapeutics. 2017;7(6):125-131.
26. Heinrich M., Ankli A., Frei B., Weimann C., Sticher O. Medicinal plants in Mexico: healers' consensus and cultural importance. Social Sci Med. 1998;47(11):1859-1871.
27. Pu J., Li Linghai P.G., Liu N.H., Liu Pingsheng Y. Palmitic acid acutely stimulates glucose uptake via activation of Akt and ERK1/2 in skeletal muscle cells. J Lipid Res. 2011;527):1319-1327.
28. Kadan S., Bashar S., Yoel S., Hilal Z. In Vitro Evaluations of Cytotoxicity of Eight Antidiabetic Medicinal Plants and Their Effect on GLUT4 Translocation. Evidence-Based Complementary and Alternative Medicine. 2013;549345.
29. Anandharajan R., Jaiganesh S., Shankernarayanan N.P., Viswakarma R.A., Balakrishnan A. In vitro glucose uptake activity of Aegles marmelos and Syzygium cumini by activation of Glut-4, PI3 kinase and PPARγ in L6 myotubes. Phytomedicine. 2006;13 (6):434-441.
30. Ben Khedher M.R.M., Arch M., Jonathan R.S. Hislop David C., Wargent E.D., Edward T. Kępczyńska, Małgorzata A. Zaibi, Mohamed S. Preventive effects of Salvia officinalis leaf extract on insulin resistance and inflammation in a model of high fat diet-induced obesity in mice that responds to rosiglitazone. 2018;6:e4166.
31. Yang Q., W. Y., Shen J, Chen M.M., Wen J.H., Li Z.M., Liang Y.Z., Xia N. Guava Leaf Extract Attenuates Insulin Resistance via the PI3K/Akt Signaling Pathway in a Type 2. Diabetes Metab Syndr Obes. 2020;13:713-718.
32. Fahmida A., Islam A., Khalil I., Hua Gan S. Metabolic Control of Type 2 Diabetes by Targeting the GLUT4 Glucose Transporter: Intervention Approaches. Current Pharmaceutical Design. 2016;22:3034-3049.
33. Babu S., Madhan K., Ponnulakshmi R., Vijayalakshmi P., Vishnupriya V., Ramajayam G., Selvaraj J. Beta-sitosterol attenuates insulin resistance in adipose tissue via IRS-1/Akt mediated insulin signaling in high fat diet and sucrose induced type-2 diabetic rats. European J Pharmaco. 2020;873:173004.
34. Dai B., Qinxuan Wu., Chengxi Z., Jiani Z., Luting C., Zizeng X., Menglin Y. The effect of Liuwei Dihuang decoction on PI3K/Akt signaling pathway in liver of type 2 diabetes mellitus (T2DM) rats with insulin resistance. J Ethnopharmaco. 2016;192:382-389.
35. Boucher J., André K., Kahn C. Ronald. Insulin receptor signaling in normal and insulin-resistant states. Cold

Spring Harbor Perspectives in Biology. 2014;6 (1):a009191.
36. Fischer A.W., Kirstin A., Christian S., Frederike S., Krott Lucia M., Hartwig S., Philip G., Ludger S., Joerg H. PID1 regulates insulin-dependent glucose uptake by controlling intracellular sorting of GLUT4-storage vesicles. Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease. 2019;1865 (6):1592-1603.
37. Yang J., Hamid S., Cai J., Liu Q., Xu S., Zhang Z. Selenium deficiency-induced thioredoxin suppression and thioredoxin knock down disbalanced insulin responsiveness in chicken cardiomyocytes through PI3K/Akt pathway inhibition. Cellular Signalling. 2017;38:192-200.
38. Li R., Chen Q., Liu O., Tan B., Dong X., Chi S., Yang Q., Zhang S. Molecular characterization and expression analysis of glucose transporter 4 from Trachinotus ovatus, Rachycentron canadums and Oreochromis niloticus in response to different dietary carbohydrate-to-lipid ratios. Aquaculture. 2019;501:430-440.
39. Huang X., Liu G., Guo J.S.Z. The PI3K/AKT pathway in obesity and type 2 diabetes." Inter J Biological sci. 2018;14 (11):1483-1496.
40. Anurag M., Prasad G.G. Antidiabetic activity of Alangium Salvifolium in alloxan induced diabetic rats. Inter Res J Pharmacy. 2011;6:101-105.
41. Ezequiel V.V., Azucena O.C., Javier M.M., Pilar C.R. Antioxidant, cytotoxic and alpha-glucosidase inhibition activities from the Mexican berry “Anacahuita” (Cordia boissieri). Órgano Oficial de la Sociedad Latinoamericana de Nutrición. 2016;66 (3):211-218.
42. Jamkhande P.G., Barde S.R., Patwekar S.L., Tidke P.S. Plant profile, phytochemistry and pharmacology of *Cordia dichotoma* (Indian cherry): A review. Asian Pacific J Tropical Biomedicine. 2013;3(12):1009-1012.
43. Nazim H., Dr. Kakoti B.B. Review on ethnobotany and phytopharmacology of *Cordia dichotoma*. J Drug Delivery & Therapeutics. 2013;3(1):110-113.
44. Wang Z.F., Pan Z.Y, Xu C.S, Li Z.Q. Activation of G-protein coupled estrogen receptor 1 improves early-onset cognitive impairment via PI3K/Akt pathway in rats with traumatic brain injury. Bioch & Biophysical Res Communications. 2017;482(4):948-953.
45. Klip A., McGraw T.E., James D.E. Thirty sweet years of GLUT4. J Biological Chem. 2019;294 (30):11369-11381.