***Hibiscus sabdariffa* Linn fruit derivatives as alternative agents for diabetes mellitus care: A basic insight**

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Received 27th May 2021 / Accepted 13th September 2021

Abstract. This study discusses the effectiveness of methanolic extract and fractions (butanol, ethyl acetate, and n-hexane) of *H. sabdariffa* Linn fruit towards antidiabetic activities (*in vitro*). In order to test the efficacy, toxicity and insulin secretion capacity of rat pancreatic β -cell lines (BRIN-BD11) were tested with the methanolic extract and fractions. The outcomes showed that both the extract and the fractions demonstrated significantly lower levels of cytotoxic activities. Furthermore, the methanolic extract and fractions displayed varied sensitivity levels towards insulin release after an incubation period of 30 min. The methanolic extract, at a concentration of 300 $\mu\text{g/mL}$, significantly stimulated secretion of insulin by 2.85-fold ($p < 0.001$). In addition, butanol, ethyl acetate, and n-hexane fractions revealed a gradual increase in insulin secretion. The stimulated insulin secretion for these fractions had been recorded at 2-fold ($p < 0.01$), 2.67-fold, and 2.31-fold ($p < 0.001$), respectively, at the highest concentrations. The methanolic extract and fractions also appeared to stimulate secretion of insulin with all modulators present, for example, potassium chloride (KCl), insulin secretion inhibitor (verapamil and diazoxide), as well as insulin secretagogue (tolbutamide and isobutylmethylxanthine (IBMX)). These results indicate that *H. sabdariffa* Linn fruit methanolic extract and fractions could indeed be beneficial for future development of antidiabetic drugs.

Keywords: *Hibiscus sabdariffa* Linn, fruit, *in vitro* study, diabetes, insulin, pancreatic β -cell

INTRODUCTION

A multitude of studies has revealed that the deterioration of insulin secretion and its ensuing effects can cause persistent hyperglycaemia amongst patients suffering from Type 2 diabetes mellitus (Ahmad and Ahmed, 2006; Guo and Tabrizchi, 2006). In fact, prolonged hyperglycaemia can lead to complications in diabetic pathogenesis (Ahmad and Ahmed, 2006; Fowler, 2008). Some of these complications are known to be associated with oxidative stress and low-grade chronic inflammation (Newsholme *et al.*, 2016). Oxidative stress, an imbalance between cellular oxidant and antioxidant systems, results from the overproduction of free radicals and

associated reactive oxygen species (ROS). HG upregulates the markers of chronic inflammation and contributes to increased ROS generation, which ultimately involves diabetes mellitus complications including vascular dysfunction (Luc *et al.*, 2019). Other complications that include microvascular disease of the eye and kidney and a variety of clinical neuropathies (Clark Jr and Lee, 1995). At present, patients diagnosed with Type 2 diabetes mellitus are prescribed antidiabetic drugs as the treatment, such as meglitinides, thiazolidinedione, and sulphonylureas. Despite the accessibility of numerous antidiabetic agents, diabetes happens to remain a detrimental health

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concern across the globe (Wild *et al.*, 2004), primarily due to the adverse effects it has upon the human's health (Kirchheiner *et al.*, 2005). For instance, treatment with sulphonylureas causes weight gain and hypoglycaemia (Chehade and Mooradian, 2000; Wei *et al.*, 1998); meglitinides often sparks constant rhinitis, headache, bronchitis, and hypoglycaemia (Chehade and Mooradian, 2000); while thiazolidinedione results in fluid retention, weight gain, liver damage, and anaemia (Bell, 2002; Watkins and Whitcomb, 1998). Hence, dedicated efforts have been taken to develop alternative antidiabetic agents to overcome the shortcomings associated with the existing antidiabetic drugs prescribed as a treatment for patients diagnosed with Type 2 diabetes mellitus. Moreover, the literature has depicted that insulin secreted from pancreatic β -cells due to consumption of antidiabetic drugs may be advantageous in reducing hyperglycaemia (Bösenberg and van Zyl, 2008; Puri, 2001). The underlying mechanism that takes place through insulin secretion favours the uptake of glucose in the insulin-sensitive organs (e.g. liver and muscle), thereby diminishes hyperglycaemia (Patel and Mishra, 2008). On top of that, numerous medicinal herbs and their extracts have been introduced over the years for the treatment of diabetes (Patel and Zaveri, 2012).

The tropical plant *H. sabdariffa* Linn, which is also known as karkade or roselle, belongs to the family Malvaceae (Wang *et al.*, 2000) and is extensively applied as a traditional medicine in treating hypertension, inflammation, as well as liver disorders (Wang *et al.*, 2000). The aqueous extract of dried roselle flowers has been discovered to contain a high concentration of flavonoids that could be used for effective treatment of leukaemia and gastric carcinoma (Lin *et al.*, 2007; Tseng *et al.*, 2000). Besides, a study demonstrated (*in vitro*) that roselle leaves extract could possess some hypoglycaemic attributes (Sachdewa *et al.*, 2001). Nevertheless, the hypoglycaemic properties of *H. sabdariffa* Linn fruit extracts have yet to be determined. As such, the potency of *H. sabdariffa* Linn fruit derived from methanolic extract and fractions (*in vitro*) were examined to determine and to suggest the feasibility of using them as alternative antidiabetic agents. With that, this study assessed the toxicity

and insulin secretion ability of these derivatives by using rat pancreatic β -cell lines (BRIN BD11).

MATERIALS AND METHODS

Chemical reagents

D-glucose, Dimethyl sulfoxide (DMSO), and 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide (MTT) had been procured in analytical grade from Sigma-Aldrich Group (Selangor, Malaysia). Meanwhile, all supplements of cell culture had been obtained from Invitrogen (USA).

Plant material and extraction procedure

Some whole fruits of *H. sabdariffa* Linn were purchased from a local herbal shop (Johor Bahru, Malaysia). Running tap water. Then, the dried fruits were finely powdered using a grinder. The plant has been officially documented by the Forest Research Institute Malaysia (FRIM) with database card number 280615-14.

In order to obtain the first batch of crude extract, the powdered sample was soaked in methanol and water at a 4:1 ratio for 96 h, following the specified procedure (Bohari *et al.*, 2006). The extract, which turned gummy, was dried with a freeze dryer (Beta 2-4 LD plus LT, Martin Christ, Germany) after it was evaporated with a rotary evaporator (EYELA N-1000, EYELA, Tokyo, Japan). Following the same protocol, four batches of crude extracts were obtained and pooled (Bohari *et al.*, 2006). The pooled extracts were finally subjected to a successive solvent-solvent partitioning from the least polar solvent (n-hexane) to the highest polarities (ethyl acetate and butanol). Next, the *in vitro* study of these methanolic extracts and fractions had been evaluated by using insulin secreted from pancreatic β cells (Bohari *et al.*, 2006).

Cell culture

The cell line that secretes insulin (BRIN BD11) had been retrieved from the European Collection of Cell Cultures (ECACC, Salisbury, UK). The obtained cell had been cultured at the Roswell Park Memorial Institute (RPMI) to prepare 1640 media, which was supplemented with 1% (v/v)

penicillin/streptomycin (10,000 units/mL penicillin and 10 mg/mL streptomycin) and 10% (v/v) foetal bovine serum (FBS) in an incubator that was humidified containing 95% air and 5% CO₂ at 37°C.

Cell viability assay

The assay that determined cell viability was prepared by adhering to the procedures prescribed by Fotakis *et al.* (2006). The cells at concentration 7×10^5 had been seed onto a plate with 96 wells, which was later incubated at 37°C for overnight. Then, it was treated with *H. sabdariffa* Linn fruit methanolic extract and fractions, as well as glibenclamide (positive control drug), for 72 h. After incubation, 20 µL of MTT (5 mg/mL) had been added into every well and then, incubated again for 4 h. Subsequently, the medium from each well had been gently aspirated, while 100 µL of DMSO had been included in order to dissolve formazan crystals. After that, each plate was shaken for 5 s, whereas the absorbance rate had been recorded at 570 nm with Anthos micro-plate reader (Beckman Coulter, USA).

Insulin secretion assay

Insulin secretion activity of *H. sabdariffa* Linn fruit methanolic extract and fractions had been assessed with cells of BRIN-BD11. Besides, the fundamental features and the production of insulin-secreting cell lines have been recorded in this study (McClenaghan *et al.*, 1996). The insulin secretion assay was conducted by using a slightly modified method, where the cells at a concentration of 1×10^6 were seeded in a plate with 96 wells, which was incubated overnight at 37°C before attachment was carried out. Next, the cells had been washed thrice on the next day by using Krebs-Ringer Bicarbonate Buffer (KRB), which was later incubated again for 40 min at 37°C. After that, the cells went through incubation for additional 30 min after adding 1 mL of KRB (negative control) or 1 mL of KRB containing gradient concentrations of *H. sabdariffa* Linn fruit methanolic extract and fractions or 1 mL of KRB containing glibenclamide (positive control). All evaluations were carried out with glucose (2 mM). After the incubation process, 1 mL of aliquots taken from every well had been placed into 1.5 mL tubes and later kept at -20°C

in order to determine concentration of insulin (Gray *et al.*, 2000).

Determination of insulin secretion mechanisms of 300 µg/mL methanolic extract

The methanolic extract of 300 µg/mL had been applied in order to identify mechanisms involved in insulin secretion. The methanolic extract concentration had been selected based on the results obtained in insulin secretion assay. In order to assess the related mechanism, the extract was incubated along with some modulators that have been reckoned to influence the channel of secondary messenger found in pancreatic β-cells. These modulators, including potassium chloride (KCl), insulin secretion inhibitor (verapamil and diazoxide), as well as insulin secretagogue (tolbutamide and isobutyl methylxanthine (IBMX)), set at a depolarizing concentration, had been used. Next, the cells of BRIN-BD11 (1×10^6 cells/well) had been seeded in a plate with 96 wells and had been left at 37°C at overnight, CO₂ (5%) for attachment purpose before acute assessment. After being incubated for an overnight, the cells were washed three times using KRB, and later incubated again with KRB at 37°C for 40 min. Next, the cells went through incubation for an additional of 30 min after including 1 mL of KRB that consisted of methanolic extract (300 µg/mL) in both the presence and the absence of tolbutamide, IBMX, verapamil, KCl, and diazoxide at concentrations of 200 µM, 100 µM, 30 mM, 50 mM, and 300 µM. All the tests were performed in the presence of glucose (2 mM). Following the incubation, 1 mL of aliquots from each well was placed at -20°C in 1.5 mL tubes for insulin assay.

Statistical analysis

Each measurement was carried out thrice to calculate the average and the standard deviation (SD) values. Besides, SPSS 21 software package (Windows Evaluation Version, SPSS Inc., USA) had been employed to perform statistical analysis upon the empirical data retrieved. After that, the Shapiro-Wilk assessment had been carried out in order to ensure data normality. Furthermore, independent *t*-test and Mann-Whitney test were performed to analyses normal and abnormal data, respectively. If the value of probability (*p*)

determined is below 0.05, the data are considered as significant (Pallant, 2007).

RESULTS

Cell viability assay

Table 1 portrays the effects of *H. sabdariffa* Linn fruit (methanolic extract and fractions) and glibenclamide upon the viability of BRIN BD11 cell. The IC₅₀ value for the methanolic extract and fractions were calculated to determine their cytotoxic effect on the insulin secretion activity. As a result, it was found that n-hexane and butanol fractions displayed high cytotoxic effect upon BRIN BD11 cells with IC₅₀ values of 316 µg/mL and 181 µg/mL, respectively. Nevertheless, the methanolic extract and the ethyl acetate exhibited lower toxicity effect upon BRIN BD11 cells with IC₅₀ values of 346 µg/mL and 363 µg/mL, respectively. Next, glibenclamide was used as a standard reference to challenge the insulin secreting activity of *H. sabdariffa* Linn fruit methanolic extract and fractions against BRIN BD11 cells. The IC₅₀ value for glibenclamide was noted to be at 489 µg/mL, which signified that glibenclamide exerted lower cytotoxic effect upon BRIN BD11 cells, in comparison to that of the extract and fractions of roselle.

Insulin secretion

Figure 1 displays the impact of methanolic extract and fractions of *H. sabdariffa* Linn fruit, as well as glibenclamide, upon insulin secretion ability from BRIN BD11 cells. Meanwhile, in comparison to the negative control, methanolic extract at 300 µg/mL exhibited higher insulin secretion activity (2.85-fold, $p < 0.001$). On the other hand, the ethyl acetate fraction at concentration 350 µg/mL showed a step-wise stimulating effect on the secretion of insulin, when compared to the negative control, with the highest stimulation at 2.67-fold. Additionally, the n-hexane and butanol fractions illustrated high potential dose-dependent stimulatory effect at 2.31- ($p < 0.001$) and 2-fold ($p < 0.01$) for 300 µg/mL and 150 µg/mL concentrations, respectively. Nevertheless, all the roselle derivatives showed inhibition for insulin secretion at concentrations above IC₅₀. On the other hand, glibenclamide

appeared to have significantly stimulated insulin secretion by 3.23-fold ($p < 0.001$) at the highest concentration of 0.2 mM. From the outcomes retrieved, methanolic extract seems to be a better insulin secretion stimulator, in comparison to other fractions, although its efficacy appears lower than glibenclamide (positive control).

Insulin secretion mechanisms of 300 µg/mL methanolic extract

The impact displayed by modulators for insulin secretion upon *H. sabdariffa* methanolic extract treatment (300 µg/mL) towards BRIN BD11 cells insulin secretion had been assessed with basal glucose (2 mM) (Figure 2). The specific concentration of methanolic extract (300 µg/mL) produced a significant insulin secretion activity, as stated above. Likewise, the extract also stimulated significant insulin secretion with all modulators present, but except for verapamil (50 mM). The magnitudes of stimulation were 2- ($p < 0.001$), 2.96- ($p < 0.001$), 5.77- ($p < 0.001$), and 7.48-fold ($p < 0.001$) when tested with diazoxide (300 µM), IBMX (100 µM), tolbutamide (200 µM), as well as KCl (30 mM), respectively, in comparison to the corresponding incubation without methanolic extract (300 µg/mL). Furthermore, with KCl and tolbutamide present, the insulin secretion activity of methanolic extract (300 µg/mL) escalated by a factor of 1.96- ($p < 0.001$) and 1.56-fold ($p < 0.001$), accordingly. On the contrary, with the presence of diazoxide and verapamil have strongly inhibited the activity of insulin release for methanolic extract (300 µg/mL) by 0.49- ($p < 0.001$), as well as 0.79-fold, accordingly. In addition, the IBMX had minimal to nil effect upon insulin release for the extract.

Table 1. Cell viability of BRIN BD11 cells against varying concentrations of *H. sabdariffa* Linn fruit (methanolic extract and fractions) and glibenclamide. Mean $\pm$ SD values for three replicates, in comparison to control, are as follows: * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$.

Concentration ($\mu\text{g/mL}$)	Control	7.81	15.63	31.25	62.5	125	250	500	1000	IC ₅₀ ($\mu\text{g/mL}$)
Methanol	100 ± 0.006	***	*	***		*	***	**	*	320
		92.14 ± 0.003	85.06 ± 0.009	79.66 ± 0.007	76.91 ± 0.004	65.77 ± 0.006	53.99 ± 0.019	37.1 ± 0.218	16.91 ± 0.022	
n-hexane	100 ± 0.020	**	**	*	**	*	***	**	**	316
		94.29 ± 0.007	91.17 ± 0.022	84.5 ± 0.037	76.08 ± 0.068	68.22 ± 0.011	55.52 ± 0.013	38.08 ± 0.003	26.64 ± 0.005	
Ethyl acetate	100 ± 0.017	*	**	**	**	*	***	***	**	369
		96.43 ± 0.012	91.43 ± 0.002	84.12 ± 0.004	80.33 ± 0.006	75.16 ± 0.012	59.52 ± 0.007	42.82 ± 0.021	18.06 ± 0.022	
Butanol	100 ± 0.017	*	**	*	***	*	***	**	***	181
		94.5 ± 0.012	92.74 ± 0.002	91.25 ± 0.004	81.32 ± 0.006	63.99 ± 0.012	39.9 ± 0.007	30.69 ± 0.021	13.8 ± 0.022	
Glibenclamide	100 ± 0.013	***	*		*	*	***	**	**	464
		98.43 ± 0.017	97.12 ± 0.027	92.63 ± 0.017	83.81 ± 0.052	75.38 ± 0.047	57.83 ± 0.016	49.23 ± 0.034	27.43 ± 0.024

Figure 1. Insulin secretion activity of BRIN BD11 cells against *H. sabdariffa* Linn fruit (methanolic extract and fractions), as well as glibenclamide. The values refer to mean $\pm$ SD based on four replicates with *** $p < 0.001$, in comparison to that of control.

Figure 1 (continued). Insulin secretion activity of BRIN BD11 cells against *H. sabdariffa* Linn fruit (methanolic extract and fractions), as well as glibenclamide. The values refer to mean $\pm$ SD based on four replicates with *** $p < 0.001$, in comparison to that of control.

Figure 2. The effect exhibited by modulators of insulin secretion upon 300 µg/mL of methanolic extract towards BRIN BD11 cells insulin secretion. The values denote mean $\pm$ SD from four replicates *** $p < 0.001$, in comparison to incubations without 300 µg/mL of methanolic extract.

DISCUSSION

This research work reveals the influence of methanolic extract, as well as its fractions, from *H. sabdariffa* Linn fruit on insulin secretion capacity by using BRIN-BD11 cell lines. The outcomes strongly postulate that methanolic extract and fractions derived from *H. sabdariffa* Linn fruit can be applied as an effective antidiabetic agent, in comparison to several other reported compounds. For instance, nutrient regulation can positively induce insulin secretion in BRIN BD11 cell lines (Gray *et al.*, 2000). Also, treatments using nymphyol (Gembal *et al.*, 1992), *C. colocynthis* (Evans and Rushakoff, 2002), and *Achillea millefolium* L. (Asteraceae) (Kecskemeti *et al.*, 2002) revealed that secretion of insulin was enhanced due to glucose stimulation via *in vitro* by 2.1- ($p < 0.05$), 1.8-, and 2.3-fold ($p < 0.001$), when compared to the control group respectively. Furthermore, the addition of a dose of Hank's balanced salt solution (0.1 mg/mL) can also efficiently induce secretion of insulin. Besides, three new flavonoids isolated from *Pseudarthria hookeri* also demonstrated a mild effect on the

secretion of insulin when stimulated with glucose (Tchamgoue *et al.*, 2016). Nevertheless, the increase noted for insulin secretion in the aforementioned compounds is not as effective as that portrayed by methanolic extract from roselle.

In order to determine the insulin secretion mechanism, the methanolic extract (300 µg/mL) had been incubated in both presence and absence of modulators for the secretion of insulin. For instance, depolarization of KCl (30 mM), the insulinotropic antagonist (verapamil and diazoxide), as well as an insulinotropic agonist (IBMX and tolbutamide). The results showed that tolbutamide, which derives from the Sulphonylureas group has been reckoned to be a hypoglycaemic agent, displayed the potential to stimulate the release of insulin via channel-dependent K^+_{ATP} pathway (Chehade and Mooradian, 2000; Evans and Rushakoff, 2002; Gembal *et al.*, 1992). This is because tolbutamide closes the path of K^+_{ATP} , apart from stimulating the Ca^{2+} influx by activating voltage-dependent Ca^{2+} channel (VDCC), as well as by depolarizing plasma membrane (Kecskemeti *et al.*, 2002). As for this research work, the tolbutamide augmented the effect of secreting insulin in

methanolic extract (300 µg/mL), thus signifying the involvement of K^+_{ATP} that depends on channel (Hannan *et al.*, 2007).

On top of that, it has been reported that IBMX, which is a phosphodiesterase inhibitor, can enhance the release of insulin by escalating the levels of intracellular cAMP in pancreatic β -cells (Siegel *et al.*, 1980). Nevertheless, this study found that IBMX failed in inducing insulin secretion for methanolic extract (300 µg/mL), thus dismissing the involvement of cAMP in the process (Hannan *et al.*, 2007). The diazoxide, which has been known to open channels of K^+_{ATP} , has been reported to inhibit insulin secretion by activating the channels of K^+_{ATP} in β -cells (Gembal *et al.*, 1992; Gray *et al.*, 2000). As for this research, the diazoxide inhibited the effects of insulin secretion for methanolic extract (300 µg/mL), which points out that the release of insulin is attributable to the activated channel of K^+_{ATP} . Nonetheless, it was found that the diazoxide could only inhibit, but not halt the effect of methanolic extract (300 µg/mL) on insulin secretion, as illustrated in Figure 2. This finding suggests that the insulin-release inducing effect of methanolic extract (300 µg/mL) could have embedded the pathway of channel-dependent K^+_{ATP} (Mathews *et al.*, 2006). This notion is supported by the observation of KCl (30 mM) that ultimately had the cell membranes depolarized. The augmented insulin secretion displayed by methanolic extract (300 µg/mL) in a depolarized state signifies the involvement of K^+_{ATP} channel-independent pathway (Gray *et al.*, 2000; McClenaghan, 2007), but this does not portray the mechanism of the molecular underpinning the pathway. The trigger of insulin secretion is possible due to shifts in adenine nucleotide concentration (Sato and Henquin, 1998) or due to the stimulating effect of Ca^{2+} on the insulin-releasing process (Gembal *et al.*, 1992), which occur at the pathway of channel-independent K^+_{ATP} .

Lastly, the verapamil, which derives from phenylalkylamines class, has been reckoned to be a VDCC blocker of the L-type that inhibits the secretion of insulin by hindering influx of Ca^{2+} in cells (Lebrun *et al.*, 1997) as for this research, it was discovered that verapamil strongly inhibited, but not to the extent of totally halting the effects of methanolic extract (300 µg/mL) on the secretion of insulin in BRIN-BD11 cells. This

appears to be an indication that the effect of the extract tested upon release of insulin had been dependent on extracellular Ca^{2+} through activated VDCC. However, secretion of insulin may also take place without the presence of extracellular Ca^{2+} as the release of insulin can be enhanced by the extract due to stored intracellular Ca^{2+} mobilization (Hannan *et al.*, 2007). Additionally, another viable method to promote the release of insulin by the extract with verapamil present refers to Ca^{2+} influx, which may take place via other Ca^{2+} channels, for instance, voltage-operated Ca^{2+} channel (VOCC) and N-type Ca^{2+} channel, thus increasing the Ca^{2+} intracellular concentration (Liu *et al.*, 2003; Satin *et al.*, 1995; Vajna *et al.*, 2001).

This study demonstrated that methanolic extract (300 µg/mL) from *H. sabdariffa* Linn fruit possesses superior insulin secretion activity by a factor of 2.85-fold ($p < 0.001$), in comparison to the negative control (cells without treatment). Hence, the present observations suggest that the methanolic extract from *H. sabdariffa* Linn fruit is a suitable candidate for the development of a new oral antidiabetic drug to stimulate secretion of insulin.

CONCLUSION

This study demonstrated that the methanolic extract and fractions from *H. sabdariffa* Linn fruit at controlled concentrations successfully stimulate secretion of insulin in BRIN BD11 cells. The findings showed that the property of anti-hyperglycaemic in *H. sabdariffa* Linn fruit had been mediated by stimulation of insulin secretion from the pancreatic β -cells. In short, the systematic experimentation, as well as the in-depth analyses undertaken in this research, reveals the prospect for progression of oral hypoglycaemic drug by using methanolic extract and fractions of *H. sabdariffa* Linn fruit.

ACKNOWLEDGEMENTS

The authors wish to express their gratitude to the Ministry of Higher Education and Scientific

Research of Iraq for the financial assistance, laboratory facilities of Tissue Engineering Laboratory FS, IBD, at Universiti Teknologi Malaysia.

CONFLICT OF INTEREST

The authors have declared that no conflict of interest exists.

REFERENCES

- Ahmad, M. S. & Ahmed, N. 2006. Antiglycation properties of aged garlic extract: possible role in prevention of diabetic complications. *The Journal of Nutrition* 136(3): 796S-799S.
- Bell, D. S. 2002. current status of diabetes treatment. (Featured CME Topic: Diabetes Mellitus). *Southern Medical Journal* 95(1): 24-30.
- Bohari, M., Pauliena, S., Hamid, M., Shaari, K., & Lajis, N. 2006. Glucose uptake: Stimulatory activity of *Gynura procumbens* in 3T3-F442A adipocytes.
- Bösenberg, L. H. & van Zyl, D. G. 2008. The mechanism of action of oral antidiabetic drugs: a review of recent literature. *Journal of Endocrinology, Metabolism and Diabetes of South Africa* 13(3): 80-88.
- Chehade, J. M. & Mooradian, A. D. 2000. A rational approach to drug therapy of type 2 diabetes mellitus. *Drugs* 60(1): 95-113.
- Clark Jr, C. M. & Lee, D. A. 1995. Prevention and treatment of the complications of diabetes mellitus. *New England Journal of Medicine* 332(18): 1210-1217.
- Evans, J. & Rushakoff, R. 2002. Oral pharmacological agents for type 2 diabetes: sulfonylureas, meglitinides, metformin, thiazolidinediones, α -glucosidase inhibitors, and emerging approaches. *Diabetes and Carbohydrate Metabolism*.
- Fotakis, G. & Timbrell, J. A. 2006. *In vitro* cytotoxicity assays: comparison of LDH, neutral red, MTT and protein assay in hepatoma cell lines following exposure to cadmium chloride. *Toxicology Letters* 160(2): 171-177.
- Fowler, M. J. 2008. Microvascular and macrovascular complications of diabetes. *Clinical Diabetes* 26(2): 77-82.
- Gembal, M., Gilon, P., & Henquin, J.-C. 1992. Evidence that glucose can control insulin release independently from its action on ATP-sensitive K⁺ channels in mouse B cells. *Journal of Clinical Investigation* 89(4): 1288.
- Gray, A., Abdel-Wahab, Y., & Flatt, P. 2000. Insulin-like and insulin-releasing actions of the traditional antidiabetic plant *Sambucus nigra* (elder). *Journal of Nutrition* 130(1): 15-20.
- Guo, L. & Tabrizchi, R. 2006. Peroxisome proliferator-activated receptor gamma as a drug target in the pathogenesis of insulin resistance. *Pharmacology & Therapeutics* 111(1): 145-173.
- Hannan, J., Marenah, L., Ali, L., Rokeya, B., Flatt, P. R., & Abdel-Wahab, Y. H. 2007. Insulin secretory actions of extracts of *Asparagus racemosus* root in perfused pancreas, isolated islets and clonal pancreatic β -cells. *Journal of Endocrinology*, 192(1): 159-168.
- Kecskemeti, V., Bagi, Z., Pacher, P., Posa, I., Kocsis, E., & Koltai, M. 2002. New trends in the development of oral antidiabetic drugs. *Current Medicinal Chemistry* 9(1): 53-71.
- Kirchheiner, J., Roots, I., Goldammer, M., Rosenkranz, B., & Brockmüller, J. 2005. Effect of genetic polymorphisms in cytochrome P450 (CYP) 2C9 and CYP2C8 on the pharmacokinetics of oral antidiabetic drugs. *Clinical Pharmacokinetics* 44(12): 1209-1225.
- Lebrun, P., Antoine, M.-H., Ouedraogo, R., Pirotte, B., Herchuelz, A., Cosgrove, K. E., *et al.* 1997. Verapamil, a phenylalkylamine Ca²⁺ channel blocker, inhibits ATP-sensitive K⁺ channels in insulin-secreting cells from rats. *Diabetologia* 40(12): 1403-1410.
- Lin, T.-L., Lin, H.-H., Chen, C.-C., Lin, M.-C., Chou, M.-C., & Wang, C.-J. 2007. Hibiscus sabdariffa extract reduces serum cholesterol in men and women. *Nutrition Research* 27(3): 140-145.
- Liu, Y.-J., Cheng, H., Drought, H., MacDonald, M. J., Sharp, G. W., & Straub, S. G. 2003. Activation of the K_{ATP} channel-independent signaling pathway by the nonhydrolyzable analog of leucine, BCH. *American Journal of Physiology-Endocrinology and Metabolism* 285(2): E380-E389.
- Luc, K., Schramm-Luc, A., Guzik, T., & Mikolajczyk, T. 2019. Oxidative stress and inflammatory markers in prediabetes and diabetes. *Journal of Physiology and Pharmacology* 70(6): 809-824.
- Mathews, J. N., Flatt, P. R., & Abdel-Wahab, Y. H. 2006. *Asparagus adscendens* (*Shweta musali*) stimulates insulin secretion, insulin action and inhibits starch digestion. *British Journal of Nutrition* 95(3): 576-581.
- McClenaghan, N., Barnett, C., O'Harte, F., & Flatt, P. 1996. Mechanisms of amino acid-induced insulin secretion from the glucose-responsive BRIN-BD11 pancreatic B-cell line. *Journal of Endocrinology* 151(3): 349-357.
- McClenaghan, N. H. 2007. Physiological regulation of the pancreatic β -cell: functional insights for understanding and therapy of diabetes. *Experimental Physiology* 92(3): 481-496.
- Newsholme, P., Cruzat, V. F., Keane, K. N., Carlessi, R., & de Bittencourt Jr, P. I. H. 2016. Molecular mechanisms of ROS production and oxidative stress in diabetes. *Biochemical Journal* 473(24): 4527-4550.
- Pallant, J. 2007. SPSS survival manual: A step-by-step guide to data analysis using SPSS version 15. Maidenhead, Berkshire, England: McGraw-Hill Education.
- Patel, M. & Mishra, S. 2008. PHCOG REV.: Invited review cell lines in diabetes research: A review. *Pharmacognosy Reviews* 2(4): 188-205.
- Patel, S. S. & Zaveri, M. N. 2012. Cytotoxic activity to find bioactive compound from *Justicia gendarussa* using brine shrimp lethality assay. *Asian Journal of Traditional Medicines*, 7(3): 102-108.
- Puri, D. 2001. The insulinotropic activity of a Nepalese medicinal plant *Biophytum sensitivum*: Preliminary experimental study. *Journal of Ethnopharmacology* 78(1): 89-93.
- Sachdewa, A., Nigam, R., & Khemani, L. 2001. Hypoglycemic effect of *Hibiscus rosa sinensis* L. leaf extract in glucose and streptozotocin induced hyperglycemic rats. *Indian Journal of Experimental Biology* 39(3): 284-286.
- Satin, L., Tavalin, S., Kinard, T., & Teague, J. 1995. Contribution of L- and non-L-type calcium channels to voltage-gated calcium current and glucose-dependent insulin secretion in HIT-T15 cells. *Endocrinology* 136(10): 4589-4601.
- Sato, Y. & Henquin, J.-C. 1998. The K⁺-ATP channel-independent pathway of regulation of insulin secretion by glucose. *Diabetes* 47: 1713-1721.
- Siegel, E., Wollheim, C., Kikuchi, M., Renold, A., & Sharp, G. 1980. Dependency of cyclic AMP-induced insulin release

- on intra- and extracellular calcium in rat islets of Langerhans. *Journal of Clinical Investigation* 65(2): 233.
- Tchamgoue, J., Hafizur, R. M., Tchouankeu, J. C., Kouam, S. F., Adhikari, A., Hameed, A., *et al.* 2016. Flavonoids and other constituents with insulin secretion activity from *Pseudarthria bookeri*. *Phytochemistry Letters* 17: 181-186.
- Tseng, T.-H., Kao, T.-W., Chu, C.-Y., Chou, F.-P., Lin, W.-L., & Wang, C.-J. 2000. Induction of apoptosis by hibiscus protocatechuic acid in human leukemia cells via reduction of retinoblastoma (RB) phosphorylation and Bcl-2 expression. *Biochemical Pharmacology* 60(3): 307-315.
- Vajna, R., Klöckner, U., Pereverzev, A., Weiergräber, M., Chen, X. h., Miljanich, G., *et al.* 2001. Functional coupling between 'R-type' Ca^{2+} channels and insulin secretion in the insulinoma cell line INS-1. *The FEBS Journal* 268(4): 1066-1075.
- Wang, C.-J., Wang, J.-M., Lin, W.-L., Chu, C.-Y., Chou, F.-P., & Tseng, T.-H. 2000. Protective effect of Hibiscus anthocyanins against tert-butyl hydroperoxide-induced hepatic toxicity in rats. *Food and Chemical Toxicology* 38(5): 411-416.
- Watkins, P. B. & Whitcomb, R. W. 1998. Hepatic dysfunction associated with troglitazone. *New England Journal of Medicine* 338(13): 916-917.
- Wei, M., Gaskill, S. P., Haffner, S. M., & Stern, M. P. 1998. Effects of diabetes and level of glycemia on all-cause and cardiovascular mortality: the San Antonio Heart Study. *Diabetes Care* 21(7): 1167-1172.
- Wild, S., Roglic, G., Green, A., Sicree, R., & King, H. 2004. Global prevalence of diabetes estimates for the year 2000 and projections for 2030. *Diabetes Care* 27(5): 1047-1053.