DOI:10.24041/ejmr2020.26

HYPOGLYCEMIC ACTIVITY OF THE AQUEOUS EXTRACT OF CAESALPINIA PULCHERRIMA FLOWERS IS INDEPENDENT OF INSULIN STIMULATION AND SUPPRESSED IN THE PRESENCE OF METFORMIN

Angela B.F. Carrington-Dyall*, Ahmad Idrees Shekaib*, Nickelia E. Clarke-Jordan**

Department of Biochemistry, Genetics & Research, Department of Premedical Sciences***

American University of Barbados, School of Medicine, Wildey, St. Michael, Barbados

Received on : 26-12-2020
Accepted on : 29-12-2020

Address for correspondence
Dr. Angela B.F. Carrington-Dyall
Department of Biochemistry, Genetics & Research
American University of Barbados, School of Medicine, Wildey, St. Michael, Barbados
Email: angela.carringtondyall@gmail.com
Contact no: +1-246-428-2000

ABSTRACT

Considering the high prevalence of insulin resistance, antidiabetic strategies that enhance insulin action or act independent of insulin are desirable. *Caesalpinia pulcherrima* (CP) flowers are known to have antidiabetic properties, but more work is required with respect to this action in insulin resistant adipocytes, particularly, its dependence on insulin and its therapeutic equivalence and/or interactions with other antidiabetic drugs. The purpose of this study was therefore to investigate the insulin-dependency of the water extract of CP flowers (CP extract) hypoglycemic effects, compare its antidiabetic action in diabetic and non-diabetic glucose loads, and explore its therapeutic equivalence and interactions with metformin. CP extract was prepared by boiling the air-dried flowers in cell culture media prepared in Krebs Ringer Bicarbonate buffer for 5mins. Metformin solution was prepared from a Metformin hydrochloride extended-release tablet to obtain low and therapeutic levels of metformin. Insulin resistant (IR) adipocytes were exposed to CP extract in cell culture containing either 8mM or 18mM glucose and one of three insulin concentrations. CP extract allowed an efficient glucose disposal in the IR adipocytes in an insulin independent manner ($p<0.0001$). The percentage of glucose uptake did not significantly differ by models of diabetic and non-diabetic conditions ($p=0.4727$) although the significantly higher glucose concentration taken up by the IR adipocytes in the presence of IR adipocytes suggest an enhancement of antidiabetic action in hyperglycemic conditions. Expectedly metformin had a higher potency than the CP extract with its therapeutic dose of 1.8-2.4mg/L corresponding to 280mg/l of CP extract ($p=0.9996$). Additionally, metformin and CP extract appear to compete for similar sites which suppressed the hypoglycemic activity of CP extract.

KEYWORDS: Caesalpinia pulcherrima, Metformin, Insulin resistance, Glucose uptake, Antidiabetic, Adipocytes.

INTRODUCTION

Insulin resistance refers to an impaired response of tissues to insulin and is therefore noted in the face of a glucose challenge. It is also generally found in conditions of increased fuel stores (1) and in comorbidities of obesity particularly Type 2 diabetes mellitus and metabolic syndrome (2). Strategies that can allow the uptake of glucose independent of insulin are therefore always highly sought after. With respect to Type 2 diabetes mellitus, some of these methods include oral hypoglycemic agents and diet control. However, traditionally, medicinal plants have also been utilized in the treatment of diabetes (3-5) and might therefore hold the key to the identification of a plant extract that can significantly regulate glucose disposal by insulin resistant tissue that would function independently of insulin stimulation.

Currently, the most prescribed insulin-sensitizing, antidiabetic treatment is Metformin. Metformin or dimethyl biguanide has an impressive record spanning over 50 years of use for the treatment of Type 2 diabetes (6). Interestingly, although a synthesized compound, Metformin originated from the parent compound guanidine which was isolated from the plant, *Galega officinalis*. *Galega officinalis* was used traditionally in Europe for the treatment of diabetes (7). Metformin's mode of action has been and still is the subject of intense research, but it is accepted that its primary actions with respect to insulin resistant tissue is two-fold. Metformin has been shown to increase insulin binding, in which case its action is additive to that of insulin (8), but it also appears to act independent of insulin binding purportedly by interacting with the glucose transporter (9). Metformin's action has also been found to be enhanced in the hyperglycemic state (8).

Caesalpinia pulcherrima (CP) is a flowering shrub of immense value both for its beauty and its medicinal value. It is found in many areas ranging from India to the Caribbean and is the national flower of Barbados, which gives it the name of Pride of Barbados (10). Its high nutrient value has seen much research into its use

as a feed supplement for chickens (11) and swine and even as a possible ingredient for food formulations of humans (12). Its antidiabetic (10, 13-14) and anti-obesity (15) properties has also lent it to much research particularly with respect to its use as an alternative or supplement to traditional treatment for conditions like Type 2 diabetes and metabolic syndrome, which have at their backbone the state of insulin resistance. However, it is unknown whether CP's antidiabetic properties stem from an insulin dependent or insulin independent method of glucose disposal.

This study sought to provide insight into this using an aqueous extract of the CP flowers (CP extract), and naturally insulin-resistant chicken adipocytes (IR adipocytes). Exposure experiments were designed to determine whether CP extract could positively affect glucose uptake by the IR adipocytes in the absence of insulin while under diabetic type conditions, that is, high insulin/high glucose conditions. Whether the antidiabetic activity of CP extract varied with diabetic versus non-diabetic conditions was also considered. Finally, the antidiabetic effects of CP extract on IR adipocytes were compared to Metformin's and the combination of these two hypoglycemic agents was explored.

MATERIALS & METHODS

CP flowers, harvested during the dry season, were air-dried as per traditional methods and ground to a fine powder. Base media comprised of 135mM NaCl, 5.01mM KCl, 0.99mM $MgSO_4$, 0.99mM $CaCl_2$, 24.6mM $NaHCO_3$ and 0.17mM KH_2PO_4 in water was prepared and used to perform a 5mins decoction of the dried flowers at a concentration of 10mg/ml (CP extract). After cooling and removing sediment, the extract was filtered sterilized with a 0.22µm filter and used without further concentration.

IR adipocytes were obtained by bromelain digest at 37°C of 0.1g adipose tissue obtained from chickens, (fasted for minimum 12hrs), freshly slaughtered for normal human consumption by the local abattoir. Cell culture media was prepared by adding 10% autologous serum and 1% penicillin-streptomycin to the base media.

Experiments were conducted with 10mg/ml CP extract in 18mM and three ITS (insulin-transferrin-sodium selenite supplement) concentrations (0%, 10% and 20%) to determine the effect of insulin stimulation on the hypoglycemic character of CP extract. For comparative studies, IR adipocytes were also exposed to 8mM glucose which was a nondiabetic postprandial glucose level. Treatment media were made by adding dilutions of the CP extract or metformin as required for the condition under investigation.

For the purposes of this study, metformin refers to the powder obtained from one Metformin Hydrochloride Extended Release tablet. The coating of the tablet was removed by a scalpel and the released tablet was ground to a fine powder. 0.1g was then dissolved in base media, filtered through a 1µm filter to remove any undissolved particles and filter sterilized via a 0.22µm filter prior to use. Based on the properties of metformin which has an oral bioavailability of 40-60%, it is estimated that 40-60% of the metformin would be present in the filtrate (16). The results are therefore presented as a range of 0.8 – 1.2mg/l metformin (met 2) and 1.6 – 2.4mg/l metformin (met 4).

Treatment or control media (100µl) was added to each well of a sterile 96 well cell culture plate containing 2 x 10^4 IR adipocytes for 30mins and/or 60mins. The infranatant fluid was tested for glucose using a FreeStyle Optium Neo glucometer (Abbott) and the percentage glucose depletion recorded as the indicator of glucose uptake (% glucose uptake).

Statistical analysis of the three experiments conducted in triplicate was done using GraphPad Prism 9 with $p<0.05$ deemed significant.

RESULTS

The results are presented as answers to the questions under investigation.

Are CP extract's antidiabetic effects dependent on Insulin?

Under conditions of high glucose (18mM), IR adipocytes were exposed to 10mg/ml CP extract in the presence of 0%, 10% or 20% ITS for an incubation period of 1hr. CP extract increased glucose uptake compared to Control, that is, IR adipocytes incubated in the absence of extract (11.4% ± 1.33% versus 2.9% ± 1.62%; $p<0.0001$) in the absence of insulin. The addition of insulin to the cell culture media of the IR adipocytes did not increase glucose uptake into the cells compared to 0% insulin even at concentrations as high as 20% ITS (Figure 1).

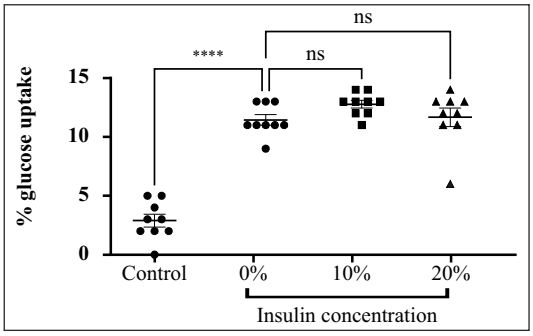

Fig. 1: Insulin resistant adipocytes (IR adipocytes) were exposed to cell culture media prepared in modified Krebs Ringers Bicarbonate buffer containing 18mM glucose, 10.0mg/ml CP extract and 1% penicillin-streptomycin with or without insulin (ITS) supplement) and incubated at 37°C for 1hr. Glucose concentration in the cell culture

media before and after incubation was measured using the FreeStyle Optium Neo glucometer and used to calculate the percentage of glucose depleted from the media which, as movement of glucose across cell membranes is via facilitative transport, corresponds to the amount of glucose taken up by the cells. CP extract increased glucose uptake in 60mins in the absence of insulin (p<0.0001). Insulin did not stimulate any further increase in glucose uptake beyond that obtained with CP extract. (ns= not statistically significant). Bars indicate mean and SEM. (CP extract = aqueous extract of Caesalpinia pulcherrima flowers, ITS = Insulin-transferrin-sodium selenite supplement, **** = p<0.0001)

Are CP extract's antidiabetic properties enhanced in diabetic conditions?

Some diabetic medication most notably, Metformin can influence glucose uptake under diabetic (blood glucose>11.1mM) and non-diabetic (blood glucose <10mM) postprandial conditions, but Metformin's action is enhanced in the hyperglycemic state. Investigation of this characteristic in CP extract, via the addition of 10mg/ml CP extract to cell culture media, did not result in a significantly different % glucose uptake by the IR adipocytes at 8mM compared to 18mM (11% ± 4.0% vs 13% vs 1.0%, p=0.4727) but a significant increase in the concentration of glucose taken up by the adipocytes (2.3mM ± 1.69mM vs 1.5mM ± 0.93mM, p=0.0010) was noted at 18mM compared to 8mM (Figure 2).

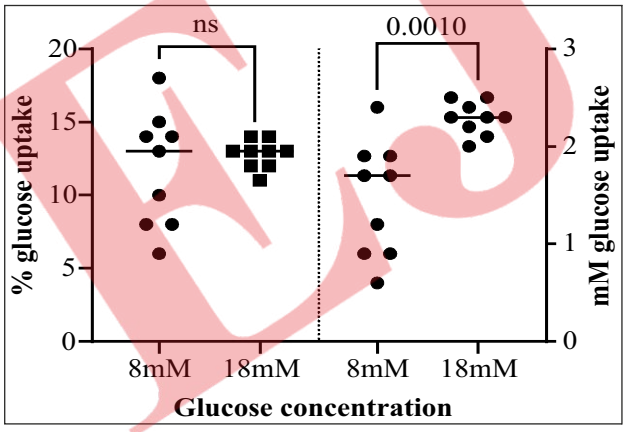

Fig. 2: In the presence of high insulin concentration, IR adipocytes were exposed to 10mg/ml CP extract in cell culture media containing either 8mM or 18mM glucose. After a 1hr incubation at 37°C, glucose measurements were taken and used to calculate the quantity of glucose taken up by the cells (mM glucose uptake) and the percentage of the added glucose that was taken up by the cells (% glucose uptake). Although there was no significant change in the % glucose uptake, a significant increase in the quantity of glucose taken into the cell (p=0.0010) was noted. Bars indicate mean ± SEM; ns = not statistically significant

How does CP extract's antidiabetic effects on IR adipocytes compare to Metformin's and can they be safely used together?

As it is known that Metformin has maximal activity in a hyperglycemic/diabetic state, comparison analysis was conducted in culture media containing 18mM glucose and 20% ITS at 37°C for 1hr.

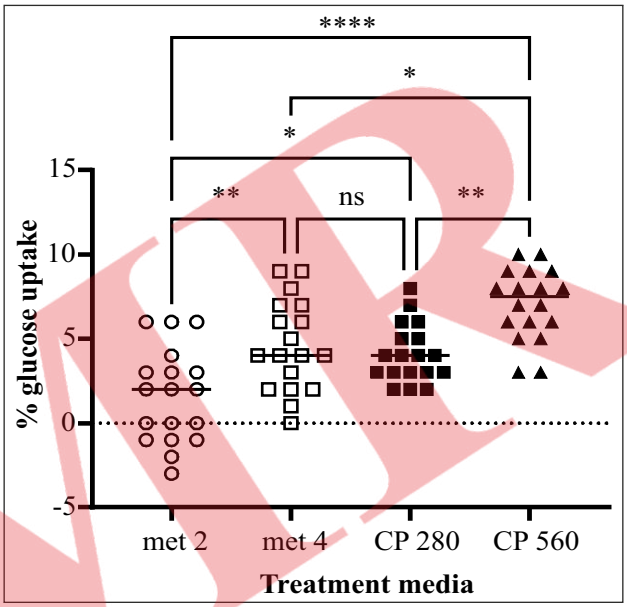

Fig. 3: Therapeutic dose of metformin (met 4) was found to be comparable in activity to 280mg/l CP extract (CP280) with a p-value of 0.9996. Low dose of metformin (met 2) had very limited hypoglycemic activity whereas 560mg/l CP extract (CP560) had the highest activity of the four concentrations. Bars indicate mean ± SEM, ns= not statistically significant, *=p<0.05, ** = p<0.01, ***= p<0.001, ****=p<0.0001)

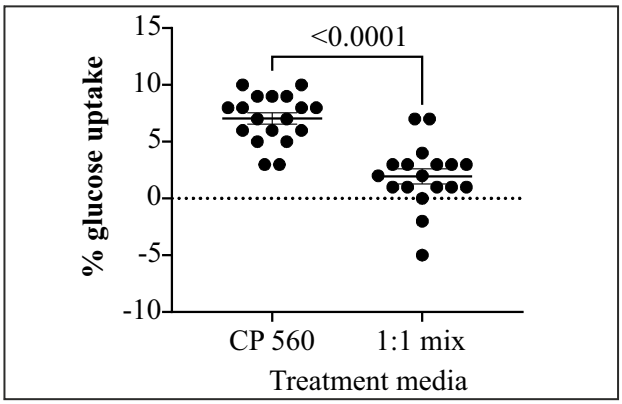

Fig. 4: 560mg/l CP extract (CP560) stimulated a 7% increase in glucose uptake in IR adipocytes whereas, met 2, the low dose of metformin had no significant stimulation of glucose uptake. Incubation of IR adipocytes in the presence of a combination of these two agents (1:1 mix) showed a significant reduction in glucose uptake by the IR adipocytes (p<0.0001). Bars indicate mean ± SEM

As expected, metformin resulted in increased glucose uptake in much lower concentration compared to CP extract with the hypoglycemic effect of 1.6 – 2.4mg/l metformin corresponding to similar activity in 280mg/l CP extract (Figure 3). After combining equal volumes of 560mg/l CP extract and 0.8 – 1.2mg/l metformin, CP extract's hypoglycemic activity at that concentration was significantly suppressed (7.0% $\pm$ 2.13% to 1.9% $\pm$ 2.80%; $p<0.0001$) (Figure 4).

DISCUSSION

With the high prevalence of insulin resistance in conditions like Type 2 diabetes mellitus, the identification of treatment options that can overcome or bypass the need for insulin during glucose disposal is warranted. *Caesalpinia pulcherrima* is an accessible shrub with antidiabetic properties in many parts of the plant. However, much is still unknown with regards to its antidiabetic activity particularly with respect to its action on insulin resistant adipocytes.

This study showed that an aqueous extract of *Caesalpinia pulcherrima* flowers not only afforded a more efficient glucose disposal in chicken insulin resistant adipocytes, but it appears to do so in an insulin independent manner. Chicken adipocytes lack Glut-4, the insulin-dependent glucose transporter, but like all other adipocytes, they also possess Glut-1 transporters which dispose of glucose independently of insulin (17). Although Glut-1 in the chicken is reported to be responsive to insulin (18), no additive action was noted in the presence of insulin, irrespective of insulin concentration. This is highly promising but it must be noted that although improving glucose disposal is desired, it has been proposed that disposal via Glut-1 instead of Glut-4 could actually increase insulin resistance as the glucose becomes available for more pathways, particularly the hexosamine pathway (19).

Nevertheless, CP extract should not be sidelined as it is known that the hypoglycemic agent, metformin also has the ability to improve glucose disposal by cells via insulin independent pathways (9) and yet it is the most recommended treatment for Type 2 diabetes mellitus based on its efficacy. CP extract, even in the unconcentrated form, resulted in glucose uptake quantities and rates in insulin resistant adipocytes that were comparable to metformin. Consideration must also be given to the fact that the quantity of glucose removed under diabetic conditions was significantly larger than that removed under non-diabetic conditions. This would seem to suggest that the active ingredient in the CP extract, like metformin, had enhanced activity in the hyperglycemic state. However, the fact that similar increases in percent glucose uptake was noted when IR adipocytes were exposed to 8mM and 18mM glucose might require increased monitoring of persons, especially non-diabetics, who use the extract.

With such similarities between them, it was considered that metformin and CP extract might compete for similar sites and therefore negate their individual hypoglycemic activity. This was indeed shown to be the case with metformin completely suppressing the hypoglycemic activity of the CP extract at therapeutic dose.

Further work is needed that could explore the mechanism of action of the CP extract.

CONCLUSION

Although the antidiabetic properties of *Caesalpinia pulcherrima* had previously been identified, to the knowledge of the authors, this is the first work that reports that the hypoglycemic activity of a 5mins aqueous decoction of its flowers, operates via an insulin independent pathway that can be suppressed by therapeutic doses of metformin.

REFERENCES

1. Kahn BB, Flier JS. Obesity and insulin resistance. The Journal of Clinical Investigation. 2000; 106(4):473-481.
2. Seidell JC. Obesity, insulin resistance and diabetes - a worldwide epidemic. British Journal of Nutrition. 2000; 83(S1): S5-S8.
3. Yoshioka H, Mori M, Fujii H, et al. Sasa veitchii extract reduces obesity-induced insulin resistance and hepatic steatosis in obese mice fed a high-fat diet. Nagoya J Med Sci. 2017; 79(3): 279-290.
4. Balasundaram M, Dhanesh Gandhi A, Kavitha P, et al. Effect of Caesalpinia pulcherrima (L.) Sw. seeds on serum glucose and other metabolic parameters of normal and alloxan - induced diabetic rats. 2018;4(4): 22.
5. Wright CM, Bezabhe W, Fitton JH, et al. Effect of a Fucoidan Extract on Insulin Resistance and Cardiometabolic Markers in Obese, Nondiabetic Subjects: A Randomized, Controlled Trial. J Altern Complement Med. 2019; 25(3): 346-352.
6. Rena G, Pearson ER, Sakamoto K. Molecular mechanism of action of metformin: old or new insights?. Diabetologia. 2013; 56(9): 1898-906.
7. He L, Wondisford Fredric E. Metformin Action: Concentrations Matter. Cell Metabolism. 2015; 21(2): 159-162.
8. Klip A, Leiter LA. Cellular Mechanism of Action of Metformin. Diabetes Care. 1990;13(6):696.

9. Fantus IG, Brosseau R. Mechanism of Action of Metformin: Insulin Receptor and Postreceptor Effects in Vitro and in Vivo*. The Journal of Clinical Endocrinology & Metabolism. 1986; 63(4): 898-905.
10. Balasubramanian V, Seetaram P, Gayasuddin M, et al. Demonstration of β-cell regeneration and anti-diabetic activity of Caesalpinia Pulcherrima flower extract in alloxan induced diabetic rats. Der Pharmacia Lettre. 2012; 4(6): 6.
11. Ogunbode AA, Ogungbenro SD, Raji MO, et al. Performance and nutrient digestibility of broiler fed graded levels of raw pride of Barbados seed meal. International Journal of Agriculture and Biosciences. 2014; 3(3): 123-126.
12. Akoja SS. Studies on the physical and functional properties of Pride of Barbados (Caesalpinia pulcherima) seed flour. Journal of Global Biosciences. 2017; 1(1): 4643-4651.
13. da Cunha Jácome Marques F, da Silva Pantoja P, Matos VEA, et al. Galactomannan from the seeds of Caesalpinia pulcherrima prevents indomethacin-induced gastrointestinal damage via neutrophil migration. Int J Biol Macromol. 2019; 141: 68-75.
14. Kumar MP, Sankeshi V, Naik RR, et al. The inhibitory effect of Isoflavones isolated from Caesalpinia pulcherrima on aldose reductase in STZ induced diabetic rats. Chemico-Biological Interactions. 2015; 237: 18-24.
15. Chichioco-Hernandez CL, Leonido FMG. Weight-lowering effects of Caesalpinia pulcherrima, Cassia fistula and Senna alata leaf extracts. Journal of Medicinal Plants Research. 2011; 5(3): 452-455.
16. Scheen AJ. Clinical pharmacokinetics of metformin. Clin Pharmacokinet. 1996; 30(5): 359-371.
17. Seki Y, Sato K, Kono T, et al. Broiler chickens (Ross strain) lack insulin-responsive glucose transporter GLUT4 and have GLUT8 cDNA. General and Comparative Endocrinology. 2003; 133(1): 80-87.
18. Shimamoto S, Nakashima K, Kamimura R, et al. Insulin acutely increases glucose transporter 1 on plasma membranes and glucose uptake in an AKT-dependent manner in chicken adipocytes. General and Comparative Endocrinology. 2019; 283: 113232.
19. Ebeling P, Koistinen HA, Koivisto VA. Insulin-independent glucose transport regulates insulin sensitivity. FEBS Letters. 1998; 436(3): 301-303.

How to cite this article : Dyall A.B.F.C., Shekaib A.I., Clarke-Jordan N.E. Hypoglycemic Activity Of The Aqueous Extract Of Caesalpinia Pulcherrima Flowers Is Independent Of Insulin Stimulation And Suppressed In The Presence Of Metformin. Era J. Med. Res. 2020; 7(2): 152-156.