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WATER EXTRACT OF *CAESALPINIA PULCHERRIMA* FLOWERS INCREASES GLUCOSE UPTAKE IN INSULIN RESISTANT ADIPOCYTES IN A DOSE-DEPENDENT MANNER

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ABSTRACT

Caesalpinia pulcherrima (CP) flower extract has been shown to have antidiabetic effects on pancreatic beta cell regeneration and improved skeletal muscle glucose utilisation. However, little is known about its effect on adipocytes, particularly insulin resistant adipocytes. This study investigated the possibility of improving glucose uptake in adipocytes obtained from chickens which are naturally insulin resistant via direct effects of water extract of *Caesalpinia pulcherrima* flowers. The extract was prepared by boiling air dried CP flowers in a water based modified Krebs Ringer Bicarbonate buffer for 5mins and sterilizing the filtrate via vacuum filtration. A glucose load of 18mM and 10% ITS was added to the extract. In the absence of adipocytes, the glucose concentration of the CP extract remained unchanged ($p=0.5087$). Insulin resistance in the chicken adipocytes was confirmed by delayed and limited glucose uptake (maximum $2.9\% \pm 1.62\%$ in 60mins). This was reversed via the addition of the water extract of the CP flowers as concentrations as low as 2.8mg/ml doubled glucose uptake and concentrations as low as 5.6mg/ml increased the rate of glucose uptake to that of insulin sensitive cells. These results suggest that water extract of CP flowers directly modulate glucose uptake into insulin resistant cells at concentrations as low as 2.8mg/ml following a 5mins decoction preparation.

KEYWORDS: Caesalpinia pulcherrima, Obesity relat ed insulin resistance, Antidiabetic, Adipocytes, Glucose uptake.

INTRODUCTION

Obesity related insulin resistance (Ob-IR) refers to the diminished response of insulin sensitive tissue to insulin and is a state that is commonly noted in individuals with increased adipose tissue stores (1-2). This Ob-IR is associated with chronic non-communicable diseases most notably Type 2 diabetes mellitus (3) in which hyperglycemia is noted in both the fasted and postprandial states. With Ob-IR closely involved in the etiology of these conditions, treatment strategies that target Ob-IR would be useful in providing a basis for decreasing their prevalence and overall morbidity. Investigation of such strategies have been afforded both via in vivo models which allows a whole body analysis of events and in vitro methods which assist in ascertaining changes that occur at the organ specific level (4-6). With the strong association of diet to conditions associated with obesity, it is becoming more prevalent to utilize strategies involving the use of plants and plant extracts (5, 7-9).

Caesalpinia pulcherrima (CP) also known as Flower Fence, Guletura, Peacock Flower and Pride of Barbados is a thorny flowering shrub whose inflorescence is the national flower of Barbados (10). It is also a plant with immense medicinal properties ranging from anti-microbial (11) and anti-inflammatory (12) to anti-diabetic (13). It is unknown whether CP's antidiabetic properties include a negative effect on Ob-IR. Extracts of the CP seed in animal models have shown reduced hyperglycemia in diabetic mice (10). In an effort to identify the organ(s) involved, Balasubramanian et al (14) reported increased regeneration of pancreatic beta cells but their work was completed using ethanolic extract of the CP flowers.

However, little to no research has been done on aqueous extracts of CP flowers on Ob-IR by exploring any direct effects on adipocytes. The aims of this study were therefore to ascertain whether the antidiabetic effects of CP flowers extended to a direct effect on adipocytes, if these properties could be identified in the simple water extraction traditionally prepared and if these antidiabetic effects could overpower the insulin resistance of chicken adipocytes even at low concentrations.

MATERIALS & METHODS

Preparation of the water extract of the Caesalpinia pulcherrima flowers

Caesalpinia pulcherrima (CP) flowers were harvested during the dry season and air-dried in a brown paper bag as per traditional methods. The dried flowers were then placed in a blender and ground to a fine powder. The powder was then stored in 50ml falcon tubes until use. The extract was prepared with 10mg/ml of the dried powder boiled in base media (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) for 5 mins with constant stirring. Water lost by evaporation was replaced. After cooling, the tea was filtered using a100µm filter to remove sediment and sterile filtered through a 0.22µm filter prior to use. To mimic traditional methods, the CP extract was used without further concentration.

Isolation and primary culture of insulin resistant adipocytes

Adipose tissue and autologous blood of chickens freshly slaughtered for normal human consumption were obtained from the local abattoir. As standard for slaughtering purposes, all chickens had been held in a fasted state overnight (minimum 12hrs) prior to slaughter. Adipocytes were isolated from 0.1g of tissue at 37°C for 45- 60mins, washed in PBS and resuspended in culture media (base media with 10% autologous serum and 1% penicillin-streptomycin) for counting.

EXPOSURE EXPERIMENT

Exposure media were made by preparing dilutions of the CP extract in base medium with added 10% autologous serum, 18mM glucose, 1% penicillin-streptomycin and 10% ITS (insulin-transferrin-sodium selenite media supplement- Sigma).

2 x 10^4 adipocytes were loaded into each well of a sterile 96-well cell culture plate and exposed to 100µl of the exposure media. Following the specified incubation period, the infranatant fluid was transferred to another plate for testing. Glucose measurements were taken at 0mins and every 30mins thereafter for up to 2hrs, using a Free Style Neo Optium glucometer (Abbott). Glucose uptake was recorded as the percentage glucose depletion (% glucose uptake) from the cell culture media in the presence of the adipocytes. CP exposure medium in the absence of cells was used to determine whether CP's action was as a result of a direct effect on adipocytes.

Statistical analysis

All experiments were conducted in triplicate, in replicates of three and the data was analysed using GraphPad Prism 9 with $p<0.05$ deemed significant.

RESULTS

The preparation of the water extract resulted in the collection of two fractions; a translucent, dark brown, aromatic liquid (CP extract) and a sticky, thick, dark brown-black residue that passed easily through the 100µm but could not pass through the 0.22µm filter even under vacuum pressure. Only the CP extract was used in the experiments. Even after allowing to sit overnight, no further sediment was noted in the CP extract. This ensured that there would be reduced likelihood of fouling of the glucometer strip during testing.

After the addition of 18mM glucose, the glucose level in the CP extract was tested following a 2hr incubation period and found to be unchanged with a mean ± SD of 18.16mM ± 0.675mM ($p=0.5087$). This suggested that not only was there no detectable glucose in the CP extract, but any effects noted in the presence of CP extract and cells would be due to CP extract's effect on the cells and not of the CP extract alone.

Chicken adipocytes responds to high glucose challenge under post prandial conditions

Repeated measures one-way ANOVA of the time course experiment of the chicken adipocytes in the presence of insulin and 18mM glucose, showed the utilization of glucose with maximum glucose uptake of 2.9% ± 1.62% (adjusted $p = 0.0420$) noted in 60mins (Figure 1).

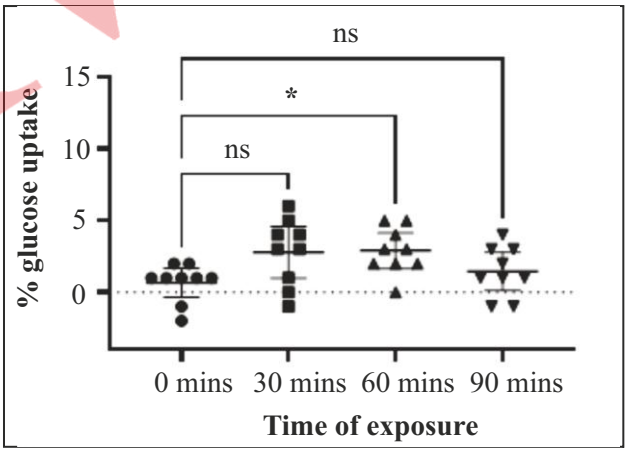

Fig. 1: Insulin Resistant Adipocytes Have A Delayed Glucose Uptake Response With Maximum Glucose Uptake Noted In 60mins (* = P<0.05, Ns= Not Statistically Significant). Bars Indicate Mean And SEM

Water extract of the CP flower increases glucose uptake by IR adipocytes

Increasing concentrations of CP extract increased glucose uptake in insulin resistant adipocytes compared to the control (Figure 2) in a dose dependent manner in 60mins. CP extracts of 5.6 mg/ml to 10 mg/ml also increased the rate of glucose uptake with significantly

higher glucose uptake levels noted in 30mins. This suggests that CP extract at these concentrations overcame the insulin resistance of the adipocytes returning them to an insulin sensitive rate of glucose uptake.

collection methods, quantities, drying time and grinding processes for future work. However, it must be noted, that this work was not designed to identify the active ingredient(s) in the extract nor to detail the mechanism by

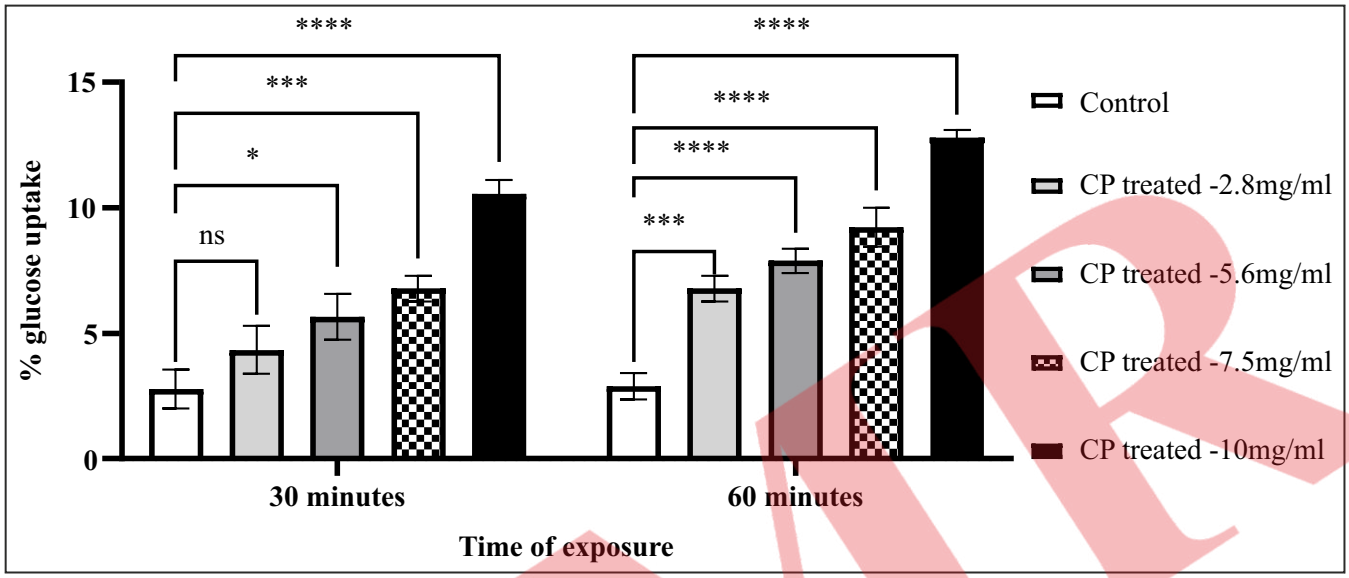

Fig. 2: Glucose Uptake In Insulin Resistant (IR) chicken adipocytes exposed to increasing concentrations of CP Extract In Cell Culture Media (Krebs Ringer Bicarbonate Buffer With Autologous Serum, 10% Insulin-Transferrin-selenite, 1% Penicillin/streptomycin and 18mM Glucose) at 37°C for 30 Mins And 1hr. (ns= not Statistically Significant, * = p<0.05, ***=p<0.01, **** = p<0.001). Bars Indicate Mean And SEM

DISCUSSION

Elucidation of treatment options that can directly affect Ob-IR could offer significant hope for individuals with chronic noncommunicable diseases such as metabolic syndrome and Type 2 diabetes. *Caesalpenia pulcherimma* is a shrub renown not only for its beauty, but its medicinal properties, including its antidiabetic properties. With limited studies conducted on the medicinal aspects of the water extract of its flowers, particularly the paucity of information relating to its effects on insulin resistant adipocytes, an exploration of these areas was the focus of this study.

Although lacking the most common mammalian glucose transporter, Glut-4 (15), adipocytes from chicken, a naturally insulin resistant animal as characterized by its hyperglycemia and hyperinsulinemia, possess Glut-1 (which has been found to be insulin responsive in chickens (16) and insulin responsive Glut-8 (15) and are therefore capable of utilizing glucose in a post-prandial state albeit in a less insulin sensitive fashion. Utilising these cells should therefore provide much insight into the use of CP extract in insulin resistant conditions.

One major challenge associated with the use of traditional herbal preparations is the inconsistency of preparation for study. This study used the traditional method of preparing the extract but with standardized

which any activities might occur but rather to determine whether the water extract of CP flowers warranted further study as a potential antidiabetic candidate that improves obesity insulin resistance in its action.

It was successful in its aims as the water extract of dried CP flowers increased the quantity of glucose taken up by insulin resistant adipocytes within 1hr in concentrations as low as 2.8mg/ml.

However, CP extract did not only increase glucose uptake. The exposure experiments mimicked the second half of a standard oral glucose tolerance curve where the glucose is removed into insulin sensitive cells between 30 – 60mins. As expected, IR adipocytes in the presence of hyperglycemic, postprandial conditions resulted in a flattening of that curve as they were only capable of removing a significant quantity of glucose in 60mins. When these IR adipocytes were exposed to CP extract, the rate at which the glucose was removed into the cell also increased (within 30 mins) at CP extract concentrations as low as 5.6mg/ml. This suggested that CP extract improved the rate of glucose uptake to that of an insulin sensitive cell.

To the knowledge of the authors, this is the first report of the ability of a traditionally prepared, water extract of dried flowers of the *Caesalpenia pulcherimma* plant to exert antidiabetic effects by directly overcoming the

insulin resistance of insulin resistant adipocytes. The standardised method for the preparation of the extract also serves as a base for future studies. Considering the cytotoxicity commonly noted with *Caesalpenia pulcherimma (17)*, the possibility of using such a low concentration of material to exert an antidiabetic effect that overcomes insulin resistance is heartening.

CONCLUSION

The water extract of *Caesalpenia pulcherimma* flowers has been shown to have potent antidiabetic properties at concentrations as low as 2.8mg/ml without further isolation or concentration of the active ingredient. This CP extract allowed chicken adipocytes to overcome their natural insulin resistance increasing both the rate and quantity of glucose taken into the cell.

REFERENCES

1. Xu H, Barnes GT, Yang Q, et al. Chronic inflammation in fat plays a crucial role in the development of obesity-related insulin resistance. The Journal of Clinical Investigation. 2003;112(12):1821-1830.
2. Kahn BB, Flier JS. Obesity and insulin resistance. The Journal of Clinical Investigation. 2000; 106(4): 473-481.
3. Seidell JC. Obesity, insulin resistance and diabetes — a worldwide epidemic. British Journal of Nutrition. 2000; 83(S1): S5-S8.
4. Gnasso A, Cacia M, Cortese C, et al. No effect on the short-term of a decrease in blood viscosity on insulin resistance. Clin Hemorheol Microcirc. 2018; 68(1): 45-50.
5. Malin SK, Kullman EL, Scelsi AR, et al. A whole-grain diet reduces peripheral insulin resistance and improves glucose kinetics in obese adults: A randomized-controlled trial. Metabolism. 2018; 82: 111-117.
6. Martinez B, Peplow PV. Treatment of insulin resistance by acupuncture: a review of human and animal studies. Acupunct Med. 2016; 34(4): 310-319.
7. 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): 346-52.
8. Xian HM, Che H, Qin Y, et al. Coriolus versicolor aqueous extract ameliorates insulin resistance with PI3K/Akt and p38 MAPK signaling pathways involved in diabetic skeletal muscle. Phytother Res. 2018; 32(3): 551-560.
9. 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): 279-90.
10. 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.
11. Sudhakar M, Rao CV, Rao PM, et al. Antimicrobial activity of Caesalpinia pulcherrima, Euphorbia hirta and Asystasia gangeticum. Fitoterapia. 2006; 77(5): 378-380.
12. 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.
13. 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.
14. 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.
15. 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.
16. 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.
17. Srinivas KVNS, Koteswara Rao Y, Mahender I, et al. Flavanoids from Caesalpinia pulcherrima. Phytochemistry. 2003;63(7):789-793.

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