TRANSDERMAL DELIVERY OF *SYZYGIUM AROMATICUM*-DERIVED OLEANOLIC ACID BY DERMAL PATCHES IN STREPTOZOTOCIN-INDUCED DIABETIC RATS: EFFECTS ON SOME SELECTED METABOLIC PARAMETERS

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INTRODUCTION

Medical plants believed to be safe and cost effective than synthetic hypoglycaemic agents play important roles in the management of diabetes mellitus in developing countries where resources are meagre. We have isolated triterpenes from *Syzygium aromaticum* as the bioactive compounds that possesses hypoglycaemic effects in experimental diabetes. However, the poor water solubility of triterpenes observed in oral administration has necessitated the evaluation of alternative methods of administration for effective diabetes management. Accordingly, the aim of this study was to investigate whether transdermal application of *Syzygium aromaticum*-derived oleanolic acid patch (P-OA) formulations sustain controlled release of oleanolic acid (OA) into the bloodstream of STZ-induced diabetic rats with concomitant alleviation of some of the complications associated with diabetes.

OBJECTIVES

The objectives of this study were to investigate whether topically applied P-OA amidated matrix patch can:

i. sustain controlled OA release into the bloodstream

ii. control some selected deranged metabolic parameters in experimental diabetes.

MATERIALS AND METHODS

Patch preparation

Amidated pectin hydrogel matrix patches were prepared using a previously described protocol by Hadebe *et al*. 2014 with slight modifications that involved liquefying OA [1]. Briefly, various amounts of OA (200, 400, 800 mg) were dissolved in separate beakers containing 3 mL of dimethyl sulfoxide (DMSO) overnight. Subsequently, the dissolved OA was transferred to separate beakers containing amidated low methoxyl pectin dissolved in deionized water (4 g / 100 mL) and mixed with agitation. Aliquots (11 mL) were transferred to petri dishes and subjected to solidification with 2% $CaCl_2$. Patches with measured widths containing 5.24, 10.48 and 20.95 mg of OA translating to dosages of 21, 42 and 84 mg/kg, respectively were cut out and placed on hydrofilm that served as backing material.

Study design

The study was designed to establish the effects of P-OA hydrogel matrix patch formulation on selected metabolic parameters in experimental diabetes.

Acute studies

Oral glucose tolerance (OGT) responses

OGT responses were evaluated in separate groups of non-diabetic and STZ-induced diabetic groups of rats following topical application of P-OA matrix patch on the back of the neck. The animals were fasted overnight (18 h), followed by measuring blood glucose (time 0). Subsequently, OGT responses to topically applied P-OA hydrogel patches at various doses of OA (21, 42 and 84 mg/kg) were monitored. Rats sham treated with drug free pectin hydrogel matrix patches and insulin (175 µg/kg, s.c.) served as negative and positive controls, respectively. Blood glucose was measured before glucose loading and at 15 minutes intervals for the first hour and then hourly for the subsequent 5 hours after glucose-loading.

Short-term effects

Short-term (5 weeks) effects were assessed in animals applied thrice daily 8 hours apart with topical P-OA patches containing various doses of OA (21, 42 and 84 mg/kg). Animals treated with drug-free pectin and insulin (175µg/kg, s.c.) acted as negative and positive controls, respectively. Blood samples and tissue samples were collected for the measurement of selected biochemical parameters.

Statistical analysis

All data were expressed as means ± standard error of means (S.E.M.). The $AUC_{0\text{-}360min}$ values were calculated using blood glucose concentrations following topical application of P-OA matrix patch. Statistical comparison of the differences between the control means and experimental groups was performed with GraphPad InStat Software (version 5.00, GraphPad Software, San Diego, California, USA), using one-way analysis of variance (ANOVA), followed by Tukey-Kramer multiple comparison test. A value of $p<0.05$ was considered significant.

RESULTS

OGT responses (Figure1)

OGT responses and the area under the glucose curve (AUC) of STZ-induced diabetic rats topically applied with P-OA hydrogel patches on the skin at various doses of OA are shown in Figure 1.

As can be seen by Figure 1, P-OA patch treatment on diabetic rats resulted in a statistically significant decrease in blood glucose at all-time points. In addition, the blood glucose AUC was smaller in P-OA hydrogel treated animals compared with respective control. Administration of s.c. insulin demonstrated blood glucose-lowering effects in STZ-induced diabetic rats.

A dose-dependent effect on the magnitude of POA-induced blood glucose lowering was not statistically significant.

In summary, the OGT responses and $AUC_{glucose}$ were not significantly different from those observed with s.c. insulin.

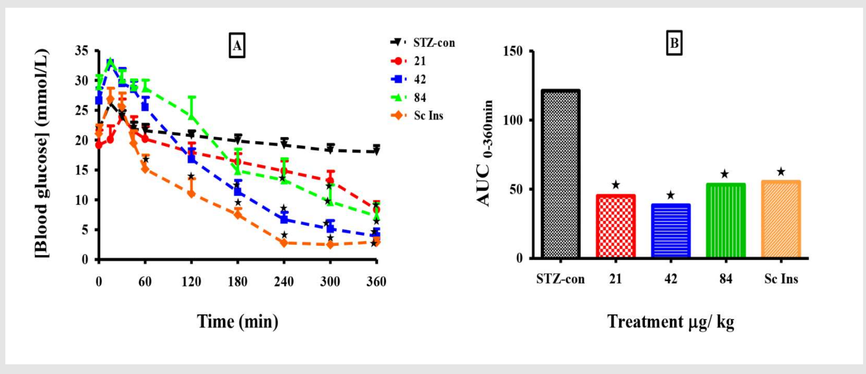

Figure 1: Comparisons of the effects of OA-containing dermal matrix patches of different OA concentrations on OGT responses (A) and $AUC_{glucose\ 0\text{-}360min}$ (B) in STZ-induced diabetic rats with untreated STZ-induced diabetic rats, control non-diabetic (ND) and s.c insulin treated animals.

★ = $p<0.05$ by comparison with untreated animals.

Short-term studies

Effects of dermal patches on IRS

To establish whether OA was transported across the skin of STZ-induced diabetic rats following topical application of POA-containing dermal matrix patches, we monitored the density of phosphorylated insulin receptor substrates (IRS) in skin tissues by immunohistochemical staining.

The method control skin section showed faint negative immune-reactivity (Figure 2A). Untreated non-diabetic rat skin sections exhibited intense widespread localization of IRS (Figure 2C) compared to faint staining of untreated STZ-induced diabetic control rats (Figure 2B).

Topical application of POA-containing dermal patches also demonstrated widespread localization of IRS in cell bodies of the dermis, collagen and subcutaneous layer (Figure 2D) which were significantly different from the faint staining of untreated STZ-induced diabetic rats but comparable to the intense staining of the subcutaneously treated rats (Figure 2E) and non-diabetic control animals.

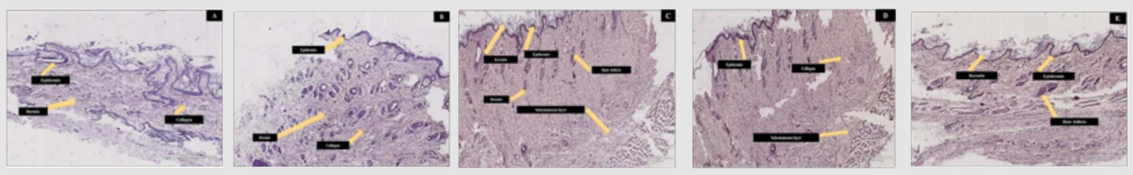

Figure 2: **Immunohistochemical micrographs illustrating the effects of P-OA dermal patches on the expression of insulin receptor (IR) in skin sections of the method control (A), non-diabetic control rat (C), untreated STZ-induced diabetic rat (B), transdermal OA treated rat (D) and subcutaneous insulin treated rat (E).**

Metabolic parameters

Untreated STZ-induced diabetic rats exhibited extensive hyperglycaemia, depletion of liver and muscle glycogen concentrations by the end of the 5-week experimental period (Figure 3 and Table 1).

Treatment with s.c. insulin (175 µg/ kg) and various doses of topically applied P-OA hydrogel matrix patch for 5 weeks reduced blood glucose concentrations and restored the depleted glycogen concentrations to levels that were comparable to the non-diabetic control animals (Figure 3 and Table 1).

The P-OA treated groups showed no dose-dependent effects, however, the effects of P-OA were comparable to those of the s.c. treated animals.

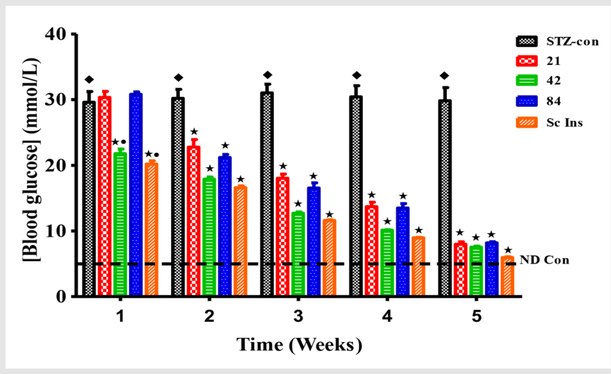

Figure 3: **Comparison of the effects of transdermally delivered OA on blood glucose concentration in STZ-induced diabetic rats with untreated diabetic rats, control non-diabetic (ND) and sc insulin treated animals.**

★ = $p<0.05$ by comparison with respective control animals;
◆ = $p < 0.05$ by comparison with non-diabetic control animals;
● = $p<0.05$ by comparison to the lowest and highest dose.

Table 1: **Comparisons of the effects of POA–containing dermal patches on hepatic and muscle glycogen concentrations in STZ-induced diabetic rats with untreated diabetic rats, control non-diabetic (ND) animals and subcutaneous (sc) insulin treated animals.**

	Glucose mmol/L	Glycogen	µg/100g/tissue
		Hepatic	Skeletal muscle
Non-diabetic control	4.51 ± 0.01	28.42 ± 0.41	2.62 ± 0.32
STZ-untreated	29.83 ± 2.01◆	12.36 ± 0.72◆	1.02 ± 0.21◆
STZ-induced 21	7.98 ± 0.18*	18.64 ± 0.76*◆	2.00 ± 0.11*
STZ-induced 42	7.54 ± 0.15*	18.90 ± 0.97*◆	2.20 ± 0.13*
STZ-induced 84	8.19 ± 0.36*	17.74 ± 0.53*◆	1.83 ± 0.16*
STZ-induced sc ins	5.95 ± 0.11*	21.28 ± 0.94*	2.36 ± 0.21*

* = $p<0.05$ by comparison with untreated STZ-induced diabetic animals
◆ = $p<0.05$ by comparison with non-diabetic animals

Effects on plasma insulin concentrations

The possible mechanisms responsible for the hypoglycaemic effects exerted by topical application of OA-containing dermal patches were investigated. Plasma insulin concentrations were measured from samples collected after 6 hours (acute) and after 5 weeks (chronic) of experimental period.

There was no change in plasma insulin concentrations of STZ-induced diabetic rats following acute and short-term daily treatment with OA-containing dermal patches.

Interestingly, the reduced plasma insulin concentrations following the chronic P-OA transdermal treatment were not statistically significant in comparison to the s.c. treated group.

Overall results showed that OA-containing dermal patches did not alter the plasma insulin concentrations of STZ-induced diabetic rats.

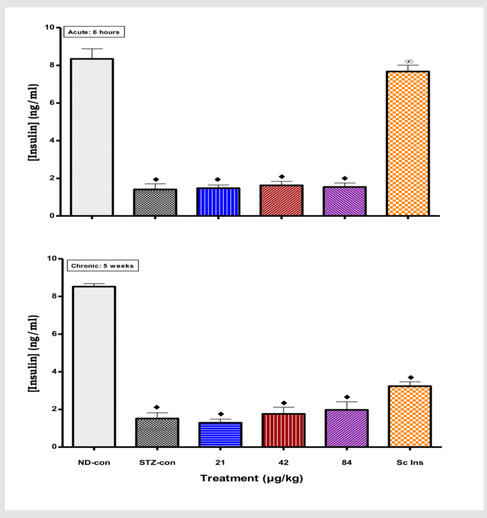

Figure 4: **Comparison of the effects of OA matrix patches of different OA concentrations on plasma insulin concentrations in STZ-induced diabetic rats with untreated diabetic rats, control non-diabetic (ND) and sc insulin treated animals.**

◆ = $p<0.05$ by comparison with non-diabetic control;

∞ = $p<0.05$ by comparison with transdermal OA treated animals.

DISCUSSION

The aim of this study was to increase solubility of triterpenes by developing a transdermal delivery formulation for controlled sustained OA release into the bloodstream with good control of hyperglycaemia and consequent impact on alleviating complications in diabetes. A carrier for the transdermal delivery of OA made out of a cocktail comprising (a) low methoxy pectin gelled with calcium ions (b) OA (c) a transdermal transfer enhancing agent and (d) an antioxidant which sustained controlled release of OA into the bloodstream of streptozotocin (STZ)-induced diabetic rats was developed. It thus appears that these patches lower blood glucose concentration and a dose-independent effect is seen.

CONCLUSION

Pectin hydrogel OA patches transport OA through the skin and lower blood glucose concentration in diabetic rats with concomitant amelioration of some metabolic parameters. We suggest that the formulation may free diabetic patients from multiple insulin injections thereby improving patient compliance.

REFERENCES

1. Hadebe SI, Ngubane PS, Serumula MR, Musabayane CT. (2014). Transdermal delivery of insulin by amidated pectin hydrogel matrix patch in streptozotocin-induced diabetic rats: Effects on some selected metabolic parameters. PLoS One; 9:e101461.