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Oral hypoglycaemic activity *of Ipomoea aquatica* Forsk. and its active constituents

By

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PhD

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DECLARATION BY CANDIDATE

The work described in this thesis, was carried out by me; under the supervision of Prof. E. R. Jansz and Prof. (Mrs.) S.M.D.N. Wickramasinghe (Department of Biochemistry, Faculty of Medical Sciences, University of Sri Jayewardenepura) and a report on this has not been submitted in whole or in part to any University for another Degree/ Diploma.

30.12.2002
Date

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Signature of candidate

DECLARATION BY SUPERVISORS

We certify that the above statement made by the candidate is true and that this thesis is suitable for submission to the University for the purpose of evaluation.

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TO ALL MY TEACHERS

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ABBREVIATIONS

ADP	Adenosine diphosphate
ALP	Alkaline phosphatase
ALT	Alanine aminotranferase
ALX	Alloxan monohydrate
ANOVA	Analysis of variance
AST	Aspartate aminotransferase
ATP	Adenosine triphosphate
BAW	n Butanol: glacial acetic acid
BC	before Christ
BEN	n Butanol: ethanol: ammonia = 7:2:5 ratio
Co-A	Co-enzyme A
CPT	Carnitine palmitoyltransferase
D	Dextro
DM	Diabetes mellitus
DP	Dipeptidyl peptidase
EC	Enzyme classification
ELIZA	Enzyme linked immunosorbent assay
Forsk.	Forskal
γ GT	Gamma glutamyl transpeptidase
GDM	Gestational diabetes mellitus
GIP	Glucose-dependent insulinotropic polypeptide
GLP	Glucagon-like peptide
GLUT	Glucose transporter

GSK	Glycogen synthase kinase
H	Hour
HSL	Hormone sensitive lipase
IDDM	Insulin dependent diabetes mellitus
IDF	Insoluble dietary fibre
IGT	Impaired glucose tolerance
IR	Infra red
L	Laevo
Linn.	Linnaeus
MPLC	Medium pressure liquid chromatography
NAD^+	Nicotinamide adenine dinucleotide$^+$
NADH	Nicotinamide adenine dinucleotide
NIDDM	Non-insulin dependent diabetes mellitus
PDH	Pyruvate dehydrogenase kinase
SDF	Soluble dietary fibre
SEM	Standard error of mean
STZ	Streptozotocin
TLC	Thin layer chromatography
WE	Whole extract

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ABSTRACT

Ipomoea aquatica Forsk. (Convolvulaceae) is a common green leafy vegetable, which has been in human consumption since antiquity. According to the indigenous medicinal system, the plant is supposed to possess an insulin-like principle. A study was done to determine the oral hypoglycaemic activity of the plant in healthy and diabetic Wistar rats as well as Type II diabetic patients. Activity directed fractionation was also carried out. A single as well as multiple doses of the aqueous, whole extract effectively reduced serum glucose concentration of healthy Wistar rats subjected to a glucose challenge. Multiple doses of the fresh, edible portion of the plant exerted a statistically significant oral hypoglycaemic effect in streptozotocin and alloxan-induced diabetic Wistar rats. The optimal dose in the rats was 3.4 g/kg and the optimal time of activity was 2 h after the administration of the extract.

The oral hypoglycaemic activity exerted by the plant was comparable to that of tolbutamide. This hypoglycaemic effect was significantly higher than the effect of the soluble and insoluble dietary fibre extracted from *I. aquatica*. The active constituents were contained in the ethanol extract of the fresh, edible portion.

The results showed that the long-term consumption of *I. aquatica* has no possible toxicity on the liver and kidney. Toxicity studies which were carried out for 8 weeks did not show any increase in the levels of key hepatic enzymes *viz*; alkaline phosphatase, alanine amino transferase, aspartate amino transferase and γ-glutamyl transpeptidase. Nevertheless, there was a significant reduction in the serum alkaline phosphatase level of the Test group when

compared with the Control. Uric acids levels in the Test and Control groups were not significantly different from each other indicating there was no possible renal damage. Glucose challenge studies with Type II diabetics showed a significant reduction in the serum glucose levels 2 h post glucose load when administered the aqueous, whole extract. Fractionation of the ethanol extract by gel filtration chromatography with Sephadex G_{25} yielded 2 oral hypoglycaemic fractions when tested on rats. When further purified, the active fraction appeared to contain flavonoids. These flavonoids comprising flavones and flavanols were found to separate into 5 sub bands on preparative TLC out of which 4 were oral hypoglycaemic in rats. MPLC on with a solvent gradient of, hexane $\rightarrow$ chloroform $\rightarrow$ ethylacetate $\rightarrow$ methanol and water with a dilution factor of 6, yielded MPLC 1 with methanol and water in the ratio of 99.168: 0.832, while MPLC II was eluted with ethyl acetate and methanol in the ratio of 93.7: 6.3. Infra red spectroscopy of the 2 compounds along with standard indicated that MPLC I was a flavone glycoside while MPLC II was a flavanol glycoside. Enzyme hydrolysis and TLC of the compounds showed the presence of glucose and rhamnose in MPLC 1 and rhamnose only in MPLC II The sugar moeties were necessary for the oral hypoglycaemic activity of MPLC II as shown by the inactivity of the aglycone of the compund. The infra red spectra of the aglycone indicate the presence of a tri hydroxy flavone. Studies directed at the mechanism of action showed that the extract enhanced the absorption of glucose in the intestine in the rats; and at the same time removed the absorbed glucose efficiently from circulation. The extract has enhanced the uptake of glucose by the peripheral tissues. The extract lowered the serum insulin levels of the Type II diabetics subjected to a glucose challenge, indicating that the extract may have increased the receptor sensitivity of insulin. The mechanism of action of the plant extract may be by enhancing the tissue uptake of glucose, which could be mediated via an increase in the sensitization of the receptors for insulin.