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ORAL HYPOGLYCAEMIC AND ANTI-INFLAMMATORY ACTIVITY OF *Pleurotus* MUSHROOMS

By

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Thesis submitted to the University of Sri Jayewardenepura for the award of the Degree of Doctor of Philosophy in Biochemistry on 'Oral hypoglycaemic and anti-inflammatory activity of *Pleurotus* mushrooms', on 30th September 2013.

DECLARATION BY CANDIDATE

The work described in this thesis was carried out by me under the supervision of Dr. T.S. Suresh (Department of Biochemistry, Faculty of Medical Sciences, University of Sri Jayewardenepura), Prof. D.T.U. Abeytunga (Department of Chemistry, Faculty of Science, University of Colombo), Prof. G.H. Fernando (Department of Pharmacology, Faculty of Medical Sciences, University of Sri Jayewardenepura) and Dr. C.A. Wanigatunga (Department of Pharmacology, 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.

08/04/2014

Date

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 MY PARENTS

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IV. ABBREVIATIONS

AE	Acetone extract
ABM	*Agaricus blazei* Murill
ALT	Alanine amino transferase
ALP	Alkaline phosphatase
AqFrA	Aqueous fraction of acetone extract
AST	Aspartate amino transferase
BB	Broad band
BSA	Bovine serum albumin
b.w.	Body weight
Con A	Concanavalin A
COSY	Correlation spectroscopy
COX	Cyclooxygenase
DPP-4	Dipeptidyl peptidase-4
DEPT	Distortionless enhancement by polarization transfer
DMFrA	Dichloromethane fraction of acetone extract
EAF	Ethyl acetate fraction
ELISA	Enzyme-linked immunosorbent assay
FPG	Fasting plasma glucose
γ-GT	Gamma glutamyl transferase
GIP	Glucagon–like insulinotropic peptide
GLP-1	Glucagon-like peptide -1

GK	Glucokinase
GKA	Glucokinase activators
GLUT	Glucose transporter
GSK	Glycogen synthase kinase
GSKI	Glycogen synthase kinase inhibitors
Hb	Haemoglobin
HbA1c	Glycated haemoglobin
HBSS	Hank's balanced salt solution
HeFrA	Hexane fraction of acetone extract
HMBC	Heteronuclear multiple-bond correlation spectroscopy
HOMA-IR	Homeostasis model assessment of insulin resistance
HPLC	High performance liquid chromatography
HRP	Horseradish peroxidase
HSQC	Heteronuclear single quantum coherence spectroscopy
IDDM	Insulin dependent diabetes mellitus
IFG	Impaired fasting glycaemia
IFN	Interferon
IGT	Impaired glucose tolerance
IL	Interleukin
iNOS	Inducible nitric oxide synthase
i.p.	Intraperitoneal
LPS	Lipopolysaccharide
NF-κB	Nuclear transcription factor

NIDDM	Non–insulin-dependent diabetes
NMR	Nuclear magnetic resonance
NO	Nitric oxide
NOESY	Nuclear overhauser effect spectroscopy
NSAIDs	Non-steroidal anti-inflammatory drugs
NSS	Normal sterile saline
OD	Optical density
OGTT	Oral glucose tolerance test
PBS	Phosphate buffered saline
PG	Prostaglandin
Pp-aqu	Aqueous extract of *Pleurotus pulmonarius*
RBC	Red blood cell
RLU	Relatively light unit
ROS	Reactive oxygen species
SFDP	Suspensions of freeze dried and powdered
SOZ	Serum opsonized zymosan
TNF-α	Tumour necrosis factor-α

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ABSTRACT

This study investigates the oral hypoglycaemic activity of the culinary mushrooms *Pleurotus ostreatus* and *P. cystidiosus* and the anti-inflammatory activity of *P. ostreatus*. Oral hypoglycaemic activity of *P. ostreatus* and *P. cystidiosus* was investigated in normal and alloxan-induced diabetic Wistar rats. The suspensions of freeze-dried and powdered *P. ostreatus* and *P. cystidiosus* possess significant hypoglycaemic activity which was comparable with metformin and glibenclamide. The maximally effective dose was 500 mg/kg for both mushrooms. Optimal time of activity was 120 minutes after administration of suspensions of *P. ostreatus* and *P. cystidiosus*. Hypoglycaemic effect of *P. ostreatus* and *P. cystidiosus* (500 mg/kg) was investigated using diabetic female rats at different stages of the oestrous cycle. The highest hypoglycaemic effect was found at the proestrous stage for both mushrooms. Both short and long term administration of suspensions of freeze-dried and powdered *P. ostreatus* and *P. cystidiosus* exerted an apparent control on the homeostasis of blood glucose of diabetic rats. Severe reduction of weight which is characteristic of uncontrolled diabetes was significantly reduced in mushroom groups ($p < 0.05$). The hypoglycaemic effect was retained in the acetone extract which reduced the postprandial serum glucose levels significantly ($p < 0.05$) in normal and diabetic rats.

Experiments were directed to elucidate the mechanism of action of hypoglycaemic activity. Administration of suspensions of *P. ostreatus* and *P. cystidiosus* to diabetic rats increased the intestinal absorption of glucose but simultaneously reduced the serum glucose levels. The mushrooms reduced the serum levels of glycogen synthase kinase and promoted insulin secretion when compared with the Control group ($p < 0.05$). Further, there was a significant increase in serum levels of glucokinase in *P. cystidiosus* group when compared with the Control group ($p = 0.02$). Thus, it could be postulated that *P. ostreatus* and *P. cystidiosus* exert the oral hypoglycaemic activity via several possible mechanisms *viz* increasing glucokinase activity, increasing insulin secretion, promoting glycogen synthesis and increasing glucose utilization by peripheral tissues. Long term feeding of mushrooms to rats did not show signs of liver and renal toxicity.

This study was focused further to determine the efficacy and safety of *P. ostreatus* and *P. cystidiosus* in healthy volunteers and patients with Type 2 diabetes on diet control. Administration of mushroom suspensions at daily dosages of 50 mg/kg for 2 weeks showed a significant reduction ($p < 0.05$) in fasting and postprandial serum glucose levels of healthy volunteers. *Pleurotus ostreatus* and *P. cystidiosus* (50 mg/kg) significantly ($p < 0.01$) reduced the postprandial serum glucose levels and increased the serum insulin levels ($p < 0.05$) of patients with Type 2 diabetes on diet control. Healthy volunteers and patients with Type 2 diabetes on diet control were monitored for one month after receiving *P. ostreatus* and *P. cystidiosus* for any adverse effects. There were no significant difference ($p > 0.05$) in the serum levels of liver enzymes and creatinine as well as estimated creatinine clearance of subjects after one month.

The anti-inflammatory potential of *P. ostreatus* in normal and alloxan- induced diabetic Wistar rats was investigated using the carrageenan-induced rat paw oedema model. The suspensions of freeze-dried and powdered *P. ostreatus* and acetone extract of *P. ostreatus* significantly ($p < 0.05$) inhibited the carrageenan induced paw oedema and the effect was comparable to that of indomethacin.

Anti-inflammatory activity of *P. ostreatus* in rats is mediated via multiple mechanisms *viz* antihistamine and membrane stabilizing activity, inhibition of cell migration to the site of inflammation and inhibition of nitric oxide production.

The acetone extract of *P. ostreatus* was subjected to an anti-inflammatory activity guided fractionation to identify the fraction having the highest activity. Extracts and fractions were tested for anti-inflammatory activity using the carrageenan-induced paw oedema assay in rats and the *in vitro* Luminol based chemiluminescence assay. The acetone extarct of *P. ostreatus* was sequentially fractionated with solvents with increasing polarity. The residual aqueous fraction of the acetone extract showed significant ($p < 0.05$) anti-inflammatory activity in both models. The active fractions were identified and the compounds were isolated by size exclusion, normal and reverse phase column chromatography and recycling HPLC techniques. Spectroscopic analysis of the isolated compounds were done by MS, ^{1}HNMR, ^{13}CNMR and 2DNMR (COSY, NOESY, HMBC, HMQC). A disaccharide, a derivative of uridine, N^{10} isopentenyl adenosine, niacinamide and uracil were isolated from *P. ostreatus*. The derivative of uridine and novel isolated compound N^{10} isopentenyl adenosine compounds possess significant ($p < 0.05$) anti-inflammatory activity in rats.

In conclusion, this study suggests that consumption of *P. ostreatus* and *P. cystidiosus* will bring health benefits to mankind and these culinary mushrooms can be popularized as functional foods.