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Comparison of hypoglycaemic, hepatoprotective and anti-inflammatory activities of natural plant and callus cultures of *Munroinia pinnata*

By

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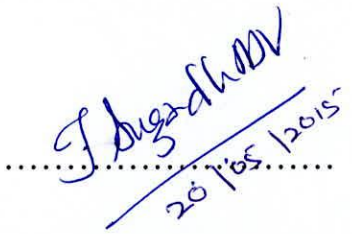
We certify that the candidate has incorporated all corrections, amendments and additions recommended by the examiners.



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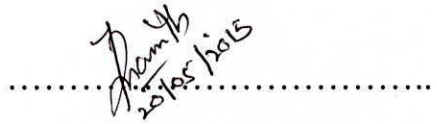

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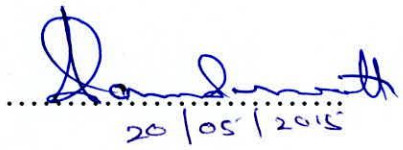

Dr. S. Handunnetti

DECLARATION BY CANDIDATE

"The work described in this thesis was carried out by me under the supervision of Dr. Sugandhika Suresh (Department of Biochemistry, Faculty of Medical Sciences, University of Sri Jayewardenepura), Prof. W.T.P.S.K. Senarath Department of Botany, Faculty of Applied Sciences, University of Sri Jayewardenepura) and Dr. S. Handunnetti (Institute of Biochemistry, Molecular Biology and Biotchnology, University of Colombo) and a report on this has not been submitted in whole or in part to any university or any other institution for another Degree/ Diploma".

20/05/2015 .....

Date

..... Signature of Candidate

DECLARATION BY SUPERVISORS

"I/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".

....

Dr. Sugandhika Suresh Prof. W.T.P.S.K. Senarath

.....

Dr. S. Handunnetti

TO MY FAMILY

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2, 4 D	2-4 – dicloroxyphenoxyacetic acid
y-GT	Gamma glutamyl transferase
ALT	Alanine amino transferase
ALP	Alkaline phosphatase
ANOVA	Analysis of varience
AST	Asparate amino transferase
BAP	6 - benzylaminopurine
CC	column chromatography
CCM	Complete Culture Medium
COX	Cyclooxygenase
^{13}C-NMR	carbon-nuclear magnetic
CRD	Completely Randomized Design
ELISA	Enzyme linked immunosorbant assay
HbAlc	Glycated haemoglobin
^{1}H-NMR	proton-nuclear magnetic resonance

iNOS	Inducible nitric oxide synthase
i.p	Intraperitoneal
LSD	Least Significance Difference
MS	Murashige and Skoog medium
NIDDM	Non -insulin -dependent diabetes
NMMA	1 mM n-monoethyl- L- arginine acetate salt
OD	Optical density
OECD	Organization of Economic Co-operation Development
OPD	Out Patient Department
NMR	Nuclear overhauser effect spectroscopy
NSAIDs	Non-steroidal anti-inflammatory drugs
PBS	Posphate-Buffered Saline
PG	Prostaglandin
RBC	Red blood cell
WHO	World health organization

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Swarna Damayanthi Hapuarachchi

Comparison of hypoglycaemic, hepatoprotective and anti-inflammatory activities of natural plant and callus cultures of *Munronia pinnata*

By Swarna Damayanthi Hapuarachchi

ABSTRACT

Munronia pinnata [(Wall) Theob (Meliaceae) – (*Bin kohomba*)] is a rare medicinal herb in Sri Lanka. Traditional physicians claim that it has been used for many diseases in the form of powder and decoction in folk medicinal practices of Sri Lanka. *In-vitro* propagation techniques have been applied as an attempt to meet the increasing demand for this plant. The possible use of calli as a substitute for whole plants has been querried. Despite its long record of usage in indigenous clinical practices, there is a paucity of published studies on the biological activities and active ingredients of this herb. The objective of the present study was thus to investigate the hypoglycaemic, hepatoprotective and anti-inflammatory activities of natural plants and compare such activities with the callus cultures of *M. pinnata.*

Leaf explants were cultured on Murashige and Skoog medium supplemented with 1.1 mg/L 2-4 – dicloroxyphenoxyacetic acid (2,4-D) and 0.3 mg/L 6 - benzylaminopurine (BAP) to obtain callus cultures. Three leaflet type of natural plants and 3- months' old calli of *M. pinnata* were used for the preparation of ethanol extracts and aqueous extracts. Experimental studies with Wistar rats (healthy and diabetic) and clinical studies with diabetic patients were carried out.

A single as well as multiple doses of aqueous and ethanol extracts of the natural plants and calli significantly reduced the serum glucose concentrations in healthy and diabetic rats in a dose

dependant manner. The optimal dose of the water extract of the natural plants was 2.0g/kg and it was 0.7 g/kg for the ethanol extract. The optimal time of activity for natural plants and calli was 2 h after the administration of the aqueous extract and was 3 h for ethanol extract. Oral hypoglycaemic activity exerted by both extracts was comparable to the time course of that of standard synthetic anti diabetic drugs. Toxicity studies revealed that neither ethanol nor aqueous extracts of test samples exert any adverse effects. This is an important finding, as standard hypoglycaemic drugs, have to be administered over a relatively long period of time in the therapeutic practices and absence of adverse effects is an advantage.

Glucose challenge studies with healthy subjects and Type 2 diabetics showed a significant reduction (23.30%, $p < 0.01$) in the serum glucose concentration levels when administered with aqueous extract of natural plants. Though the aqueous extract of calli reduced the serum glucose concentration in Type 2 diabetics, it was not as high as the aqueous extract (6.60%, $p < 0.05$). Studies on the mechanism of action, showed a significant reduction in intestinal glucose absorption and serum glucose concentration when compared to control group (distilled water) after glucose challenge. Thus, it could be suggested that *M. pinnata* appears to exert its oral hypoglycaemic activity via several possible mechanisms. These might include the inhibition of intestinal glucose absorption, possible stimulation of insulin secretion from the beta cells of islets of Langerhans and facilitation of glucose uptake and utilization by peripheral tissues.

The hepatoprotective activity in healthy Wistar rats was investigated against $CC1_4$ induced lipid peroxidation and hepatic injury. Results revealed that there is hepatoprotective activity against the toxic effect of $CC1_4$, which was also supported by histological studies. Consequently, it explains that either ethanol or aqueous extracts and not only of natural plants but also of the calli

could be used as a therapeutic regime in treatment of some hepatic disorders without any side effects.

The aqueous and ethanol extracts of both natural plants and calli possess anti-inflammatory activity in carrageenan-induced rat paw oedema model compared to the control (distilled water), in diabetic as well as healthy Wistar rats. Anti-inflammatory effect was dose-dependent and the optimal dose was 2.0g/kg of aqueous extract while it was 0.5 g/kg of ethanol extract. Aqueous extracts of both natural plants and calli significantly inhibit the peritoneal phagocytic cell infiltration and impair nitric oxide production in peritoneal cells. These results indicated that anti inflammatory activity of *M. pinnata* is mediated through inhibition of NO production, phagocytic cell infiltration and anti-histamine effect. As decoction of *M. pinnata* exhibit significant anti - inflammatory activity in the tested models, it may prove the scientific rationale for the use of this species in folk medicine as an anti – inflammatory agent.

During activity guided fractionation, methanol extracts of natural plants and calli were partitioned in to water and ethyl acetated TLC was done on each fraction using ethyl acetate: hexane (4:6) solvent system. The ethyl acetate extract of both natural plants and calli showed the highest reduction of serum glucose in glucose challenge studies and showed anti-inflammatory activity in healthy Wistar rats. Further fractionation of ethyl acetate extract by column chromatography with Sephadex LH_{20} yielded two oral hypoglycaemic fractions of natural plants and one fraction of calli. Similar R_f value was obtained from one compound of the fraction 2 of ethyl acetate fraction of natural plants and calli. When compared with the TLC analysis in ethyl acetate: hexane (4:6) solvent system and these compounds appeared to contain terpenoids. Among the tested fractions, known sesquiterpene, senecrassidiol (55.0 mg; w/w) was isolated from the ethyl acetate fraction

of methanol extract of MP and at a dose of 2.0 mg/mL exhibited significant hypoglycaemic and anti–inflammatory effects in healthy rats. The results of this study provide the scientific rationale for the use of *M. pinnata* in folk medicine.