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Evaluation of the anti-inflammatory properties of a complex homeopathic product Modul8® using the BALB/c murine asthmatic animal model

By

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Department of Anatomy

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Supervisor: **Prof E. Pretorius**

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Department: Anatomy

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Abstract

Modul8® is a complex homeopathic product which acts as an immunomodulator by enhancing the immunity of an individual in order to favour a particular immunological response. Asthma is a chronic inflammatory disease to which many different treatment approaches have been applied. The aim of the current study was to investigate the effect of this natural, homeopathic product on ultrastructural, immunological and haematological parameters in the treatment of asthma by using the BALB/c asthmatic animal model.

Mice have traditionally been used as experimental animals in studying human diseases and although there is no animal model that is an exact replica of the human disease, they have helped to understand some of the basic mechanisms involved as well as the development of new therapies. Since there is a correlation between allergic mice and human asthmatics, the BALB/c murine asthmatic animal model was implemented and used in the current study.

Implementation of the BALB/c murine asthmatic animal model involved the sensitization, nebulization and treatment of mice over a period of 41 days. Mice were randomly divided into four experimental groups including a control group, an asthmatic group receiving no treatment, an asthmatic group exposed to physiological comparable levels of Modul8® and lastly an asthmatic group exposed to hydrocortisone as positive control. The animals were also weighed on specific reference days throughout the study to determine if any significant changes exist between the experimental groups' weights over the experimental period.

Haematological analysis revealed that Modul8® possesses the ability to alter the asthmatic profile of the white blood cell counts and stabilize it to levels comparable with the control group. In the ultrastructural analysis of platelets and fibrin networks it seemed that Modul8® stabilized platelet and fibrin morphology to a profile similar to that of control animals. Comparison of the ultrastructure of platelets and fibrin networks of the BALB/c asthmatic animals to that of human asthmatics revealed that this animal model adequately mimics the human disease on this specific level. Modul8® was also shown to activate mice peritoneal macrophages *in vitro* and this was linked to the important role of activated macrophages in the immune response. Histological and ultrastructural analysis of the lungs showed that Modul8® has the ability to stabilize the changes and remodelling caused in the lungs by the inflammatory process involved in asthma.

It is therefore concluded that this homeopathic immunomodulator has the ability to positively influence the chosen parameters of the inflammatory response involved in asthma and therefore might successfully be used as an adjuvant therapeutic approach to the known anti-asthmatic and anti-inflammatory treatments.

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Declaration

I, Hester Magdalena Oberholzer, hereby declare that this thesis entitled:

“Evaluation of the anti-inflammatory properties of a complex homeopathic product Modul8® using the BALB/c murine asthmatic animal model”

Which I herewith submit to the University of Pretoria for the Degree of Doctor of Philosophy in Anatomy, is my own original work and has never been submitted for any academic award to any other tertiary institution for any degree.

_____ _____

Date **H. M. Oberholzer**

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"I can do all things through Him who gives me strength"

-Philippians 4:13-

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Articles Published

- **Pretorius E., Oberholzer H. M.** Ultrastructural changes of platelets and fibrin networks in human asthma: a qualitative case study. *Blood coagulation and fibrinolysis* 2009; **20**: 146-149

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- **Oberholzer H. M., Pretorius E.** Investigating lung remodeling in MODUL8® - treated BALB/c asthmatic animals. *Micron* 2009; **40**: 775-782

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LIST OF ABBREVIATIONS AND SYMBOLS

AD	Anno Domini
AHR	Airway hyperreactivity/ hyperresponsiveness
AIP	Actin interacting protein
$Al(OH)_3$	Aluminum Hydroxide
ANOVA	Analysis of Variance
APC	Antigen presenting cell
ASM	Airway smooth muscle
BC	Before Christ
BMI	Body mass index
$CaCO_3$	Calcium carbonate
CAM	Complementary and alternative medicine
CD	Cluster of differentiation
COX	Cyclooxygenase
CRTH	Chemo attractant receptor homologous molecule expressed on TH_2 cells
CysLTs	Cystenyl leukotrienes
CXCL	Chemokine ligand
D	Declared potencies
DPBS	Dulbecco's phosphate buffered saline
EAR	Early-phase asthmatic reaction
EEG	Electroencephalogram
EGF	Epidermal growth factor

ELISA	Enzyme-linked immunosorbent assay
ERK1/2	Extracellular signal-regulated kinases 1 and 2
FGF	Fibroblast growth factor
GM-CSF	Granulocyte macrophage-colony stimulating factor
HbsAG	Hepatitis B surface antigen
HCV	Hepatitis C virus
HIV	Human immunodeficiency virus
HMDS	Hexamethyldisilazane
ICS	Inhaled corticosteroids
IES	Inhalation exposure system
IFN-γ	Interferon gamma
IgE	Immunoglobulin E
IGF	Insulin-like growth factor 1
IL	Interleukin
JNK	c-JunN-terminal kinase
LAR	Late-phase asthmatic reaction
LABA	Long acting beta agonist
LPS	Lipopolisaccharide
LTB_4	Leukotriene B_4
LTC_4	Leukotriene C_4
LTD_4	Leukotriene D_4
LTRAs	Leukotriene receptor antagonists

MAPK	Mitogen-activated protein kinase
MCP	Monocyte chemotactic protein
MDC	Macrophage- derived chemokine
MHC	Major histocompatibility complex
MIP	Macrophage inflammatory protein
NCSS	Number Cruncher Statistical System
NF-κB	Nuclear factor- kappa B
NHLBI	National Heart Lung and Blood Institute
NK	Natural killer
NO	Nitric oxide
NOS	Nitric oxide synthase
OsO_4	Osmium tetraoxide
OVA	Ovalbumin
PAF	Platelet activating factor
PBS	Phosphate buffered saline
PDGF	Platelet derived growth factor
PGD_2	Prostaglandin D_2
PGE_2	Prostaglandin E_2
PGI	Prostacyclin
pH	Measure of the acidity or basicity
PRP	Platelet rich plasma
RANTES	Regulated upon activation, normal T-cell expressed and released

RCF	Relative centrifugal force
ROS	Reactive oxygen species
RPM	Refs per minute
SABA	Short acting beta-agonist
SD	Standard deviation
SSE	*Sam so Eum*
SEM	Scanning electron microscope
TCD_4	Helper T lymphocyte
TEM	Transmission electron microscope
TGF	Transforming growth factor
Th	T helper cell
TLR	Toll- like receptor
TNF-α	Tumour necrosis factor alpha
UPBRC	University of Pretoria Biomedical Research Centre
VCAM	Vascular cell adhesion molecule
%	Percentage
°C	Degrees centigrade
hr	Hour
G	Gram
mg	Milligram
ml	Millilitre
µl	Microlitre

kg	Kilogram
mg/kg	Milligram per kilogram
µl/kg	Microlitre per kilogram
U/ml	Unit per millilitre
µm	Micrometer
nm	Nanometre
G	Gauge
X	Times