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The *in vitro* and *in vivo* anti-inflammatory properties and cytotoxicity of extracts of *Euphorbia hirta*

By

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ABSTRACT

Asthma is considered one of the most common respiratory complaints in the world today but a medical cure for this condition is currently not available. The use of herbal medicines to treat asthma has however been reported and *Euphorbia hirta* is one such herb. The alkaloids, flavonoids, glycosides, sterols, tannins and triterpenoids in *E. hirta* appear to exert the anti-asthma effects reported.

In the first part of this study, the aqueous, acetone, dichloromethane and hexane extracts of *E. hirta* were evaluated for their effects on the lysosomal membrane integrity, cell viability and cell number of MRC-5 cell-line using the NR/MTT/CV assay. Hydrocortisone was used as a pharmaceutical control. The differences between the effects of the different extracts were investigated and the effects of the extracts were compared with hydrocortisone. Results obtained showed that hydrocortisone was relatively toxic to the MRC-5 cells whereas all four extracts studied showed very limited cytotoxic effects, with the aqueous extracts generally exhibiting the least effects.

In the second part of this study, the effects of the aqueous *E. hirta* extract on the blood coagulation system and general airway wall microstructure and ultrastructure were investigated using the BALB/c mouse asthma model. Hydrocortisone was also used as a pharmaceutical control. Parameters studied included inflammatory cell population in peripheral blood and their migration into the lung parenchyma; platelet aggregation and fibrin fibre morphology; fibroblast and mucous cell proliferation; alveolar cell numbers, lamellar body formation as

well as filopodia formation. The animal weights were continuously being monitored throughout the study.

Results from the animal studies showed that the aqueous extract of *E. hirta* had limited effects on changes in the animal weights and did not cause fragility of blood fibrin fibres nor change the integrity and morphology of the platelets in the mice as seen in those treated with hydrocortisone. *E. hirta* extracts also significantly reduced the number of active inflammatory cells (especially neutrophils, eosinophils and basophils); restored the histological alterations observed in respiratory structures studied and had diverse, dose-dependent beneficial ultrastructural effects like reduction of smooth muscle hypertrophy, inhibition of macrophages into the airway parenchyma, among others.

The final judgment and conclusion of this study was that the aqueous *E. hirta* extract did not show cytotoxic effects and could be used for the treatment of asthma in the BALB/c mice at doses ranging 25-62.5mg/kg. Further research leading to clinical trials is recommended after testing the potency of equivalent doses of this extract in other animal asthma models.

DECLARATION

I, Okobi Ekpo hereby declare that this thesis entitled:

"The *in vitro* and *in vivo* anti-inflammatory properties and cytotoxicity of extracts of *Euphorbia Hirta*"

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 **Okobi Ekpo**

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LIST OF PUBLICATIONS

Pretorius E, Ekpo OE, Smit E (2007): Comparative ultrastructural analyses platelets and fibrin networks using the murine model of asthma. Experimental and Toxicologic Pathology 59 (2): 105-114.

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Ekpo OE and Pretorius E (2007): Euphorbia hirta and its anti-inflammatory properties: news and views. South African Journal of Science 103 (5, 6): 201-203.

Submitted for Publication

Oberholzer HM, Pretorius E, Smit E, Ekpo OE, Humphries P, Auer RE (2007): Investigating the effect of Withania somnifera, selenium and hydrocortisone on blood count and bronchial lavage of Balb/c mice. (*Scandinavian Journal of Laboratory Animal Science*)

Ekpo OE and Pretorius E (2007): Using the Balb/c asthmatic mouse model to investigate the effects of hydrocortisone and an herbal asthma medicine on animal weight. (*Scandinavian Journal of Laboratory Animal Science*)

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AHR	Airway hyperresponsiveness
ASM	Airway smooth muscle
Al $(OH)_3$	Aluminium trioxide
ANOVA	Analysis of Variance
AS	Asthma
ACD	Atopic contact dermatitis
PWDs	Percentage weight differences
BTG	Beta-thromboglobulin
BALF	Bronchoalveolar lavage fluid
Ca^{2+}	Calcium ion
CD4+	Cluster of differentiation 4
cm^2	Centimetre squared
CAM	Complimentary and Alternative Medicine
Conc.	Concentration
CT	Control
CV	Crystal Violet
DCM	Dichloromethane
DMSO	DImethyl sulphoxide
DNA	Deoxyribonucleic acid
DPBS	Dulbecco's Phosphate Buffered Saline
ddH_2O	Double distilled and deionized water
EMEM	Eagles Minimum Essential Medium
EIA	Exercise induced asthma
ECP	Eosinophil cationic protein

EDTA	Ethylene diamine tetra acetate
ECM	Extracellular matrix
FCS	Foetal Calf Serum
FER	Food efficiency ratio
GM-CSF	Granulocyte monocyte colony stimulating factor
HBSS	Hanks Balanced salt solution
HC	Hydrocortisone
HASMC	Human airway smooth muscle cells
Hu-PBMC	Human peripheral blood Mononuclear cells
IFN-γ	Interferon-gamma
IL	Interleukin
IgE	Immunoglobulin E
ICS	Inhaled corticosteroids
IFN-a	Interferon-alpha
KCl	Potassium chloride
KH_2PO_4	Potassium dihydrogen phosphate
Kg	Kilogram
LT	Long-term
LEH	Low EH
LHC	Low HC
M	Molar
MIP	Macrophage inflammatory protein
MBP	Major basic protein
MPM	Malleable Protein Matrix
Mg/ml	Milligram/Millilitre

MTT	[3-(4, 5-dimethylthiazol-zyl) 2, 5-dimethyl tetrazolium bromide]
NR	Neutral Red
NA	Not available
NOS	Nitric Oxide Synthase
NSD	No significant difference
Na_2HPO_4	Disodium hydrogen phosphate
$NaHCO_3$	Sodium hydrogen carbonate
OsO_4	Osmium tetraoxide
OVA	Ovalbumin
PAI_{act}	Plasminogen activator inhibitor
pH	Measure of the acidity or basicity
PBS	Phosphate Buffered Saline
PHA	Phytohemagglutinin
PA	Plasminogen activator
PRP	Platelet rich plasma
PAF	Platelet-activating factor
PF	Pulmonary fibrosis
SEM	Scanning Electron Microscope
ST	Short-term
SD	Standard deviation
SD	Significant difference
SO	Superoxide
Th1	T helper type 1 lymphocytes
TNF-α	Tumour Necrosis Factor-α
TEM	Transmission Electron Microscope

TGF	Transforming Growth Factor
µ	Micro
β	Beta
µl	Micro litre
g	Gram
%	Percent
≤	Equal or less than
µg/mL	Microgram per millilitre
^{0}C	Degree Celsius