EVALUATION OF THE HEPATOPROTECTIVE ACTIVITY OF A TRIHERBAL FORMULATION (*Gongronema latifolia, Ocimum gratissimum* and *Vernonia amygdalina*)

BY

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CERTIFICATION

This is to certify that the Thesis:

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DEDICATION

To my loving husband, Ogbonnaya Chukwuemeka Iroanya and our beautiful children Nma Zoe Angel and Ifeanyichukwu Jeffery Ikechukwu for the joy they have brought to my life.

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LIST OF ACRONMYS AND ABBREVIATIONS

Acronym	Term
ADP	Adenosine Diphosphate
ALB	Albumin
ALP	Alkaline Phosphatase
ALT	Alanine Aminotransferase
APAP	Acetaminophen
AST	Aspartate Aminotransferase
BUN	Blood Urea Nitrogen
CAT	Catalase
CCl_4	Carbon tetrachloride
CHO	Cholesterol
CREA	Creatinine
DAP	Dihydroxyacetone Phosphate
D-GaIN	D- Galactosamine
G3P	Glycerol-3-Phosphate
GGT	L-Gamma Glutamyltransferase
GK	Glycerol Kinase
GPO	Glycerol Phosphate Dehydrogenase
GPx	Glutathione Peroxidase
GSH	Reduced Glutathione
GST	Glutathione-S-Transferase
H_2O_2	Hydrogen Peroxide
Hb	Haemoglobin
HPLC	High Performance Liquid Chromatography

HPTLC	High Performance Thin Layer Chromatography
LDH	Lactate Dehydrogenase
LPL	Lipoprotein Lipase
MCH	Mean Cell Haemoglobin
MCHC	Mean Cell Haemoglobin Concentration
MCV	Mean Cell Volume
MDA	Malondialdehyde
NADH	Nicotinamide-Adenine Dinucleotide
NAPQI	*N*-acetyl-*p*-benzoquinone imine
PBS	Phosphate Buffered Saline
PCV	Packed Cell Volume
RBC	Red Cell Count
RNS	Reactive Nitrogen Species
ROS	Reactive Oxygen Species
SOD	Superoxide Dismutase
STD-NMR	The Saturation-Transfer Difference – Nuclear Magnetic Resonance Spectroscopy Based Screening.
TBA	Thiobarbituric Acid
TBARS	Thiobarbituric Acid Reactive Substances
TCA	Trichloroacetic Acid
TG	Triglycerides
TLC	Thin Layer Chromatography
TP	Total Protein
WBC	White Blood Cell

ABSTRACT

The aim of this study is to investigate the hepatoprotective, anti-apoptotic, antioxidant and antiproliferative properties of a tri-herbal formulation made from 50 % ethanolic extract of the leaves of *Gongronema latifolia* Benth, *Ocimum gratissimum* Linn. and *Vernonia amygdalina* Del. (GOV). The phytochemical constituents, safe dose, analgesic and anti-inflammatory activities of GOV were also ascertained.

Wistar albino rats were treated with different doses of GOV (2, 4 and 8 g kg^{-1} b. wt. p.o.) for 14 days. At the end of the experimental period, hepatotoxicity was induced using different toxins e.g. acetaminophen, alcohol, carbon tetrachloride and D-galactosamine. The hepatoprotective, antioxidant, and anti-apoptotic activities of GOV was determined by, aascertaining its effect on haematologic indices and serum liver marker enzymes, evaluating its antioxidant potentials using serum, liver and kidney homogenates and profiling the anti-apoptotic activities of GOV using leukocytes from whole blood to ascertain the fold-increase in caspase-2, 3 and 9 activities. The analgesic and anti-inflammatory activities of GOV was investigated using preliminary pharmacologic assays. The hepatoprotective, anti-apoptotic, antioxidant, analgesic and anti-inflammatory activities of GOV were compared to standard reference drugs e.g. Silymarin, Liv 52®, indomethacin, dexamethasone, Morphine and Acetylsalicylic acid. The antiproliferative activity of GOV toward human hepatocellular liver carcinoma cell line (Hep G2 cells) and nasopharyngeal cancer cells (CNE2 and SUME –α- nasopharyngeal cells) were evaluated. *In vitro* antioxidant potential of GOV was determined using brain homogenates of Wistar albino rats and erythrocyte of Swiss mice. Acute and sub chronic toxicity tests were carried out to determine the safety of GOV while preliminary phytochemical analysis, thin layer chromatography (TLC), high performance thin layer chromatography (HPTLC) and saturation-

transfer difference – nuclear magnetic resonance spectroscopy (STD-NMR) were used to determine its phytochemical constituents.

Stigmasterol, β-sitosterol, Rutin, Hyperoside, Eugenol and Ascorbic acid are suspected to be present from phytochemical screenings. On administration of GOV at 16 g kg^{-1} orally and 2.5 g kg^{-1} intraperitoneally, it proved to be safe. It dose dependently showed significant antinociceptive and anti-inflammatory activity, increased most of the haematologic indices, attenuated the activities of serum liver marker enzymes and improved the concentration of chemical analytes compared to the control groups. GOV offered protection against free radical-mediated oxidative stress in serum, liver and kidney of the experimental animals and also exhibited *in vitro* antioxidant activities in, brain homogenates of Wistar albino rats and inhibition of erythrocyte haemolysis using Swiss mice. It lowered the extent of release of pro-apoptotic proteins with subsequent decrease in caspase activity and inhibited cancer cells proliferation in HepG2 cells.

The ability of a hepatoprotective agent to reduce the injurious effects, or to preserve the normal hepatic physiologic mechanism which have been disturbed by a hepatotoxin, is an index of its protective effects. These findings show the prophylactic efficacy of GOV in maintaining the integrity and functional status of hepatocytes in rats and its antiproliferative property on Hep G2 cells. The individual or combined action of these bioactive constituents in GOV may be the contributing factor towards its hepatoprotective, antioxidant, antiproliferative, analgesics and anti-inflammatory activities. The present findings provide scientific evidence to the ethno-medicinal use of this triherbal formulation by the tribal group of Eastern Nigeria in treating liver diseases.

Keywords: Triherbal formulation, *Gongronema latifolia*, *Ocimum gratissimum*, *Vernonia amygdalina*, hepatoprotective, anti-apoptotic, antioxidant and antiproliferative.