

**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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TABLE OF CONTENTS

CONTENTS	PAGES
Title Page	i.
Certification	ii.
Dedication	iii.
Acknowledgement	iv.
Table of Contents	viii.
List of Figures	xvii.
List of Tables	xviii.
List of Abbreviations and Acronyms	xxii.
Abstract	xxv.
1. INTRODUCTION	1
1.1. Background of the Study	1
1.2. Statement of Problem	3
1.3. Purpose of Study	4
1.4. Objectives of the Study	5
1.5. Research Questions and / or Hypotheses.	6
1.6. Significance of Study	7
1.7. Operational Definition of Terms	9
2. LITERATURE REVIEW	12
2.1 Medicinal Plants	12

2.1.1	<i>Gongronema latifolia</i> Benth	14
2.1.2	<i>Ocimum gratissimum</i> Linn	18
2.1.3	<i>Vernonia amygdalina</i> Del.	21
2.2	The Mammalian Liver	25
2.2.1	Diseases of the Liver	28
2.2.2.	Hepatitis	29
2.2.3.	Causes of Hepatitis	30
2.2.3.1.	Viral Hepatitis	31
2.2.3.2.	Autoimmune Chronic Hepatitis	33
2.2.3.3.	Nonalcoholic Fatty Liver Disease (NAFLD)	33
2.2.3.4.	Inherited forms of Hepatitis	34
2.3.	Hepatotoxicity	34
2.4.	Pharmacological Models for Inducing Hepatitis	37
2.4.1.	Alcohol	38
2.4.2.	Acetaminophen (APAP)	42
2.4.3.	Carbon tetrachloride (CCl ₄)	43
2.4.4.	D- Galactosamine (D-GaIN)	47
2.5.	The Reference Drugs	50
2.5.1.	Liv 52 [®]	50
2.5.2.	Silymarin	51
3.	RESEARCH MATERIALS AND METHODS	54
3.1.	Collection and Identification of Plant Materials	54
3.2.	Extraction and Fractionation of Plant Materials	54

3.2.1.	Preparation of the 50 % Ethanolic Extract of <i>Gongronema latifolia</i> , <i>Ocimum gratissimum</i> and <i>Vernonia amygdalina</i> (GOV).	54
3.2.2.	Preparation of the Ethanolic Extract of <i>Gongronema latifolia</i> , <i>Ocimum gratissimum</i> and <i>Vernonia amygdalina</i> .	55
3.2.3.	Preparation of the Aqueous Extract of <i>Gongronema latifolia</i> , <i>Ocimum gratissimum</i> and <i>Vernonia amygdalina</i> .	55
3.2.4	Fractionation of GOV	56
3.3.	Preliminary Phytochemical Assays	56
3.3.1.	Test for Tannins	56
3.3.2.	Test for Alkaloids	57
3.3.3.	Test for Phlobatannins	57
3.3.4.	Test for Saponins	58
3.3.5.	Test for Anthraquinones	59
3.3.6.	Tests for Cardiac Glycosides	60
3.3.7.	Tests for Cyanogenetic Glycosides	61
3.3.8	Identification of Flavonosides	61
3.3.9.	Tests for Reducing Compounds	61
3.4.	Thin Layer Chromatography (TLC)	62
3.5.	Flash Column Chromatography	63
3.6.	Reverse Phase Chromatography	65
3.7.	High Performance Liquid Chromatography (HPLC)	65
3.8.	High Performance Thin Layer Chromatography (HPTLC) Analysis	65
3.9.	The Saturation-Transfer Difference – Nuclear Magnetic Resonance	67

Spectroscopy (STD-NMR) Based Screening.

3.10.	Animal Care	68
3.11.	Toxicity studies	68
3.11.1.	Acute Toxicity Test	68
3.11.2.	Subchronic Toxicity Test	69
3.12.	Preliminary Pharmacological Assays	69
3.12.1.	Analgesic Activity	69
3.12.1.1.	Hot Plate Test	69
3.12.1.2.	Mouse Writhing Assay	70
3.12.1.3.	Formalin Test	71
3.12.2.	Anti-inflammatory Activity	71
3.12.2.1.	Xylene Induced Ear Oedema	71
3.12.2.2.	Carrageenan Induced Rat Paw Oedema	72
3.13.	Pharmacological Models of Inducing Hepatotoxicity	73
3.13.1.	Drug Treatment Protocol for Alcohol Induced Hepatotoxicity	73
3.13.2.	The Effect of GOV on D-galactosamine Induced Hepatotoxicity	74
3.13.3.	The Effect of GOV on Acetaminophen Induced Hepatotoxicity	76
3.13.4.	Drug Treatment Protocol for Carbon Tetrachloride Induced Hepatotoxicity	77
3.14.	Screening for Hepatoprotective Activity of GOV	79
3.14.1.	Haematologic indices	79
3.14.1.1.	Packed Cell Volume (PCV)	79
3.14.1.2.	Haemoglobin, (Hb)	80

3.14.1.3.	Red Cell Count (RBC)	80
3.14.1.4.	Mean Cell Volume or Mean Corpuscular Volume (MCV)	82
3.14.1.5.	Mean Cell Haemoglobin or Mean Corpuscular Haemoglobin (MCH)	82
3.14.1.6.	Mean Cell Haemoglobin Concentration or Mean Corpuscular Haemoglobin Concentration (MCHC)	83
3.14.1.7.	White Cell (Leucocyte) Count	83
3.14.1.8.	Leucocyte (White Cell) Differential Count	84
3.14.1.9.	Platelet Count (Thrombocyte Count)	86
3.14.2.	Biochemical Assays	87
3.14.2.1.	Alkaline Phosphatase (ALP)	88
3.14.2.2.	Alanine Aminotransferase (ALT)	88
3.14.2.3.	Aspartate Aminotransferase (AST)	89
3.14.2.4.	Lactate Dehydrogenase (LDH)	90
3.14.2.5.	L- γ -glutamyltransferase (GGT)	91
3.14.3.	Antioxidant assays	91
3.14.3.1.	Estimation of Lipid Peroxidation Product (TBARS)	92
3.14.3.2.	Estimation of Reduced Glutathione (GSH)	93
3.14.3.3.	Glutathione Peroxidase (GPx)	94
3.14.3.4.	Glutathione S-Transferases (GST)	95
3.14.3.5.	Catalase (CAT)	95
3.14.3.6.	Determination of Superoxide Dismutase (SOD) Activity	96
3.14.4.	Chemical Analytes Assay	97
3.14.4.1.	Total Protein (TP)	97

3.14.4.2.	Albumin (ALB)	98
3.14.4.3.	Creatinine (CREA)	99
3.14.4.4.	Triglycerides (TG)	100
3.14.4.5.	Blood Urea Nitrogen (BUN)	101
3.14.5.	Caspase Activity Assay	102
3.14.5.1.	Isolation of Leukocytes from Whole Blood	102
3.14.5.2.	Caspase-2 Assay Procedure	103
3.14.5.3.	Caspase-3 Assay Procedure	104
3.14.5.4.	Caspase-9 Assay Procedure	104
3.14.6.	Histopathology	105
3.15.	<i>in vitro</i> Antioxidant Assays	106
3.15.1	Assay of Lipid Peroxidation using Brain Homogenates	106
3.15.2.	Assay of Inhibition of Erythrocyte Haemolysis	106
3.16.	Antiproliferative Activity toward Human Hepatocellular Liver Carcinoma Cell Line (HepG2 cells) and Nasopharyngeal Cancer Cells (CNE2 and SUME – α - Nasopharyngeal Cells)	107
3.17	Statistical analysis	108
4	RESULTS	109
4.1.	Extraction of plant materials	109
4.2.	Fractionation of plant materials	109
4.3.	Phytochemical analysis	109
4.4.	Thin Layer Chromatogram of GOV	111
4.5.	High Performance Thin Layer Chromatograms (HPTLC) of GOV Extract	115

4.6.	NMR Spectra for the Binding Study of GOV and its Fractions with Dihydrodipicolinate Reductase (DHPR)	118
4.7.	Toxicity Tests	126
4.7.1	Acute Toxicity	126
4.7.2.	Sub-chronic toxicity	126
4.7.2.1.	Sub-chronic Effect on Haematologic Indices	126
4.7.2.2.	Sub-chronic effect of GOV on serum marker enzymes and chemical analytes	129
4.7.2.3.	Sub-chronic Effect on Serum Antioxidant Enzymes	132
4.7.2.4.	The Histopathology of Normal Liver of Rats Treated with GOV	134
4.8.	Preliminary Pharmacological Assays	136
4.8.1.	Antinociceptive Activities of GOV	136
4.8.2.	Anti-Inflammatory Activities of GOV	142
4.9.	Alcohol Induced Hepatotoxicity	146
4.9.1.	Effect of GOV on the Haematologic Indices on Alcohol Induced Hepatotoxicity on Rats.	146
4.9.2.	Effect of GOV on Serum Hepatic Enzymes and Chemical Analytes on Alcohol intoxicated rats	149
4.9.3.	The Effects of Alcohol on Antioxidant Defence Enzymes of Albino Rats	152
4.9.4.	Effect of Alcohol on Caspase Activities	158
4.9.5.	Histopathology of Liver of Rats Intoxicated with Alcohol	160
4.10.	D- galactosamine (D-GaIN) Induced Hepatotoxicity	162

4.10.1	Effect of GOV on the Haematologic Indices on D-GaIN Induced Hepatotoxicity on Rats.	162
4.10.2.	Effect of GOV on Serum Hepatic Enzymes and Chemical Analytes on D-GaIN intoxicated rats	165
4.10.3.	Effect of D-GaIN on Antioxidant Enzymes	168
4.10.4.	Effect of D-GaIN on Caspase Activities	174
4.10.5.	Histopathology of rats pretreated with GOV before D-GaIN damage	176
4.11.	Acetaminophen Induced Hepatotoxicity	178
4.11.1.	Effect of GOV on Hematological Parameter Acetaminophen-Induced Hepatotoxic Rats	178
4.11.2.	Effect of GOV on Serum Hepatic Enzymes and Chemical Analytes on Acetaminophen intoxicated rats	181
4.11.3	The Effects of APAP on Antioxidant Defence Enzymes of Albino Rats	184
4.11.4	Colorimetric assay of caspase - 2, 3 and 9 activities in the white blood cell of rats treated with Acetaminophen	190
4.11.5.	Histopathology of Liver of Rats Intoxicated with APAP	192
4.12.	Carbon Tetrachloride Induced Hepatotoxicity (CCl ₄)	194
4.12.1.	Effect of pretreatment with GOV on the hematological parameter in rats with CCl ₄ induced hepatotoxicity	194
4.12.2.	Effect of GOV on Serum Hepatic Enzymes and Chemical Analytes on CCl ₄ intoxicated rats	197
4.12.3.	The effect of CCl ₄ damage on serum antioxidant enzymes in rats	200

	pretreated with GOV	
4.12.4.	Colorimetric assay of caspase - 2, 3 and 9 activities in the white blood cell of rats treated with CCl ₄	206
4.12.5.	Histopathology of rats pretreated with GOV before CCl ₄ damage	208
4.13.	Antiproliferative activity of GOV towards HepG2 hepatoma, CNE2 and SUME- α nasopharyngeal cells (<i>in vitro</i> studies)	210
4.14.	<i>in vitro</i> antioxidant assays	212
4.14.1.	Inhibition of erythrocyte haemolysis using Swiss mice	212
4.14.2.	Lipid Peroxidative Activity of GOV using Brain Homogenates of Wistar Albino Rats	214
5.	DISCUSSION	216
	Conclusions	231
	Contributions to Knowledge	233
	References	234

LIST OF FIGURES

Figure 1:	Picture of <i>Gongronema latifolia</i> Benth	17
Figure 2:	Picture of <i>Ocimum gratissimum</i> Linn.	20
Figure 3:	Picture of <i>Vernonia amygdalina</i> Del.	24
Figure 4:	Diagram of the Liver	27
Figure 5:	A) STD difference spectrum of GOV with DHPR. B) Proton NMR spectrum of GOV in the presence of DHPR.	119
Figure 6:	A) STD difference spectrum of the ethyl acetate fraction of GOV with DHPR. B) Proton NMR spectrum of the ethyl acetate fraction of GOV in the presence of DHPR.	120
Figure 7:	A) STD difference spectrum of the ethanol extract of a mixture of <i>Gongronema latifolia</i> , <i>Ocimum gratissimum</i> and <i>Vernonia amygdalina</i> with DHPR. B) Proton NMR spectrum of the ethanol extract of a mixture of <i>G. latifolia</i> , <i>O. gratissimum</i> and <i>V. amygdalina</i> in the presence of DHPR.	121
Figure 8:	A) STD difference spectrum of the butanol fraction of GOV with DHPR. B) Proton NMR spectrum of the butanol fraction GOV in the presence of DHPR.	122
Figure 9:	A) STD difference spectrum of the chloroform fraction of GOV with DHPR. B) Proton NMR spectrum of the chloroform fraction of GOV in the presence of DHPR.	123
Figure 10:	A) STD difference spectrum of the water fraction of GOV with DHPR. B) Proton NMR spectrum of the water fraction of GOV in the presence of DHPR.	124
Figure 11:	A) STD difference spectrum of the hexane fraction of GOV with DHPR. B) Proton NMR spectrum of the hexane fraction of GOV in the presence of DHPR.	122

List of Tables

Table 1:	Preliminary phytochemical analysis of 50% ethanolic extract of <i>Gongronema latifolia</i> Benth., <i>Ocimum gratissimum</i> Linn. and <i>Vernonia amygdalina</i> Del.(GOV)	110
Table 2:	The Rf values of the test samples and standard reference compounds	114
Table 3a:	Effect of GOV on the hematologic indices in rats	127
Table 3b:	Effect of GOV on the hematologic indices in rats	128
Table 4a:	Serum levels of ALT, AST, ALP, LDH and GGT of rats treated with GOV	130
Table 4b:	Serum levels of albumin, cholesterol, creatinine, total protein, triglyceride and urea of rats treated with GOV.	131
Table 5:	The effect of GOV on serum antioxidant enzymes in rats	133
Table 6:	Effect of the GOV on acetic acid induced writhing in mice	137
Table 7:	Effect of GOV on formalin induced pain	139
Table 8:	Effect of GOV on hot plate test	141
Table 9:	Effect of GOV on carrageenan-induced rat paw oedema	143
Table 10:	Effect of the GOV on xylene induced ear oedema in mice	145
Table 11a:	Effect of pretreatment with GOV on the hematologic indices in rats with alcohol induced hepatotoxicity.	147
Table 11b:	Effect of pretreatment with GOV on the blood hematologic indices in rats with alcohol induced hepatotoxicity.	148
Table 12a:	Serum levels of ALT, AST, ALP, LDH and GGT in rats pretreated with GOV before alcohol damage.	150
Table 12b:	Serum levels of albumin, cholesterol, creatinine, total protein, triglyceride and urea in rats pretreated with GOV before alcohol damage	151
Table 13:	The effect of alcohol damage on serum antioxidant enzymes in rats pretreated with GOV	153
Table 14:	The effect of alcohol damage on liver antioxidant enzymes in rats pretreated with GOV.	155
Table 15:	The effect of alcohol damage on kidney antioxidant enzymes in rats pretreated with GOV	157

Table 16:	Colorimetric assay of caspase - 2, 3 and 9 activities in the white blood cell of rats treated with ethanol	159
Table 17a:	Effect of pretreatment with GOV on the hematologic indices in rats with D-GaIN induced hepatotoxicity.	163
Table 17b:	Effect of pretreatment with GOV on the hematologic indices in rats with D-GaIN induced hepatotoxicity.	164
Table 18a:	Serum levels of ALP, ALT, AST, GGT and LDH in rats pretreated with GOV before D-GaIN damage.	166
Table 18b:	Serum levels of albumin, cholesterol, creatinine, total protein, triglyceride and urea in rats pretreated with GOV before D-GaIN damage.	167
Table 19:	The effect of D-GaIN damage on serum antioxidant enzymes in rats pretreated with GOV	169
Table 20:	The effect of D-GaIN damage on liver antioxidant enzymes in rats pretreated with GOV	171
Table 21:	The effect of D-GaIN damage on kidney antioxidant enzymes in rats pretreated with GOV	173
Table 22:	Colorimetric assay of caspase - 2, 3 and 9 activities in the white blood cell of rats treated with D-GaIN	175
Table 23a:	Effect of pretreatment with GOV on the hematological parameter in rats with APAP induced hepatotoxicity	179
Table 23b:	Effect of pretreatment with GOV on the hematological parameter in rats with APAP induced hepatotoxicity	180
Table 24a:	The activities of ALT, AST, ALP, LDH and GGT in rats treated with a triherbal formulation (GOV) and a single dose of acetaminophen (APAP).	182
Table 24b:	Effect of GOV on serum ALB, CHO, CREA, TP, TG and urea concentrations in rats treated with a triherbal formulation (GOV) and a single dose of APAP.	183
Table 25:	The effect of APAP damage on serum antioxidant enzymes in rats pretreated with GOV	185
Table 26:	The effect of APAP damage on kidney antioxidant enzymes in rats pretreated with GOV	187

Table 27:	The effect of APAP damage on liver antioxidant enzymes in rats pretreated with GOV.	189
Table 28:	Colorimetric assay of caspase - 2, 3 and 9 activities in the white blood cell of rats treated with APAP	191
Table 29a:	Effect of pre-treatment with GOV on the hematologic indices in rats with CCl ₄ induced hepatotoxicity	195
Table 29b:	Effect of pre-treatment with GOV on the hematologic indices in rats with CCl ₄ induced hepatotoxicity	196
Table 30a:	Serum levels of ALP, ALT, AST, total bilirubin, GGT and LDH in rats pretreated GOV before CCl ₄ damage.	198
Table 30b:	Serum levels of albumin, cholesterol, creatinine, total protein, triglyceride and urea in rats pretreated with GOV before CCl ₄ damage.	199
Table 31:	The effect of CCl ₄ damage on serum antioxidant enzymes in rats pretreated with GOV	201
Table 32:	The effect of CCl ₄ damage on liver antioxidant enzymes in rats pretreated with GOV	203
Table 33:	The effect of CCl ₄ damage on kidney antioxidant enzymes in rats pretreated with GOV	205
Table 34:	Colorimetric assay of caspase - 2, 3 and 9 activities in the white blood cell of rats treated with CCl ₄	207
Table 35:	Antiproliferative activity of GOV towards HepG2 hepatoma cells, CNE2 and SUME- α nasopharyngeal cells	211
Table 36:	Anti-oxidant activity of GOV and its fraction using Red Blood Cell.	213
Table 37:	Lipid Peroxidative Activity of GOV using Brain Homogenates of Wistar Albino Rats	215

LIST OF ACRONYMS 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 ₂ O 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	<i>N</i> -acetyl- <i>p</i> -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, ascertaining 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.