HYPOTHALAMIC-PITUITARY-TESTICULAR-AXIS RESPONSE IN WISTAR RAT TREATED WITH CHLORPROMAZINE, *RAUWOLFIA VOMITORIA* LEAF AND COMBINATION OF RESERPINE, ASCORBATE AND ZINC

By

ADELEKE, OPEYEMI SAMSON

(12/68LD001)

B.Tech. (Ogbomoso), M.Sc. (Ilorin)

A Thesis Submitted to the Department of Anatomy, Faculty of Basic Medical Sciences, College of Health Sciences, University of Ilorin, in Partial Fulfilment of the Requirements for the Award of the Degree of Doctor of Philosophy in Anatomy

April, 2021

CERTIFICATION

This is to certify that this research work was done and written by ADELEKE Opeyemi Samson with Matric number 12/68LD001 and it has been read and approved as meeting the requirements of the Department of Anatomy, University of Ilorin, Ilorin, Nigeria for the Award of Doctor of Philosophy degree.

…………………………..……………. …………………………….
A.O OYEWOPO (B.Sc, M.Sc., Ph.D.) DATE

(Supervisor)

……………………………………...…… …………………………….
G.O OMOTOSHO (M.B;B.S, M.Sc. Ph.D.) DATE

(Head of Department)

…………………………………………… …………………………….
EXTERNAL EXAMINER DATE

DEDICATION

This thesis is dedicated to the Creator of the Universe – God Almighty –who has been helping me in all my endeavours.

DECLARATION

I, ADELEKE Opeyemi Samson with matric number 12/68LD001 hereby declare that this thesis entitled Hypothalamic-Pituitary-Testicular-Axis response in Wistar rat treated with Chlorpromazine, *Rauwolfia Vomitoria* leaf and combination of Reserpine, Ascorbate and Zinc is a record of my research work. It has neither been presented nor accepted in any previous application for a higher degree. All sources of information have been specifically acknowledged.

In addition, the research work has been ethically approved by the University Ethical Review Committee (UERC/ASN/2017/1067).

……………………………………… ……………………………

Adeleke, O.S Date

ACKNOWLEDGEMENTS

This project report was embarked upon and completed with the special grace and assistance of God Almighty; I give all glories and adoration to his name.

My great gratitude goes to my amiable supervisor Dr. A.O. Oyewopo, who through his wealth of knowledge impacted in me constructive criticism, patience and professionalism throughout the period of carrying out this project. My prayer is that God will enlarge your coast and proper you more in all your endeavors (Amen).

I am indebted to my darling wife (Mrs. Kehinde Adeleke), Children (Moyosoreoluwa and Moyinoluwa), parent (Elder. and Mrs. J.O Adeleke) and brother (Kunle Adeleke) for their immense contribution in kind, prayers and advise. I love you all.

My special thanks goes to Dr. O.O. Omotuyi of the Chemo-Genomics Research Institute, Afe Babalola University, Ado Ekiti, Ekiti State, Nigeria Phyto-Therapeutics Research Unit, Mols and Sims, Ado Ekiti, Ekiti State, Nigeria for providing the laboratory and equipment for this research.

My appreciation also goes to all the teaching and non teaching staff member of the Department of Anatomy University of Ilorin, Ilorin, Kwara State. God Bless you all (Amen).

Abstract

Synthetic antipsychotic drugs have been reported to induce reproductive toxicity while psychiatric patients treated with traditionally used herb like *Rauwolfia vomitoria* (*RV*) showed no traces of reproductive toxicity. Molecular mechanisms underlying control of Hypothalamic-Pituitary-Testicular-Axis (HPTA) by synthetic and traditionally used antipsychotic drugs are poorly understood. Thus, this study aimed to compare the effects of chlorpromazine (CPZ), *RV* leaf extracts and combination of reserpine, ascorbic acid and zinc (RAZ) on HPT-Axis of Wistar rats. The objectives of the study were to determine: (i) histological changes in the testes and hypothalamus; (ii) gonadotropin releasing hormone (GnRH), cytokeratin-18, Bcl_2 and ki-67 protein expression; (iii) expression of Cyclic-adenosine-monophosphate Responsive Element Modulator (CREM), protamine (PRM) genes in the testes (iv) andrological parameters; and (v) antioxidant status.

Seventy-two male Wistar rats (weight: 180.00$\pm$4.67g) were assigned into nine groups (A-I) (n=8). Group A (control) was administered physiological saline while rats in Groups B and C received 10 and 20 mg/kg body weight (bwt) of chlorpromazine respectively. Groups D and E received 2.5 and 5 mg/kg bwt of reserpine while Groups F and G received 150 and 300 mg/kg bwt of *RV* leaf extract respectively. Groups H and I received (2.5:5:100) mg/kg bwt and (5:10:200) mg/kg of combination of RAZ respectively. The administration lasted for 56 days. On the 57th day, the rats were sacrificed, hypothalamus and testes were excised for histological, genes and immunohistochemical examinations while serum was used for hormonal analysis (follicle stimulating hormone (FSH), luteinizing hormone (LH), testosterone, sperm count, motility and morphology) and biochemical analysis (glutathione peroxidase (Gpx), superoxide dismutase (SOD) and malondialdehyde). Data analyses were done by Analysis of Variance followed by *Tukey's post-hoc* test at $P<0.05$ level of significant.

The findings of the study were that:

i. chlorpromazine and reserpine treated rats showed hypothalamic arcuate neurons and testicular germ cells degeneration;
ii. chlorpromazine and reserpine treated rats showed negative immunoreactivity to GnRH and ki-67 and weak positive immunoreactivity to cytokeratin and Bcl_2 proteins while

RV and combination of RAZ treated rats showed weak positive immunoreactivity to all the proteins;

iii. chlorpromazine and reserpine treated rats when compared with control and RAZ groups showed significant ($p<0.001$) down regulation of CREM (0.32±0.05), protamine-I (0.14±0.02) and II (0.13±0.02) genes expression;

iv. serum FSH (0.19±0.03 ng/ml), LH (0.33±0.06 ng/ml), testosterone (0.15±0.02 ng/ml), percentage of normal sperm count (17.40±2.59), motility (19.60±2.86) and morphology (16.60±2.91) were significantly ($p<0.001$) decreased in chlorpromazine and reserpine treated animals while prolactin (0.11±0.03 ng/ml) level was significantly ($p<0.01$) increased when compared with control and RAZ groups; and

v. serum GPx (21.00±3.50 U/L), SOD (0.54±0.12 u/mL) levels were significantly ($p<0.001$) reduced in chlorpromazine and reserpine treated rats while malondialdehyde (0.79±0.15) level was significantly ($p<0.001$) increased compared with control and RAZ groups.

The study concluded that HPT-Axis was impaired by chlorpromazine and reserpine while *RV* and combination of RAZ (2.5:5:100) mg/kg bwt administration enhanced the axis. The study recommended that combination of RAZ should be prescribed in order to improve reproductive toxicity associated with antipsychotic drugs.

TABLE OF CONTENTS

LIST OF FIGURES

List of Abbreviations

5-HT1 and 5-HT2	Serotonin receptors
ACT	Activator of CREM in testis
AFR	ascorbate free radical
AMH	anti-müllerian hormone
Arc	arcuate
AVPV	anteroventral periventricular
Bwt	Body weight
cAMP	Cyclic adenosine monophosphate
COMT	Catechol-O-methyltransferase
CPZ	Chlorpromazine
CRE	cAMP responsive elements
CREB1	cAMP response element-binding protein
CREM	Cyclic-adenosine-monophosphate Responsive Element Modulator
CVD	cardiovascular disease
D	Dopamine
DHA	Dehydroascorbic acid
DHT	dihydrotestosterone
DMSO	dimethyl sulfoxide
DNA	Deoxyribonucleic acid
ELISA	Enzyme-linked immunosorbent assay
Fe2+	ferrous

Fe^{3+}	ferric
FSH	Follicle stimulating hormone
FSHR	Follicle stimulating hormone receptor
GATA4	GATA Binding Protein 4
GLUT	glucose transporter
GnRH	Gonadotrophin releasing hormone
GPx	Glutathione peroxidase
H_2O_2	hydrogen peroxide
HCG	human chorionic gonadotropin
H & E	Hematoxyline and Eosin
HED	human equivalent dose
HIV	human immunodeficiency virus
HPTA	Hypothalamic-Pituitary-Testicular-Axis
HRP	Horse Radish Peroxidase
KIF17	Kinesin family member 17
LDL	low density lipoproteins
L-DOPA	levodopa
LH	Luteinizing hormone
MAO	monoamine oxidase
MDA	Malondialdehyde
NO	nitric oxide
oxLDL	oxidatively modified low density lipoprotein

PKA	Protein Kinase A
pNPP	p-nitrophenylphosphate
POA	preoptic area
PRL	prolactine
RAZ	Reserpine, Ascorbate and Zinc
RDA	Recommended Dietary Allowance
REM	Rapid eye movement
RES	Reserpine
RNA	ribonucleic acid
ROS	reactive oxygen species
RT-PCR	Reverse Transcriptase Polymerase Chain Reaction Analysis
RV	*Rauwolfia vomitoria*
SLE	systemic lupus erythematosus
SOD	Superoxide dismutase
SOX9	sex determining region Y
SRY	Sex region Y
SVCT	sodium-dependent vitamin C transporter-type 1 ascorbate transporter
TH	tyrosine hydroxylase
TSH	thyroid-stimulating hormone
TUNEL	Terminal deoxynucleotidyl Transferase Biotin-dUTP Nick End Labeling
UERC	University of Ilorin ethical review committee
Vit C	Vitamin C

VMAT	vesicular monoamine transporter
VSMC	Vascular smooth muscle cell
Zfp	zinc finger protein
Zn	Zinc