UNIVERSITI PUTRA MALAYSIA

PROTECTIVE EFFECT OF Nigella sativa L. (BLACK CUMIN) SEED OIL ON SODIUM VALPROATE-INDUCED NEURAL TUBE DEFECTS AND BEHAVIOUR IN MICE MODEL

BELLO SIRAJO SHIITU

FPSK(P) 2017 18

PROTECTIVE EFFECT OF *Nigella sativa* L. (BLACK CUMIN) SEED OIL ON SODIUM VALPROATE-INDUCED NEURAL TUBE DEFECTS AND BEHAVIOUR IN MICE MODEL

By

BELLO SIRAJO SHIITU

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Doctor of Philosophy

August 2017

COPYRIGHT

DEDICATION

I dedicate this thesis dissertation to Almighty Allah my creator, my source of inspiration, knowledge and wisdom. May His grace and benefaction be upon the noble prophet Muhammad (SAW), His household and close associates I also dedicate this work to my parents, siblings, wife and children, I pray that Allah Almighty bless you all.

Abstract of thesis presented to the senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy

PROTECTIVE EFFECT OF *Nigella sativa* L. (BLACK CUMIN) SEED OIL ON SODIUM VALPROATE-INDUCED NEURAL TUBE DEFECTS AND BEHAVIOUR IN MICE MODEL

By

BELLO SIRAJO SHIITU

August 2017

Chairman : Professor Fauziah Othman, PhD
Faculty : Medicine and Health Sciences

Neural tube defects (NTDs) are the major congenital malformations of the central nervous system in humans. The universal occurrence of neural tube defects (NTDs) ranges from 0.5 to 12 per 1000 live births depending on the countries, with higher incidence in the developing countries; this accounts for 400,000 live births globally per annum. Women of childbearing age with epilepsy are often concerned about hazards of drug exposure during pregnancy and lactation. Valproic acid (VPA) an antiepileptic drug causes teratogenicity and embryo toxicity, when taken during pregnancy. Plant extracts has been in use to prevent and treat different diseases, *Nigella sativa* L. (NS) is a family member of the Ranunculaceae that has been in use as food additives to prevent different types of diseases. Effect of NS extract on central nervous system has been studied by many researchers and most of the studies have shown positive outcome and good benefit on the central nervous system. This study was done to evaluate the effects of NS oil extract on the prevention of sodium valproate-induced neural tube defects in mice, and the developmental impairment induced by sodium valproate exposure during intrauterine stage. Seventy five (60 female and 15 male), non-gravid ICR mice were used for the study. The mice at 7 weeks of age were allowed two weeks of adjustment in the animal house. In the first study, gravid mice were divided into five groups of six pregnant mice each. Group 1 was treated with VPA only at a dose of 600mg/kg daily, groups 2, 3 and 4 received VPA at a dose of 600mg/kg/day + 0.2ml of NS oil extract, VPA at a dose of 600mg/kg/day + 0.1ml of NS oil extract and 600mg/kg/day + FA 400µg, respectively. Group 5 mice were administered with 0.9% saline, and served as control. The dams were sacrificed on gestational day 15 and the embryos were harvested for physical examination and laboratory evaluation. The treatment regimens for Groups 1, 2, 4 and 5 were repeated in the second study, with the exclusion of Group 3. The offspring from these groups were assessed at 3-7 weeks

old for physical developmental milestones and evaluated for various behavioural tests. Muscle weakness, memory impairment and anxiety were ameliorated in the offspring that had been treated with NS oil extract plus VPA, compared to those treated with VPA only, and VPA + FA. Animals in the Control group did not have any evidence of muscular and memory deficits. Light microscopy histological evaluation illustrated that there were significant distortion in the architecture of the cell in the spinal cord in VPA only, and VPA + FA groups. There was no significant distortion observed in VPA + NS 0.2 ml group. Nuclear swelling and necrosis associated with VPA exposure seen on TEM images was significantly reduced by NS oil extract supplemented at 0.2 ml. The inhibition of histone deacetylase (HDAC) by VPA was significantly reversed in the VPA + NS 0.2 ml treatment group. In conclusion, this study demonstrated that administration of NS oil extract improved memory and learning abilities in offspring that had shown disabilities associated with prenatal exposure to sodium valproate. The NS oil extract is also beneficial in improving the muscular strength, and reversal of anxiety and behavioural deficits associated with *in utero* VPA exposure. It is postulated that the administration of NS oil extract prevented NTDs associated with prenatal VPA exposure, by reducing the inhibition of HDAC activity associated with VPA exposure.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

KESAN PELINDUNGAN MINYAK BIJI *Nigella sativa* L. (BLACK CUMIN) KE ATAS KECACATAN TIUB SARAF DAN TINGKAH LAKU TERARUH NATRIUM VALPROATE PADA MODEL MENCIT

Oleh

BELLO SIRAJO SHIITU

Ogos 2017

Pengerusi : Profesor Fauziah Othman, PhD
Fakulti : Perubatan dan Sains Kesihatan

Kecacatan tiub saraf (NTDs) adalah kecacatan kongenital sistem saraf pusat pada manusia. Kejadian sejagat NTD adalah sekitar 0.5 ke 12 insiden untuk setiap 1000 kelahiran, bergantung kepada negara, dengan insiden lebih tinggi di negara membangun. atau setara dengan 400,000 kelahiran global setahun. Wanita pada umur boleh mengandung yang mengidap epilepsi sering mengambil berat tentang bahaya dari ubat-ubatan yang diambil dalam tempoh mengandung dan penyusuan. Asid valproik (VPA), adalah sejenis ubat antiepileptik yang teratogenik dan boleh menyebabkan keracunan embrio jika diambil ketika mengandung. Pati tumbuhan telah digunakan untuk mencegah dan merawat pelbagai penyakit. *Nigella sativa* L. (NS) dari keluarga Ranunculaceae telah digunakan sebagai penokok diet untuk mencegah pelbagai jenis penyakit. Kesan pati NS atas sistem saraf pusat telah dikaji oleh ramai pengkaji, dan kebanyakan kajian menunjukkan kesan positif dan manfaat terhadap sistem saraf pusat. Kajian ini dijalankan untuk menilai kesan pati minyak NS dalam mencegah kecacatan tiub neural teraruh natrium valproate pada mencit, dan kecacatan perkembangan yang diaruh oleh pendedahan terhadap natrium valproat ketika dalam rahim. Tujuh puluh lima (60 betina dan 15 jantan) mencit ICR tak gravid digunakan dalam kajian ini. Mencit berumur 7 minggu dibiarkan untuk beradaptasi selama dua minggu di rumah haiwan. Kajian pertama menggunakan lima kumpulan rawatan yang terdiri daripada enam mencit bunting dalam setiap kumpulan. Kumpulan 1 diberi VPA sahaja pada dos 600mg/kg sehari, kumpulan 2, 3 dan 4 masing-masing diberi VPA pada dos 600mg/kg/hari + 0.2ml pati minyak NS, VPA pada dos 600mg/kg/hari + 0.1ml pati minyak NS dan VPA pada dos 600mg/kg/hari + FA 400µg. Mencit dari kumpulan 5 diberi 0.9% larutan salin dan bertindak sebagai kumpulan kawalan. Mencit betina dikorbankan pada hari bunting ke-15 dan embrio diambil untuk pemeriksaan fizikal dan penilaian makmal. Untuk kajian kedua, rawatan yang serupa dengan kajian pertama diberikan, tanpa kumpulan

yang dirawat dengan 0.1ml pati minyak NS. Anak dari kumpulan-kumpulan ini dinilai pada umur 3-7 hingga minggu untuk melihat tahap perkembangan fizikal dan untuk tujuan ujian tingkah laku. Kelemahan otot, gangguan ingatan dan kerisauan didapati berkurangan pada anak mencit yang mengambil minyak NS dengan VPA, berbanding dengan kumpulan yang hanya mengambil VPA dan VPA + FA. Sementara itu kumpulan kawalan tidak menunjukkan sebarang susutan kekuatan otot dan gangguan ingatan. Pemeriksaan histologi menunjukkan pengherotan yang siginifikan pada bentuk sel saraf tunjang pada kumpulan VPA dan VPA + FA. Tiada pengherotan yang signifikan dicerap pada kumpulan VPA + NS 0.2ml. Pembengkakan nuklear dan nekrosis berikutan pendedahan kepada VPA yang dicerap dengan TEM berkurang dengan signifikan selepas rawatan dengan pati minyak NS pada 0.2ml. Rencatan histone deasetilase (HDAC) oleh VPA berbalik kepada paras normal dalam kumpulan VPA + NS 0.2ml. Kesimpulannya, kajian ini menunjukkan rawatan dengan pati minyak NS telah memberi kesan positif terhadap pemulihan keupayaan pembelajaran dan ingatan anak mencit yang didedahkan kepada natrium valproate semasa pranatal. Ia turut menunjukkan manfaat pati minyak NS dalam memperbaiki kelemahan otot, mengurangkan tahap kerisauan serta defisit perilaku yang berkait dengan autisme, selepas pendedahan pranatal kepada VPA. Rawatan dengan pati minyak NS telah mencegah NTD yang disebabkan oleh pendedahan pranatal kepada VPA, dengan mengurangkan rencatan aktiviti HDAC yang disebabkan oleh VPA.

ACKNOWLEDGEMENTS

All thanks, glorifications and total submissions are due to Allah alone, the Creator of the universe, the Preserver and Maintainer of all its affairs, the Administrator and Sustainer of all its workings, the Will behind its phenomena and Disposer of all activities to its successful completion. May peace and blessings of Allah (SWT) be upon our leader, Muhammad (SAW), the seal of the prophets, illustrious lamp and guide to the righteous path, and upon his house-hold and companions, till the Day of Judgment.

The most grateful of people to Allah are those who are most grateful to other people in life. It is with great pleasure that I wish to thank all the people who supported me and were involved in my journey toward the acquisition of PhD. Human Anatomy and in preparation of this dissertation. I will start by thanking my parents for bringing me to this world, nurturing and showing me the right direction, May Allah Almighty reward you with Aljanna Firdausi.

I would like to express my sincere gratitude and appreciations to my supervisor, Prof. Dr. Fauziah Othman for her untiring and dedicated selfless support and contributions towards the successful actualization of this work, without which this project would not have been possible, and her kind cooperation in all steps of my study. Surely her undeniable assistance has lifted my spirit high and made me complete this important task. I am forever indebted to your wonderful care and kind gestures.

I also thank and appreciate the marvelous support and contributions of my Co-Supervisors; Professor Hamidon Basri, Associate Prof. Dr. Faez Firdaus Jesse Abdullah and Dr. Zolkapli Eshak for their useful advice, corrections and encouragement towards my successful completion of this course.

The role and contributions of Dr. Michael Ling, Associate Prof. Dr. Cheah Pike See, Dr. Usman Bala, Dr. Dahiru Sani and Dr. Ibrahim Suleiman with respect to helping with some aspect of the work, advice, suggestions and encouragement during this research work is worth mentioning. I am highly indebted to you all, and no word can quantify the measure of my thankfulness.

I shall not conclude this acknowledgement without mention the remarkable and inspiring friends/colleagues like, Drs Aliyu Mamuda, Bashir Bello, Abubakar, Danmaigoro, Musa Samaila and several others so numerous to mention for their patience, understanding and moral support despite odd circumstances towards the realization of this goals and aims. May Allah (SWT) bless you all, to my wife and children I say thank you for your understanding. Finally, I pray that our ends be justified with the garden of endless bliss.

I certify that a Thesis Examination Committee has met on 4 August 2017 to conduct the final examination of Bello Sirajo Shiitu on his thesis entitled "Protective Effect of *Nigella sativa* L. (Black Cumin) Seed Oil on Sodium Valproate-Induced Neural Tube Defects and Behaviour in Mice Model" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Doctor of Philosophy.

Members of the Thesis Examination Committee were as follows:

Mohd Nasir bin Mohd Desa, PhD
Associate Professor
Faculty of Medicine and Health Sciences
Universiti Putra Malaysia
(Chairman)

Roslida binti Abd Hamid @ Abdul Razak, PhD
Associate Professor
Faculty of Medicine and Health Sciences
Universiti Putra Malaysia
(Internal Examiner)

Goh Yong Meng, PhD
Associate Professor
Faculty of Veterinary Medicine
Universiti Putra Malaysia
(Internal Examiner)

Bay Boon Huat, PhD
Professor
National University of Singapore
Singapore
(External Examiner)

NOR AINI AB. SHUKOR, PhD
Professor and Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 30 November 2017

This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Doctor of Philosophy. The members of the Supervisory Committee were as follows:

Fauziah Binti Othman, PhD
Professor
Faculty of Medicine and Health Sciences
Universiti Putra Malaysia
(Chairman)

Hamidon Bin Basri, PhD
Professor
Faculty of Medicine and Health Sciences
Universiti Putra Malaysia
(Member)

Faez Firdaus Jesse Abdullah, PhD
Associate Professor
Faculty of Veterinary Medicine
Universiti Putra Malaysia
(Member)

Zolkapli Eshak, PhD
Lecturer
Faculty of Pharmacy
Universiti Teknologi Mara
(Member)

ROBIAH BINTI YUNUS, PhD
Professor and Dean
School of Graduate Studies,
Universiti Putra Malaysia

Date:

Declaration by graduate student

I hereby confirm that:

- this thesis is my original work;
- quotations, illustrations and citations have been duly referenced;
- this thesis has not been submitted previously or concurrently for any other degree at any institutions;
- intellectual property from the thesis and copyright of thesis are fully-owned by Universiti Putra Malaysia, as according to the Universiti Putra Malaysia (Research) Rules 2012;
- written permission must be obtained from supervisor and the office of Deputy Vice-Chancellor (Research and innovation) before thesis is published (in the form of written, printed or in electronic form) including books, journals, modules, proceedings, popular writings, seminar papers, manuscripts, posters, reports, lecture notes, learning modules or any other materials as stated in the Universiti Putra Malaysia (Research) Rules 2012;
- there is no plagiarism or data falsification/fabrication in the thesis, and scholarly integrity is upheld as according to the Universiti Putra Malaysia (Graduate Studies) Rules 2003 (Revision 2012-2013) and the Universiti Putra Malaysia (Research) Rules 2012. The thesis has undergone plagiarism detection software

Signature: _____ Date: _____

Name and Matric No. Bello Sirajo Shiitu, GS43080

Declaration by Members of Supervisory Committee

This is to confirm that:

- the research conducted and the writing of this thesis was under our supervision;
- Supervision responsibilities as stated in the Universiti Putra Malaysia (Graduate Studies) Rules 2003 (Revision 2012-2013) were adhered to.

Signature: _____
Name of Chairman of Supervisory Committee: Professor Dr. Fauziah Binti Othman

Signature: _____
Name of Member of Supervisory Committee: Professor Dr. Hamidon Bin Basri

Signature: _____
Name of Member of Supervisory Committee: Associate Professor Dr. Faez Firdaus Jesse Abdullah

Signature: _____
Name of Member of Supervisory Committee: Dr. Zolkapli Eshak

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

AA	Arachidonic acid
AChE	Acetylcholinesterase
AEDs	Antiepileptic drugs
AFP	Alfa fitoprotein
AMT	Amino methyl transferase
ANOVA	Analysis of variance
ASD	Autism spectrum disorder
AtSD	Atrial septal defect
BCA	Bicinchoninic acid
BDMA	Benzyl dimethylamine
BMP4	Bone morphogenetic protein 4
BT-474	Breast ductal carcinoma cell
cDNA	complementary de-oxyribonucleic acid
CELSR1	Cadherin EGF LAG Seven-Pass G-Type Receptor 1
CNS	Central nervous system
CO2	Carbon dioxide
CP	Crossing point
CSF	Cerebrospinal fluid
CYP450	Cytochrome P450
DDSA	Dodecenyl succinic anhydride
DEPC	Diethylpyrocarbonate
DHA	Decosahexanoic acid (DHA)
DNA	Deoxyribonucleic acid
DTQ	Dithymoquinone.
EM	Electron microscopy
ELISA	enzyme linked immunosorbent assay
EPA	Eicosapentanoic acid
EPM	Elevated plus maze
EURAP	Prospective observational study of pregnancies with antiepileptic drugs
FA	Folic acid
FDA	Food and Drug Administration
FPSK	Faculti Perubatan dan Sains Kesihatan
FVS	Foetal Valproic syndrome
FZD3	Frizzled-3
GABA	Gamma-Aminobutyric acid
GADD45α	Growth arrest and DNA damage 45α
gas5	hypoxanthine-guanine-phosphoribosyl-transferase
GC	Gray Colum
GC-MS	Gas chromatography mass spectrometry

GD	Gestational day
GLDC	Glycine dehydroxylase
H_2O_2	Hydrogen peroxide
H3	Histone 3
H4	Histone 4
HATs	Histone acetyltransferases
HDAC	Histone deacetylases
H&E	Haematoxylin and Eosin
HSR	Hypersensitivity syndrome reaction
IACUC	Institutional Animal Care and Use Committee
ICR	Institute of Cancer Research
IP	Intraperitoneal
IQ	intelligence quotient
IUGR	Intrauterine growth restriction
MCF-7	Michigan Cancer Foundation-7
MDA-MB-231	Invasive ductal carcinoma cell line
MMC	Myelomeningocele
MNA	Methyl nadic anhydride
Mn	Manganese
MNNR	Malaysian national neonatal registry
MRI	Magnetic resonance imaging
MTHFDIL	Methylenetetrahydrofolate Dehydrogenase
MWM	Morris water maze
NaVP	Sodium valproate
NOR	Novel object recognition
Nrf2	NF-E2-related factor 2
NS	*Nigella sativa*
NTDs	Neural tube defects
NCBI	National Centre for Biotechnology Information
O_2^-	superoxide radical
OD	optical density
OH	hydroxyl radicals
Pax-3	Paired Box 3
P53 protein	Phosphoprotein 53
PCR	polymerase chain reaction
PBUH	Peace be upon him
Pgk1	Phosphoglycerate kinase 1
PH	potential of hydrogen
PND	Postnatal day
Psmb2	Proteasome Subunit Beta 2
PTZ	Pentylenetetrazole
PVDF	polyvinylididine difluoride

Psa	pneumococcal surface adhesin
PTK7	Protein Tyrosine Kinase 7
RBC	Red blood cell
ROS	Reactive oxygen species
RNA	ribonucleic acid
RNase	Ribonuclease
rRNA	ribosomal ribonucleic acid
RT-PCR	reverse transcription polymerase chain reaction
SAS	Subarachnoid space
SOD	superoxide dismutase
TEA	Triethanolamine
TEM	Transmission electron microscope
THir	Tyrosine hydroxylase immune-reactive
Tm	Annealing temperature
TQ	Thymoquinone
UCP2	Uncoupling protein 2
UK	United Kingdom
USA	United State of America
USD	United State dollar
UV	Ultra-violent
VANGL2	Van Gogh-Like 2
VPA	Valproic acid
VSD	Ventricular septal defect

CHAPTER 1

INTRODUCTION

Neural tube defects (NTDs) are the major congenital malformations of the central nervous system in humans. The commonest forms of NTDs in humans are spinal bifida and anencephaly which occur due to the absence of closure of posterior and anterior neuropore respectively (Detrait *et al*., 2005). Spinal bifida is sub-divided into: spinal bifida occulta, meningocele and mylomeningocele. The universal occurrence of NTDs ranges from 0.5 to 12 per 1000 live births depending on the country with higher incidence in the developing countries; this accounts for 400,000 live births globally per annum (Copp & Greene, 2010). Anencephaly, which is the second form of NTD in human is mostly incompatible with life and leads to stillbirth or early neonatal death, approximately half of the babies born with anencephalic have a life expectancy of few minutes to one day, only about one-quarter of the babies could live up to 10 days (Bower *et al*., 2004). Risk factors of neural tube defects include; diabetes or obesity, and maternal use of medications such as antiepileptic drugs during early pregnancy (Grewal *et al*., 2009). The aetiology of NTDs are complex, these involve a combination of genetic, nutrient, and environmental factors. Valproic acid (VPA) which is use in the treatment of epilepsy, migraines, and bipolar affective disorder. Is a well-established human teratogen (Bowden, 2009). Folic acid deficiency is also a known risk factor, and folate supplementation before and during early pregnancy may results in a decrease risk of neural tube defects (Ray *et al*., 2002). The financial implications of managing neural tube defects pose a great burden on the families and patients affected. Women of childbearing age with chronic medical conditions such as epilepsy are often concerned about hazards of drug exposure during pregnancy and lactation. The avoidance of any medication after conception may often be unwise for maternal well-being due to the fact that the drug is beneficial for her condition. Proper prescription of drugs in pregnancy is a challenge and should provide maximal safety to the foetus as well as therapeutic benefit to the mother. Teratogens act with specificity in that they produce specific abnormalities at specific times during gestation (Adebisi, 2011). For instance, thalidomide produces limb defect (phocomelia), while valproic acid and carbamazepine produce neural tube defects (e.g. spinal bifida) (Adebisi, 2011). Teratogenic specificity also applies to species, for example, aspirin and corticosteroids have been found to be teratogenic in mice and rats but appear to be safe in humans (van Gelder *et al*., 2010). The organization of proliferation and differentiation of the neuroepithelial cells is highly required for proper neural tube closure. Consequently, exposure to an agent that interferes with rapid cellular proliferation and interrupts discrete regions of differentiation and apoptosis of the neuroepithelial cells can possibly induce a NTDs. VPA is a histone deacetylase inhibitor; it is presently in clinical trials as an anticancer agent (Batty *et al*., 2009). Its properties as an anticancer agent differ for different cell types and these include: growth arrest through arrest of cell cycle and decreased proliferation, apoptosis and anti-angiogenesis (Ellis *et al*., 2009). Microarray studies of VPA intake during pregnancy have demonstrated an increase in the expression of genes

responsible for growth-arrest, such as *gadd45α* in the head region of the embryo. These suggest that neural tube defects may arise from inhibition of cell growth and induction of apoptosis in the neuroepithelial cells (Okada *et al*., 2005). Additionally, a study previously shows that embryonic *p53* protein expression increases just 3 hours after maternal VPA administration, this was localized to the embryonic somites (Di Renzo *et al*., 2010). Some previous studies on VPA, shows that it increases reactive oxygen species (ROS) formation. ROS induces DNA double strand breakage in an in vitro study (Sha & Winn, 2010). ROS can cause macromolecular damage to some components like lipids, proteins, and DNA and alteration in embryonic signalling pathways, in the cells (Wells *et al*., 2009).

Nigella sativa (NS) is a native annual flowering plant found in some part of Asia and southern Europe. The flower of NS contain some small black seeds and the seeds are the source of pharmacologically active component of NS (Keyhanmanesh *et al*., 2013). These components include thymoquinone (TQ), ditimoquinone (DTQ) and nigellin, with thymoquinone been highest in concentration. In traditional medicine, NS has been used in the treatment of different type of sickness, such as hypertension, obesity, dysentery, fever, headache, and gastrointestinal problems. The prophet of Islam Muhammad (PBUH) said that NS can be used in the treatment of all kind of diseases except death and old age (Ali & Blunden, 2003). Previous experimental studies have shown that the extract of NS seeds have anti-inflammatory and anticancer activities. It was also found that extract of NS is protective against oxidative damage in isolated rat hepatocytes (Kanter *et al*., 2006a). Feeding neonate and juvenile with NS extract at an early stage is associated with improved memory and learning in rats (Beheshti *et al*., 2015).

1.1 Problem statement

The universal occurrence of neural tube defects (NTDs) ranges from 0.5 to 12 per 1000 live births depending on the countries, with higher incidence in the developing countries; this accounts for 400,000 live births globally per annum. Women of childbearing age with epilepsy are often concerned about hazards of drug exposure during pregnancy and lactation. VPA, an antiepileptic drug that effective in women of child bearing age, has been well documented as a teratogen that causes NTDs on exposed foetus.

1.2 Rationale of the study

Pregnant woman that has chronic illness and are on medication has the risk of having a malformed foetus. Drugs such as sodium valproate use in the treatment of epilepsy can cause neural tube defects (NTDs). This study is to investigate the preventive effect of NS oil extract on neural tube defects induced by sodium valproate administration.

1.3 Research questions

1. Can co-administration of NS oil extract with sodium valproate prevent neural tube defect that can be induced by sodium valproate?
2. What will be the effect of NS oil extract on motor, memory and cognitive developmental impairment in the offspring induced by sodium valproate exposure during intra uterine life?

1.4 Research objectives

1.5 General Objectives

To study the effect of NS oil extract on prevention of sodium valproate induced neural tube defects in ICR mice.

1.6 Specific objectives

1. To investigate the effect of administering NS oil with Sodium valproate in prevention of Sodium valproate induced neural tube defect in ICR mice.
2. To determine the effect of NS oil on placental tissue damage induced by sodium valproate administration during pregnancy in mice.
3. To determine the effect of NS oil in reducing histone deacetylase inhibition activity in the embryo exposed to sodium valproate during pregnancy.
4. To evaluate the effect of NS oil on histological changes on neonatal neural tissue due to maternal sodium valproate exposure during pregnancy.
5. To determine the effect of NS oil in decreasing *gadd45α* gene expression in the embryo exposed to sodium valproate during pregnancy.
6. To evaluate the effect of NS oil on motor, memory and mental developmental impairment induced by sodium valproate exposure during intra uterine life.

1.7 Research Hypothesis

1. NS oil has no preventive effect on Sodium valproate induced neural tube defect when administered to pregnant ICR mice.
2. NS oil has no preventive effect on mental retardation associated with exposure to sodium valproate during intra uterine development on ICR mice offspring.

REFERENCES

Aaku-Saraste, E., Hellwig, a, & Huttner, W. B. (1996). Loss of occludin and functional tight junctions, but not ZO-1, during neural tube closure--remodeling of the neuroepithelium prior to neurogenesis. *Developmental Biology*, *180*(2), 664–679.

Abdollahi, A., Lord, K. A., Hoffman-Liebermann, B., & Liebermann, D. A. (1991). Sequence and expression of a cDNA encoding MyD118: a novel myeloid differentiation primary response gene induced by multiple cytokines. *Oncogene*, *6*(1), 165–167.

Abramova, M. V., Pospelova, T. V., Nikulenkov, F. P., Hollander, C. M., Fornace, A. J., & Pospelov, V. A. (2006). G1/S arrest induced by histone deacetylase inhibitor sodium butyrate in E1A + Ras-transformed cells is mediated through down-regulation of E2F activity and stabilization of ??-catenin. *Journal of Biological Chemistry*, *281*(30), 21040–21051.

Abramova, M. V., Zatulovskiy, E. A., Svetlikova, S. B., & Pospelov, V. A. (2010). HDAC inhibitor-induced activation of NF-B prevents apoptotic response of E1A+Ras-transformed cells to proapoptotic stimuli. *International Journal of Biochemistry and Cell Biology*, *42*(11), 1847–1855.

Abu El Ezz, N. M. T. (2005). Effect of Nigella sativa and Allium cepa oils on Trichinella spiralis in experimentally infected rats. *Journal of the Egyptian Society of Parasitology*, *35*(2), 511–23.

Adebisi S. S. (2011). The knowledge of teratogenicity in the prevention of congenital anomalies. *Kuwait Medical Journal,* 43(2), 89-98.

Adzick, N. S. (2012). Fetal surgery for myelomeningocele: Trials and tribulations. *Journal of Pediatric Surgery*, *47*(2), 273–281.

Adzick, N. S., Thom, E. A., Spong, C. Y., John W. Brock III, Burrows, P. K., Johnson, M. P., Sutt, L. N. (2011). A Randomized Trial of Prenatal versus Postnatal Repair of Myelomeningocele. *The New England Journal of Medicine*, *364*(11), 877–889.

Akhtar, M., Khanam, R., Mohd Aqil, Maikiyo, A., Mohd Mujeeb, & Najmi, A. (2012). Ameliorating effects of two extracts of Nigella sativa in middle cerebral artery occluded rat. *Journal of Pharmacy and Bioallied Sciences,* 4(1), 70-75.

Al-Ghamdi, M. S. (2001). The anti-inflammatory, analgesic and antipyretic activity of Nigella sativa. *Journal of Ethnopharmacology*, *76*(1), 45–48.

Al-Nazawi, M. H., & El-Bahr, S. M. (2012). Hypolipidemic and Hypocholestrolemic Effect of Medicinal Plant Combination in the Diet of Rats: Black Cumin Seeds (Nigella sativa) and Turmeric (Curcumin). *J. Anim. Vet. Adv*, 12(11), 2013-19.

Alhebshi, A. H., Gotoh, M., & Suzuki, I. (2013). Thymoquinone protects cultured rat primary neurons against amyloid β-induced neurotoxicity. *Biochemical and Biophysical Research Communications*, *433*(4), 362–367.

Ali, B. H., & Blunden, G. (2003). Pharmacological and toxicological properties of Nigella sativa. *Phytotherapy Research*, *17*(4), 299–305.

Ali, E. H. A., & Elgoly, A. H. M. (2013). Combined prenatal and postnatal butyl paraben exposure produces autism-like symptoms in offspring: Comparison with valproic acid autistic model. *Pharmacology Biochemistry and Behavior*, *111*, 102–110.

Alimohammadi, S., Hobbenaghi, R., Javanbakht, J., Kheradmand, D., Mortezaee, R., Tavakoli, M., Akbari, H. (2013). Protective and antidiabetic effects of extract from Nigella sativa on blood glucose concentrations against streptozotocin (STZ)-induced diabetic in rats: an experimental study with histopathological evaluation. *Diagnostic Pathology*, *8*, 137-43.

Alonso-Aperte, E., Ubeda, N., Achon, M., Perez-Miguelsanz, J., & Varela-Moreiras, G. (1999). Impaired methionine synthesis and hypomethylation in rats exposed to valproate during gestation. *Neurology*, *52*(4), 750–756.

Alsdorf, R., & Wyszynski, D. F. (2005). Teratogenicity of sodium valproate. *Expert Opinion on Drug Safety*, *4*(2), 345–53.

Anlar, B., Atilla, P., Cakar, N., Tombakoglu, M., & Bulun, A. (2003). Apoptosis in the developing human brain: A preliminary study of the frontal region. *Early Human Development*, *71*(1), 53–60.

Assayed, M. E. (2010). Radioprotective effects of black seed (Nigella sativa) oil against hemopoietic damage and immunosuppression in gamma-irradiated rats. *Immunopharmacology and Immunotoxicology*, *32*(2), 284–96.

Atkinson, D. E., Brice-Bennett, S., & D'Souza, S. W. (2007). Antiepileptic medication during pregnancy: Does fetal genotype affect outcome? *Pediatric Research*, 62(2), 120-127.

Azizi-Malekabadi, H., Pourganji, M., Zabihi, H., Saeedjalali, M., & Hosseini, M. (2015). Tamoxifen antagonizes the effects of ovarian hormones to induce anxiety and depression-like behavior in rats. *Arq Neuropsiquiatr*, *73*(2), 132–139.

Bäckström, T., Zetterlund, B., Blom, S., & Romano, M. (1984). Effects of intravenous progesterone infusions on the epileptic discharge frequency in women with partial epilepsy. *Acta Neurologica Scandinavica*, *69*(4), 240–8.

Bakathir, H. A., & Abbas, N. A. (2011). Detection of the antibacterial effect of Nigella sativa ground seeds with water. *African Journal of Traditional, Complementary, and Alternative Medicines : AJTCAM / African Networks on Ethnomedicines*, *8*(2), 159–64.

Baliga, M. S., Bhat, H. P., Baliga, B. R. V., Wilson, R., & Palatty, P. L. (2011). Phytochemistry, traditional uses and pharmacology of Eugenia jambolana Lam. (black plum): A review. *Food Research International*, *44*(7), 1776–1789.

Bannister, C. M. (2006). Neural Tube Defects – From Origin to Treatment. *Cerebrospinal Fluid Research*, *3*, 6-17.

Bath, K. G., & Scharfman, H. E. (2013). Impact of early life exposure to antiepileptic drugs on neurobehavioral outcomes based on laboratory animal and clinical research. *Epilepsy & Behavior*, *26*(3), 427–439.

Battino, D., Estienne, M., & Avanzini, G. (1995). Clinical Pharmacokinetics of Antiepileptic Drugs in Paediatric Patients: Part I: Phenobarbital, Primidone, Valproic Acid, Ethosuximide and Mesuximide. *Clinical Pharmacokinetics*, 29(4), 257-286.

Batty, N., Malouf, G. G., & Issa, J. P. J. (2009). Histone deacetylase inhibitors as anti-neoplastic agents. *Cancer Letters*, *280*(2), 192–200.

Bea Munoz, M., Diaz Llopis, I., Martinez Agullo, E., Lopez, A., Garcia Aymerich, V., & Forner Valero, J. V. (1994). A multicentre study of the hospital care of 1500 patients with myelomeningocele. *Paraplegia*, *32*(8), 561–564.

Beaudin, A. E., Abarinov, E. V., Noden, D. M., Perry, C. a., Chu, S., Stabler, S. P., Stover, P. J. (2011). Shmt1 and de novo thymidylate biosynthesis underlie folate-responsive neural tube defects in mice. *American Journal of Clinical Nutrition*, *93*(4), 789–798.

Beheshti, F., Hosseini, M., Shafei, M. N., Soukhtanloo, M., Ghasemi, S., Vafaee, F., & Zarepoor, L. (2014). The effects of Nigella sativa extract on hypothyroidism-associated learning and memory impairment during neonatal and juvenile growth in rats. *Nutritional Neuroscience*, *0*(0), 1–11.

Beheshti, F., Hosseini, M., Vafaee, F., Shafei, M. N., & Soukhtanloo, M. (2015). Feeding of Nigella sativa during neonatal and juvenile growth improves learning and memory of rats. *Journal of Traditional and Complementary Medicine*, 1–7.

Beheshti, F., Hosseini, M., Vafaee, F., Shafei, M. N., & Soukhtanloo, M. (2016). Feeding of Nigella sativa during neonatal and juvenile growth improves learning and memory of rats. *Journal of Traditional and Complementary Medicine*, *6*(2), 146–152.

Ben-Ari, Y. (2013). Neuropaediatric and neuroarchaeology: Understanding development to correct brain disorders. *Acta Paediatrica, International Journal of Paediatrics*, *102*(4), 331-334.

Berry, R. J., Li, Z., Erickson, J. D., Li, S., Moore, C. A., Wang, H., Correa, A. (1999). Prevention of Neural-Tube Defects With Folic Acid in China. *New England Journal of Medicine*, *341*(20), 1485–1490.

Boo, N.Y., Cheah, I. G. S., Thong, M.-K., & Registry, M. N. N. (2013). Neural Tube Defects in Malaysia: Data from the Malaysian National Neonatal Registry. *journal of tropical pediatrics*, *59*(5), 338–342.

Bowden, C. L. (2009). Anticonvulsants in bipolar disorders: current research and practice and future directions. *Bipolar Disorders*, *11 Suppl 2*, 20–33.

Bower, C., Eades, S., Payne, J., D'Antoine, H., & Stanley, F. (2004). Trends in neural tube defects in Western Australia in Indigenous and non-Indigenous populations. *Paediatric and Perinatal Epidemiology*, *18*(4), 277–80.

Braun, A. A., Skelton, M. R., Vorhees, C. V., & Williams, M. T. (2011). Comparison of the elevated plus and elevated zero mazes in treated and untreated male Sprague-Dawley rats: Effects of anxiolytic and anxiogenic agents. *Pharmacology Biochemistry and Behavior*, *97*(3), 406–415.

Bulas, D. (2010). Fetal evaluation of spine dysraphism. *Pediatric Radiology*, *40*, 1029–1037.

Burke, B., & Stewart, C. L. (2014). Functional architecture of the cell's nucleus in development, aging, and disease. *Current Topics in Developmental Biology*, *109*, 1–52.

Cameron, M., & Moran, P. (2009). Prenatal screening and diagnosis of neural tube defects. *Prenat Diagn*, *29*(4), 402–411.

Carobrez, A. P., & Bertoglio, L. J. (2005). Ethological and temporal analyses of anxiety-like behavior: The elevated plus-maze model 20 years on. In *Neuroscience and Biobehavioral Reviews* 29, 1193–1205.

Castilla, E. E., Orioli, I. M., Lopez-Camelo, J. S., Dutra, M. D. G., & Nazer-Herrera, J. (2003). Preliminary data on changes in neural tube defect prevalence rates after folic acid fortification in South America. *American Journal of Medical Genetics. Part A*, *123A*(2), 123–128.

CDC MMWR report 2015. Facts about anencephaly. Retrieved 25 August 2017 from http:// www.cdc.gov/ncbddd/birthdefects/anencephaly.html.

Chakraborty, S., Chakraborty, J., Mandal, S., & Ghosal, M. K. (2011). A rare occurrence of isolated neutropenia with valproic acid: A case report. *Journal of the Indian Medical Association*, *109*(5), 345–346.

Chan, E., & McQueen, F. (2013). Valproate-induced hyperammonaemia superimposed upon severe neuropsychiatric lupus: A case report and review of the literature. *Clinical Rheumatology*, 32(3), 403-407.

Chateauvieux, S., Morceau, F., Dicato, M., & Diederich, M. (2010). Molecular and therapeutic potential and toxicity of valproic acid. *Journal of Biomedicine & Biotechnology*, *2010*(3), 1–18.

Chen, P. S., Wang, C. C., Bortner, C. D., Peng, G. S., Wu, X., Pang, H., Hong, J. S. (2007). Valproic acid and other histone deacetylase inhibitors induce microglial apoptosis and attenuate lipopolysaccharide-induced dopaminergic neurotoxicity. *Neuroscience*, *149*(1), 203–212.

Christensen, J., Grønborg, T. K., Sørensen, M. J., Schendel, D., Parner, E. T., Pedersen, L. H., & Vestergaard, M. (2013). Prenatal valproate exposure and risk of autism spectrum disorders and childhood autism. *JAMA*, *309*(16), 1696–703.

Coleman, B. G., Langer, J. E., & Horii, S. C. (2014). The Diagnostic Features of Spina Bifida: The Role of Ultrasound. *Fetal Diagnosis and Therapy*, 37, 179-196.

Copp, A. J. (1994). Genetic models of mammalian neural tube defects. *Ciba Foundation Symposium*, *181*, 118-34-43.

Copp, A. J., & Greene, N. D. E. (2010). Genetics and development of neural tube defects. *The Journal of Pathology*, *220*(2), 217–30.

Copp, A. J., Greene, N. D. E., & Murdoch, J. N. (2003). The genetic basis of mammalian neurulation. *Nature Reviews. Genetics*, *4*(10), 784–93.

Copp, A. J., Stanier, P., & Greene, N. DE. (2013). Neural tube defects: recent advances, unsolved questions, and controversies. *The Lancet Neurology*, *12*(8), 799–810.

Copp, a J., Brook, F. a, & Roberts, H. J. (1988). A cell-type-specific abnormality of cell proliferation in mutant (curly tail) mouse embryos developing spinal neural tube defects. *Development (Cambridge, England)*, *104*, 285–295.

Correia-Pinto, J., Reis, J. L., Hutchins, G. M., Baptista, M. J., Estev??o-Costa, J., Flake, A. W., & Leite-Moreira, A. F. (2002). In utero meconium exposure increases spinal cord necrosis in a rat model of myelomeningocele. In *Journal of Pediatric Surgery*, 37, 488–492.

Costanzo, R., Watterson, R. L., & Schoenwolf, G. C. (1982). Evidence that secondary neurulation occurs autonomously in the chick embryo. *Journal of Experimental Zoology*, *219*(2), 233–240.

Crawley, J. N. (1999). Behavioral phenotyping of transgenic and knockout mice: Experimental design and evaluation of general health, sensory functions, motor abilities, and specific behavioral tests. In *Brain Research*, 835, 18–26.

Cummings, C., Stewart, M., Stevenson, M., Morrow, J., & Nelson, J. (2011). Neurodevelopment of children exposed in utero to lamotrigine, sodium valproate and carbamazepine. *Archives of Disease in Childhood*, *96*(7), 643–7.

Cutler, A. R., Wilkerson, A. E., Gingras, J. L., & Levin, E. D. (1996). Prenatal cocaine and/or nicotine exposure in rats: Preliminary findings on long-term

cognitive outcome and genital development at birth. *Neurotoxicology and Teratology*, *18*(6), 635–643.

Czeizel, A. E., & Dudas, I. (1992). Prevention of the first occurrence of neural-tube defects by periconceptional vitamin supplementation. *N Engl J Med*, *327*(26), 1832–1835.

Daniels, T., Gallagher, M., Tremblay, G., & Rodgers, R. L. (2004). Effects of valproic acid on cardiac metabolism. *Can.J.Physiol Pharmacol.*, *82*(0008–4212 (Print)), 927–933.

Date, H., Ito, C., & Numata, O. (2005). Management of myelomeningocele. *Japanese Journal of Neurosurgery*, 14(7) 442-448.

Davidson, L. a, & Keller, R. E. (1999). Neural tube closure in Xenopus laevis involves medial migration, directed protrusive activity, cell intercalation and convergent extension. *Development (Cambridge, England)*, *126*(20), 4547–4556.

De Regil, L. M., Fernández-Gaxiola, A. C., Dowswell, T., & Peña-Rosas, J. P. (2009). Effects and safety of periconceptional folate supplementation for preventing birth defects. *Cochrane Database of Systematic Reviews*, (3), 10–12.

Dehkordi, F. R., & Kamkhah, A. F. (2008). Antihypertensive effect of Nigella sativa seed extract in patients with mild hypertension. *Fundamental & Clinical Pharmacology*, *22*(4), 447–52.

Detrait, E. R., George, T. M., Etchevers, H. C., Gilbert, J. R., Vekemans, M., & Speer, M. C. (2005). Human neural tube defects: Developmental biology, epidemiology, and genetics. In *Neurotoxicology and Teratology* 27, 515–524.

Dhillon, N., & Högler, W. (2011). Fractures and Fanconi syndrome due to prolonged sodium valproate use. *Neuropediatrics*, *42*(3), 119–121.

Di Renzo, F., Broccia, M. L., Giavini, E., & Menegola, E. (2007). Relationship between embryonic histonic hyperacetylation and axial skeletal defects in mouse exposed to the three HDAC inhibitors apicidin, MS-275, and sodium butyrate. *Toxicological Sciences*, *98*(2), 582–588.

Di Renzo, F., Broccia, M. L., Giavini, E., & Menegola, E. (2010). VPA-related axial skeletal defects and apoptosis: A proposed event cascade. *Reproductive Toxicology*, *29*, 106–112.

Di renzo, F., Cappelletti, G., Broccia, M. L., Giavini, E., & Menegola, E. (2008). The inhibition of embryonic histone deacetylases as the possible mechanism accounting for axial skeletal malformations induced by sodium salicylate. *Toxicological Sciences*, *104*(2), 397–404.

Dicianno, B. E., Kurowski, B. G., Yang, J. M. J., Chancellor, M. B., Bejjani, G. K., Fairman, A. D., … Sotirake, J. (2008). Rehabilitation and medical management

of the adult with spina bifida. *American Journal of Physical Medicine & Rehabilitation / Association of Academic Physiatrists*, *87*(12), 1027–1050.

Dicianno, B. E., & Wilson, R. (2010). Hospitalizations of Adults With Spina Bifida and Congenital Spinal Cord Anomalies. *Archives of Physical Medicine and Rehabilitation*, *91*(4), 529–535.

Dinopoulos, A., Karapanou, O., Alexopoulou, E., Tzetis, M., Attilakos, A., & Fretzayas, A. (2011). VPA-induced recurrent pancreatitis in a cystic fibrosis carrier. *European Journal of Paediatric Neurology*, *15*(5), 453–455.

Dufour-Rainfray, D., Vourc'h, P., Le Guisquet, A. M., Garreau, L., Ternant, D., Bodard, S., Guilloteau, D. (2010). Behavior and serotonergic disorders in rats exposed prenatally to valproate: A model for autism. *Neuroscience Letters*, *470*(1), 55–59.

Dugoua, J. J. (2010). Herbal medicines and pregnancy. *Journal of Population Therapeutics and Clinical Pharmacology*, *17*(3), 370-378.

Edalatmanesh, M. A., Nikfarjam, H., Vafaee, F., & Moghadas, M. (2013). Increased hippocampal cell density and enhanced spatial memory in the valproic acid rat model of autism. *Brain Research*, *1526*, 15–25.

Eikel, D., Lampen, A., & Nau, H. (2006). Teratogenic effects mediated by inhibition of histone deacetylases: Evidence from quantitative structure activity relationships of 20 valproic acid derivatives. *Chemical Research in Toxicology*, *19*(2), 272–278.

Eikel, D., Lampen, A., & Nau, H. (2015). Teratogenic effects mediated by inhibition of histone deacetylases: Evidence from quantitative structure activity relationships of 20 valproic acid derivatives. *Chemical Research in Toxicology*, *19*(2), 19–20.

Elia, M. C., & Bradley, M. O. (1992). Influence of chromatin structure on the induction of DNA double strand breaks by ionizing radiation. *Cancer Research*, *52*(6), 1580–6.

Ellis, L., Hammers, H., & Pili, R. (2009). Targeting tumor angiogenesis with histone deacetylase inhibitors. *Cancer Letters*, *280*(2), 145–153.

Elmslie, J. L., Porter, R. J., Joyce, P. R., Hunt, P. J., Shand, B. I., & Scott, R. S. (2009). Comparison of insulin resistance, metabolic syndrome and adiponectin in overweight bipolar patients taking sodium valproate and controls. *The Australian and New Zealand Journal of Psychiatry*, *43*(1), 53–60.

Elul, T., Koehl, M. a, & Keller, R. (1997). Cellular mechanism underlying neural convergent extension in Xenopus laevis embryos. *Developmental Biology*, *191*(2), 243–258.

Endo, A., Fujita, Y., Fuchigami, T., Takahashi, S., & Mugishima, H. (2010). Fanconi Syndrome Caused by Valproic Acid. *Pediatric Neurology*, *42*(4), 287–290.

Ennaceur, A., & Delacour, J. (1988). A new one - trial test for neurobiological studies of memory in rats . 1 " Behavioral data. *Behavioural Brain Research*, *31*, 47–59.

Evans, M. D., Shinar, R., & Yaari, R. (2011). Reversible dementia and gait disturbance after prolonged use of valproic acid. *Seizure*, *20*(6), 509–511.

Evans, M. I., Adzick, N. S., Johnson, M. P., Flake, A. W., Quintero, R., & Harrison, M. R. (1994). Fetal therapy-1994. *Current Opinion in Obstetrics and Gynecology*, *6*(1), 58–64.

Falkenberg, K. J., & Johnstone, R. W. (2014). Histone deacetylases and their inhibitors in cancer, neurological diseases and immune disorders. *Nature Reviews. Drug Discovery*, *13*(9), 673–91.

Feldkamp, M. L., Carey, J. C., & Sadler, T. W. (2007). Development of gastroschisis: Review of hypotheses, a novel hypothesis, and implications for research. *American Journal of Medical Genetics*, 13(9), 673-91.

Finnell, R. H., Gould, A., & Spiegelstein, O. (2003). Pathobiology and genetics of neural tube defects. *Epilepsia*, *44 Suppl 3*, 14–23.

Fisher, R. S., Acevedo, C., Arzimanoglou, A., Bogacz, A., Cross, J. H., Elger, C. E., Wiebe, S. (2014). ILAE Official Report: A practical clinical definition of epilepsy. *Epilepsia*, *55*(4), 475–482.

Fornace, A. J., Alamo, I., & Hollander, M. C. (1988). DNA damage-inducible transcripts in mammalian cells. *Proceedings of the National Academy of Sciences of the United States of America*, *85*(23), 8800–4.

Fox, J. T., & Stover, P. J. (2008). Folate-mediated one-carbon metabolism. *Vitamins and Hormones*, *79*(8), 1–44.

Frisch, C., Hüsch, K., Angenstein, F., Kudin, A., Kunz, W., Elger, C. E., & Helmstaedter, C. (2009). Dose-dependent memory effects and cerebral volume changes after in utero exposure to valproate in the rat. *Epilepsia*, *50*(6), 1432–1441.

Furnari, M. A., Saw, C. L.-L., Kong, A.-N., & Wagner, G. C. (2014). Altered behavioral development in Nrf2 knockout mice following early postnatal exposure to valproic acid. *Brain Research Bulletin*, *109*, 132–42.

Furukawa, S., Hayashi, S., Usuda, K., Abe, M., Hagio, S., & Ogawa, I. (2011). Toxicological pathology in the rat placenta. *Journal of Toxicologic Pathology*, *24*(2), 95–111.

Furukawa, S., Usuda, K., Abe, M., Hayashi, S., & Ogawa, I. (2007). Busulfan-induced apoptosis in rat placenta. *Experimental and Toxicologic Pathology*, *59*(2), 97–103.

Gabory, A., Attig, L., & Junien, C. (2011). Epigenetic mechanisms involved in developmental nutritional programming. *World Journal of Diabetes*, *2*(10), 164-175.

Gao, J., Wang, X., Sun, H., Cao, Y., Liang, S., Wang, H., Wu, L. (2016a). Neuroprotective effects of docosahexaenoic acid on hippocampal cell death and learning and memory impairments in a valproic acid-induced rat autism model. *International Journal of Developmental Neuroscience*, *49*, 67–78.

Gao, J., Wu, H., Cao, Y., Liang, S., Sun, C., Wang. (2016b). Maternal DHA supplementation protects rat offspring against impairment of learning and memory following prenatal exposure to valproic acid. *The Journal of Nutritional Biochemistry*, *35*, 87–95.

Gaspari, C. N. De, & Guerreiro, C. M. (2010). Modification in body weight associated with antiepileptic drugs. *Arquivos de Neuro-Psiquiatria*, *68*(2), 277–281.

Gatto, L., Vizcaíno, J. A., Hermjakob, H., Huber, W., & Lilley, K. S. (2010). Organelle proteomics experimental designs and analysis. *Proteomics*,10 (22), 3957-3969.

Geelen, J. A., & Langman, J. (1979). Ultrastructural observations on closure of the neural tube in the mouse. *Anatomy and Embryology*, *156*(1), 73–88.

Gerstner, T., Teich, M., Bell, N., Longin, E., Dempfle, C. E., Brand, J., & König, S. (2006). Valproate-associated coagulopathies are frequent and variable in children. *Epilepsia*, *47*(7), 1136–1143.

Gilhotra, N., & Dhingra, D. (2011a). Thymoquinone produced antianxiety-like effects in mice through modulation of GABA and NO levels. *Pharmacological Reports*, *63*(3), 660–669.

Gokhin, D. S., Ward, S. R., Bremner, S. N., & Lieber, R. L. (2008). Quantitative analysis of neonatal skeletal muscle functional improvement in the mouse. *The Journal of Experimental Biology*, *211*(6), 837–43.

Gotfryd, K., Hansen, M., Kawa, A., Ellerbeck, U., Nau, H., Berezin, V., Walmod, P. S. (2011). The teratogenic potencies of valproic acid derivatives and their effects on biological end-points are related to changes in histone deacetylase and Erk1/2 activities. *Basic and Clinical Pharmacology and Toxicology*, *109*(3), 164–174.

Gottlicher, M., Minucci, S., Zhu, P., Kr??mer, O. H., Schimpf, A., Giavara, S., Heinzel, T. (2002). Valproic acid defines a novel class of HDAC inhibitors inducing differentiation of transformed cells. *EMBO Journal*, *20*(24), 6969–

6978.

Greene, N. D., & Copp, A. J. (2005). Mouse models of neural tube defects: investigating preventive mechanisms. *Am J Med Genet C Semin Med Genet*, *135*(1), 31–41.

Greene, N. D. E., & Copp, A. J. (2009). Development of the vertebrate central nervous system: Formation of the neural tube. *Prenatal Diagnosis*, 29(4) 303-311.

Grewal, J., Carmichael, S. L., Song, J., & Shaw, G. M. (2009). Neural tube defects: an analysis of neighbourhood- and individual-level socio-economic characteristics. *Paediatric and Perinatal Epidemiology*, *23*(2), 116–24.

Grosse, S. D., Ouyang, L., Collins, J. S., Green, D., Dean, J. H., & Stevenson, R. E. (2008). Economic evaluation of a neural tube defect recurrence-prevention program. *American Journal of Preventive Medicine*, *35*(6), 572–7.

Grosso, S., Mostardini, R., Piccini, B., & Balestri, P. (2009). Body mass index and serum lipid changes during treatment with valproic acid in children with epilepsy. *The Annals of Pharmacotherapy*, *43*(1), 45–50.

Guérin, P., El Mouatassim, S., & Ménézo, Y. (2001). Oxidative stress and protection against reactive oxygen species in the pre-implantation embryo and its surroundings. *Human Reproduction Update,* 7(2) 175-189.

Gustavsson, P., Greene, N. D. E., Lad, D., Pauws, E., de Castro, S. C. P., Stanier, P., & Copp, A. J. (2007). Increased expression of Grainyhead-like-3 rescues spina bifida in a folate-resistant mouse model. *Human Molecular Genetics*, *16*(21), 2640–2646.

Halder, P. P., Giri, P. P., & Ghosh, A. (2013). Fetal valproate syndrome. *Sri Lanka Journalof Child Health*, *42*(2), 97–98.

Hamdy, N. M., & Taha, R. A. (2009). Effects of Nigella sativa oil and thymoquinone on oxidative stress and neuropathy in streptozotocin-induced diabetic rats. *Pharmacology*, *84*(3), 127–34.

Harden, C. L., Pennell, P. B., Koppel, B. S., Hovinga, C. A., Gidal, B., Meador, K. J., Le Guen, C. L. (2009). Management issues for women with epilepsy--focus on pregnancy (an evidence-based review): III. Vitamin K, folic acid, blood levels, and breast-feeding: Report of the Quality Standards Subcommittee and Therapeutics and Technology Assessment Subcommittee of. *Epilepsia*, *50*(5), 1247–55.

Harden, C. L., & Sethi, N. K. (2008). Epileptic disorders in pregnancy: an overview. *Current Opinion in Obstetrics & Gynecology*, *20*(6), 557–62.

Harris, M. J., & Juriloff, D. M. (2007). Mouse mutants with neural tube closure defects and their role in understanding human neural tube defects. *Birth Defects*

Research Part A - Clinical and Molecular Teratology, *79*(3), 187–210.

Haseena, S., Aithal, M., Das, K. K., & Saheb, S. H. (2015). Effect of nigella sativa seed powder on testosterone and LH levels in sterptozotocine induced diabetes male albino rats. *Journal of Pharmaceutical Sciences and Research*, *7*(4), 234–237.

Hasnah Ibrahim, & Rahman, A. F. A. (2008). Pharmacokinetic parameters of valproic acid and carbamazepine from routinely collected data: Influence of patient characteristics, 33-42.

Hay, W. W. (1991). The placenta: Not just a conduit for maternal fuels. In *Diabetes*, 40, 44–50.

Hernandez-Diaz, S., Smith, C. R., Shen, A., Mittendorf, R., Hauser, W. A., Yerby, M.. (2012). Comparative safety of antiepileptic drugs during pregnancy. *Neurology*, *78*(21), 1692–1699.

Herzog, A G. (1991). Reproductive endocrine considerations and hormonal therapy for women with epilepsy. *Epilepsia*, 32 (6), 27–33.

Hien, V. T. T., Lam, N. T., Khan, N. C., Dung, N. T., Skeaff, C. M., Venn, B. J., Green, T. J. (2008). Folate and vitamin B12 status of women of reproductive age living in Hanoi City and Hai Duong Province of Vietnam. *Public Health Nutrition*, *12*(7), 941-6.

Hildesheim, J., & Fornace, A. J. (2002). Gadd45a: An elusive yet attractive candidate gene in pancreatic cancer. *Clinical Cancer Research*, *8*(8), 2475–2479.

Hill, D. S., Wlodarczyk, B. J., Palacios, A. M., & Finnell, R. H. (2010). Teratogenic effects of antiepileptic drugs. *Expert Review of Neurotherapeutics*, *10*(6), 943–59.

Hollander, M. C., Sheikh, M. S., Bulavin, D. V, Lundgren, K., Augeri-Henmueller, L., Shehee, R., Fornace, A. J. (1999). Genomic instability in Gadd45a-deficient mice. *Nature Genetics*, *23*(2), 176–84.

Holst, L., Nordeng, H., & Haavik, S. (2008). Use of herbal drugs during early pregnancy in relation to maternal characteristics and pregnancy outcome. *Pharmacoepidemiology and Drug Safety*, *17*(2), 151–159.

Horn, A., Dix-Peek, S., Mears, S., & Hoffman, E. B. (2014). The orthopaedic management of myelomeningocele. *South African Medical Journal*, *104*(4), 314-317.

Hossain, M. M., Nakayama, H., & Goto, N. (1995). Apoptosis in the central nervous system of developing mouse fetuses from 5-azacytidine-administered dams. *Toxicologic Pathology*, *23*(3), 367–372.

Hosseini, M., Mohammadpour, T., Karami, R., Rajaei, Z., Reza Sadeghnia, H., & Soukhtanloo, M. (2014). Effects of the hydro-alcoholic extract of Nigella sativa on scopolamine-induced spatial memory impairment in rats and its possible mechanism. *Chinese Journal of Integrative Medicine*, *21*(6), 438–444.

Hosseinzadeh, H., Parvardeh, S., Asl, M. N., Sadeghnia, H. R., & Ziaee, T. (2007). Effect of thymoquinone and Nigella sativa seeds oil on lipid peroxidation level during global cerebral ischemia-reperfusion injury in rat hippocampus. *Phytomedicine :International Journal of Phytotherapy and Phytopharmacology*, *14*(9), 621–7.

Hrubec, T. C., Prater, M. R., Toops, K. A., & Holladay, S. D. (2006). Reduction in diabetes-induced craniofacial defects by maternal immune stimulation. *Birth Defects Research Part B - Developmental and Reproductive Toxicology*, *77*(1), 1–9.

Hsiao, E. Y., & Patterson, P. H. (2012). Placental regulation of maternal-fetal interactions and brain development. *Developmental Neurobiology*, *72*(10), 1317–1326.

Hsieh, J., Nakashima, K., Kuwabara, T., Mejia, E., & Gage, F. H. (2004). Histone deacetylase inhibition-mediated neuronal differentiation of multipotent adult neural progenitor cells. *Proc Natl Acad Sci U S A*, *101*(47), 16659–16664.

Ibrahim, R. M., Hamdan, N. S., Ismail, M., Saini, S. M., Rashid, S. N. A., Latiff, L. A., & Mahmud, R. (2014). Protective effects of Nigella sativa on metabolic syndrome in menopausal women. *Advanced Pharmaceutical Bulletin*, *4*(1), 29–33.

Ijiri, K., Zerbini, L. F., Peng, H., Correa, R. G., Lu, B., Walsh, N., Goldring, M. B. (2005). A novel role for GADD45beta as a mediator of MMP-13 gene expression during chondrocyte terminal differentiation. *The Journal of Biological Chemistry*, *280*(46), 38544–55.

Imam, A., Ajao, M. S., Ajibola, M. I., Amin, A., Abdulmajeed, W. I., Lawal, A. Z., Adana, M. Y. (2016). Black seed oil ameliorated scopolamine-induced memory dysfunction and cortico-hippocampal neural alterations in male Wistar rats. *Bulletin of Faculty of Pharmacy, Cairo University,* 54(1), 49-57.

Ingram, J. L., Peckham, S. M., Tisdale, B., & Rodier, P. M. (2000). Prenatal exposure of rats to valproic acid reproduces the cerebellar anomalies associated with autism. *Neurotoxicol.Teratol.*, *22*(0892–0362 (Print)), 319–324.

Jackman, A., Alamo, A., & Fornace, F. (1994). Genotoxic Stress Confers Preferential and Coordinate Messenger RNA Stability on the Five gadd Genes. *Cancer Research*, 54(21), 5656–5662.

Jahromi, S. R., Togha, M., Fesharaki, S. H., Najafi, M., Moghadam, N. B., Kheradmand, J. A., Gorji, A. (2011). Gastrointestinal adverse effects of antiepileptic drugs in intractable epileptic patients. *Seizure*, *20*(4), 343–346.

Jena, N. R. (2012). DNA damage by reactive species: Mechanisms, mutation and repair. *Journal of Biosciences*, *37*(3), 503–17.

Johannessen, C. U. (2000). Mechanisms of action of valproate: a commentatory. *Neurochemistry International*, *37*(2–3), 103–10.

Johnston, D. (1984). Valproic Acid: Update on Its Mechanisms of Action. *Epilepsia*, *25*(1), 1–4.

Kaczorowska-Hac, B., Matheisel, A., Maciejka-Kapuscinska, L., Wisniewski, J., Alska, A., Adamkiewicz-Drozynska, E., Reszczynska, I. (2012). Anemia secondary to valproic acid therapy in a 13-year-old boy: a case report. *Journal of Medical Case Reports*, *6*(1), 239.

Kalda, A., Heidmets, L. T., Shen, H. Y., Zharkovsky, A., & Chen, J. F. (2007). Histone deacetylase inhibitors modulates the induction and expression of amphetamine-induced behavioral sensitization partially through an associated learning of the environment in mice. *Behavioural Brain Research*, *181*(1), 76–84.

Kalhan, S. C., & Marczewski, S. E. (2012). Methionine, homocysteine, one carbon metabolism and fetal growth. *Reviews in Endocrine and Metabolic Disorders*, 13 (2), 109-119.

Kanter, M., Coskun, O., & Budancamanak, M. (2005). Hepatoprotective effects of Nigella sativa L and Urtica dioica L on lipid peroxidation, antioxidant enzyme systems and liver enzymes in carbon tetrachloride-treated rats. *World Journal of Gastroenterology*, *11*(42), 6684–6688.

Kanter, M., Coskun, O., Kalayci, M., Buyukbas, S., & Cagavi, F. (2006a). Neuroprotective effects of Nigella sativa on experimental spinal cord injury in rats. *Hum Exp Toxicol*, *25*(3), 127–133.

Kanter, M., Coskun, O., & Uysal, H. (2006b). The antioxidative and antihistaminic effect of Nigella sativa and its major constituent, thymoquinone on ethanol-induced gastric mucosal damage. *Archives of Toxicology*, *80*(4), 217–224.

Kataoka, S., Takuma, K., Hara, Y., Maeda, Y., Ago, Y., & Matsuda, T. (2011). Autism-like behaviours with transient histone hyperacetylation in mice treated prenatally with valproic acid. *The International Journal of Neuropsychopharmacology*, 16 (1) 91–103.

Kazlauskas, N., Campolongo, M., Lucchina, L., Zappala, C., & Depino, A. M. (2016). Postnatal behavioral and inflammatory alterations in female pups prenatally exposed to valproic acid. *Psychoneuroendocrinology*, *72*, 11–21.

Keller, R., Shook, D., & Skoglund, P. (2008). The forces that shape embryos: physical aspects of convergent extension by cell intercalation. *Physical Biology*, *5*(1), 150-7.

Keyhanmanesh, R., Bagban, H., Nazemiyeh, H., Mirzaei Bavil, F., Alipour, M. R., & Ahmady, M. (2013). The Relaxant Effects of Different Methanolic Fractions of Nigella sativa on Guinea Pig Tracheal Chains. *Iran J Basic Med Sci*, *16*(2), 123–128.

Khan, A., Chen, H.-C., Tania, M., & Zhang, D.-Z. (2011). Anticancer Activities of *Nigella sativa* (Black Cumin). *African Journal of Traditional, Complementary and Alternative Medicines*, *8*(5S), 226–32.

Khan, M. A. U., Ashfaq, M. K., Zuberi, H. S., Mahmood, M. S., & Gilani, A. H. (2003). The in vivo antifungal activity of the aqueous extract from Nigella sativa seeds. *Phytotherapy Research* , *17*(2), 183–6.

Khor, G. L., Duraisamy, G., Loh, S. P., & Green, T. (2006). Dietary and blood folate status of Malaysian women of childbearing age. *Asia Pacific Journal of Clinical Nutrition*, *15*(3), 341–9.

Kim, K. C., Kim, P., Go, H. S., Choi, C. S., Park, J. H., Kim, H. J., Shin, C. Y. (2013). Male-specific alteration in excitatory post-synaptic development and social interaction in pre-natal valproic acid exposure model of autism spectrum disorder. *Journal of Neurochemistry*, *124*(6), 832–843.

Kimmel, C. B., Warga, R. M., & Kane, D. A. (1994). Cell cycles and clonal strings during formation of the zebrafish central nervous system. *Development {(Cambridge,} England)*, *120*(2), 265–276.

Knickmeyer, R. C., Gouttard, S., Kang, C., Evans, D., Wilber, K., Smith, J. K., Gilmore, J. H. (2008). A structural MRI study of human brain development from birth to 2 years. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *28*(47), 12176–82.

Koka, P. S., Mondal, D., Schultz, M., Abdel-Mageed, A. B., & Agrawal, K. C. (2010). Studies on molecular mechanisms of growth inhibitory effects of thymoquinone against prostate cancer cells: role of reactive oxygen species. *Experimental Biology and Medicine (Maywood, N.J.)*, *235*(6), 751–760.

Kotch, L. E., & Sulik, K. K. (1992). Patterns of ethanol-induced cell death in the developing nervous system of mice; neural fold states through the time of anterior neural tube closure. *International Journal of Developmental Neuroscience*, *10*, 273–279.

Kozma, C. (2001). Valproic acid embryopathy: report of two siblings with further expansion of the phenotypic abnormalities and a review of the literature. *American Journal of Medical Genetics*, *98*(2), 168–75.

Langen, M., Leemans, A., Johnston, P., Ecker, C., Daly, E., Murphy, C. M., Murphy, D. G. M. (2012). Fronto-striatal circuitry and inhibitory control in autism: Findings from diffusion tensor imaging tractography. *Cortex*, *48*(2), 183–193.

Levi, N., Bastuji-Garin, S., Mockenhaupt, M., Roujeau, J.-C., Flahault, A., Kelly, J. P., Maison, P. (2009). Medications as risk factors of Stevens-Johnson syndrome and toxic epidermal necrolysis in children: a pooled analysis. *Pediatrics*, *123*(2), 297-304.

Li, Z., Ren, A., Zhang, L., Ye, R., Li, S., Zheng, J., Li, Z. (2006). Extremely high prevalence of neural tube defects in a 4-county area in Shanxi Province, China. *Birth Defects Research. Part A, Clinical and Molecular Teratology*, *76*(4), 237–40.

Lin, H. C., Gean, P. W., Wang, C. C., Chan, Y. H., & Chen, P. S. (2013). The Amygdala Excitatory/Inhibitory Balance in a Valproate-Induced Rat Autism Model. *PLoS ONE*, *8*(1), 1-8.

Liu, A., & Niswander, L. A. (2005). Bone morphogenetic protein signalling and vertebrate nervous system development. *Nature Reviews. Neuroscience*, *6*(12), 945–54.

Liu, J., Zhang, L., Li, Z., Jin, L., Zhang, Y., Ye, R., Ren, A. (2016). Prevalence and trend of neural tube defects in five counties in Shanxi province of Northern China, 2000 to 2014. *Birth Defects Research Part A: Clinical and Molecular Teratology*, *106*(4), 267–274.

Loeken, M. R. (2006). Advances in understanding the molecular causes of diabetes-induced birth defects. *Journal of the Society for Gynecologic Investigation, 13*(1) 2-10.

Loft, A. G. (1995). Immunochemical determination of amniotic fluid acetylcholinesterase in the antenatal diagnosis of open neural tube defects. *Danish Medical Bulletin*, *42*(1), 54–70.

Loscher, W. (2002). Basic pharmacology of valproate: a review after 35 years of clinical use for the treatment of epilepsy. *CNS Drugs*, *16*(10), 669–694.

Lucas, A. (1994). Role of nutritional programming in determining adult morbidity. *Archives of Disease in Childhood*, *71*(4), 288–90.

Lupo, P. J., Canfield, M. a, Chapa, C., Lu, W., Agopian, a J., Mitchell, L. E., Zhu, H. (2012). Diabetes and obesity-related genes and the risk of neural tube defects in the national birth defects prevention study. *American Journal of Epidemiology*, *176*(12), 1101–9.

Lynch, S. A. (2005). Non-multifactorial neural tube defects. *American Journal of Medical Genetics. Part C, Seminars in Medical Genetics*, *135C*(1), 69–76.

Lyon, L., Saksida, L. M., & Bussey, T. J. (2012). Spontaneous object recognition and its relevance to schizophrenia: A review of findings from pharmacological, genetic, lesion and developmental rodent models. *Psychopharmacology 220*(4), 647-672.

Mabrouk, A., & Cheikh, H. Ben. (2014). Thymoquinone supplementation ameliorates lead-induced testis function impairment in adult rats. *Toxicology and Industrial Health 32*(6) 1114-21.

Machado, I. N., Martinez, S. D., & Barini, R. (2012). Anencephaly: Do the Pregnancy and Maternal Characteristics Impact the Pregnancy Outcome? *ISRN Obstetrics and Gynecology*, *2012*, 1–5.

Mallela, M., & Hrubec, T. (2012). Reduction in Valproic Acid-Induced Neural Tube Defects by Maternal Immune Stimulation: Role of Apoptosis. *Birth Defects Research Part B - Developmental and Reproductive Toxicology*, *95*(4), 296–303.

Markram, K., Rinaldi, T., La Mendola, D., Sandi, C., & Markram, H. (2008). Abnormal fear conditioning and amygdala processing in an animal model of autism. *Neuropsychopharmacology*, *33*(4), 901–12.

Marselli, L., Thorne, J., Ahn, Y.-B., Omer, A., Sgroi, D. C., Libermann, T., Weir, G. C. (2008). Gene expression of purified beta-cell tissue obtained from human pancreas with laser capture microdissection. *The Journal of Clinical Endocrinology and Metabolism*, *93*(3), 1046–53.

Masuccio, F., Verrotti, A., Chiavaroli, V., de Giorgis, T., Giannini, C., Chiarelli, F., & Mohn, A. (2010). Weight gain and insulin resistance in children treated with valproate: the influence of time. *Journal of Child Neurology*, *25*, 941–947.

McLean, M. J., & Macdonald, R. L. (1986). Sodium valproate, but not ethosuximide, produces use- and voltage-dependent limitation of high frequency repetitive firing of action potentials of mouse central neurons in cell culture. *The Journal of Pharmacology and Experimental Therapeutics*, *237*, 1001–1011.

Meador, K. J., Baker, G. A., Browning, N., Clayton-Smith, J., Combs-Cantrell, D. T., Cohen, M., Loring, D. W. (2009). Cognitive function at 3 years of age after fetal exposure to antiepileptic drugs. *The New England Journal of Medicine*, *360*(16), 1597–605.

Meador, K. J., Baker, G. A., Browning, N., Cohen, M. J., Clayton-Smith, J., Kalayjian, L. A., Loring, D. W. (2011). Foetal antiepileptic drug exposure and verbal versus non-verbal abilities at three years of age. *Brain*, *134*(2), 396–404.

Meador, K., Reynolds, M. W., Crean, S., Fahrbach, K., & Probst, C. (2008). Pregnancy outcomes in women with epilepsy: a systematic review and meta-analysis of published pregnancy registries and cohorts. *Epilepsy Res*, *81*(1), 1–13.

Medhat, A., Wassim, H. A., & Hamilton Suzanne. (2014). A Diagnosed Case of Anencephaly and Severe Neural Tube Defect at 30 Weeks , Antenatal and Intrapartum Aspects. *Obstetrics & Gynecology Int J*, *1*(3), 2–3.

Mehta, M. V., Gandal, M. J., & Siegel, S. J. (2011). mGluR5-antagonist mediated reversal of elevated stereotyped, repetitive behaviors in the VPA model of autism. *PLoS ONE*, *6*(10) 1-6.

Menegola, E., Di Renzo, F., Broccia, M. L., & Giavini, E. (2006). Inhibition of histone deacetylase as a new mechanism of teratogenesis. *Birth Defects Res C Embryo Today*, *78*(4), 345–353.

Menegola, E., Di Renzo, F., Broccia, M. L., Prudenziati, M., Minucci, S., Massa, V., & Giavini, E. (2005). Inhibition of histone deacetylase activity on specific embryonic tissues as a new mechanism for teratogenicity. *Birth Defects Research Part B - Developmental and Reproductive Toxicology*, *74*(5), 392–398.

Mikaelsson, M. A., Constância, M., Dent, C. L., Wilkinson, L. S., & Humby, T. (2013). Placental programming of anxiety in adulthood revealed by Igf2-null models. *Nature Communications*, *4*(May), 2311-2319.

Mitchell, L. E., Scott Adzick, N., Melchionne, J., Pasquariello, P. S., Sutton, L. N., & Whitehead, A. S. (2004). Spina bifida. *Lancet*, *364*(9448), 1885–1895.

Moldrich, R. X., Leanage, G., She, D., Dolan-Evans, E., Nelson, M., Reza, N., & Reutens, D. C. (2013). Inhibition of histone deacetylase in utero causes sociability deficits in postnatal mice. *Behavioural Brain Research*, *257*, 253–264.

Monti, B., Polazzi, E., & Contestabile, A. (2009). Biochemical, molecular and epigenetic mechanisms of valproic acid neuroprotection. *Current Molecular Pharmacology*, *2*(1), 95–109.

Moore, K. L., Persaud, T. V. N., & Torchia, M. G. (2004). The Developing Human: Clinically Oriented Embryology. *Clinically oriented embryology 2004*, 16-22.

Moore, S. J., Turnpenny, P. D., Quinn, A., Glover, S., Lloyd, D. J., Montgomery, T., & Dean, J. C. S. (2000). A clinical study of 57 children with fetal anticonvulsant syndromes. *Journal of Medical Genetics*, *37*(7), 489–497.

Motz, B. A., & Alberts, J. R. (2005). The validity and utility of geotaxis in young rodents. *Neurotoxicology and Teratology, 27*(4) 529-533.

Mowery, T. M., Wilson, S. M., Kostylev, P. V., Dina, B., Buchholz, J. B., Prieto, A. L., & Garraghty, P. E. (2015). Embryological exposure to valproic acid disrupts morphology of the deep cerebellar nuclei in a sexually dimorphic way. *International Journal of Developmental Neuroscience*, *40*, 15–23.

Mukhallad, A. M., Mohamad, M. M., Hatham, D., Mohammad, M., Mohamad, M. M., & Dradka, H. (2009). Effects of black seeds (Nigella Sativa) on spermatogenesis and fertility of male albino rats. *Research Journal of Medicine & Medical Sciences*, *4*(2), 386–390.

Murko, C., Lagger, S., Steiner, M., Seiser, C., Schoefer, C., & Pusch, O. (2013). Histone deacetylase inhibitor Trichostatin A induces neural tube defects and promotes neural crest specification in the chicken neural tube. *Differentiation*, *85*(1–2), 55–66.

Muskett, A., Barber, W. H., Parent, A. D., & Angel, M. F. (2012). Contemporary postnatal plastic surgical management of meningomyelocele. *Journal of Plastic, Reconstructive and Aesthetic Surgery*, *65*(5) 572-577.

Nadebaum, C., Anderson, V., Vajda, F., Reutens, D., Barton, S., & Wood, A. (2011). The Australian brain and cognition and antiepileptic drugs study: IQ in school-aged children exposed to sodium valproate and polytherapy. *Journal of the International Neuropsychological Society : JINS*, *17*(1), 133–142.

Nalivaeva, N. N., Belyaev, N. D., & Turner, A. J. (2009). Sodium valproate: an old drug with new roles. *Trends in Pharmacological Sciences*, *30*(10), 509–514.

Namazi, N., Mahdavi, R., Alizadeh, M., & Farajnia, S. (2015). Oxidative Stress Responses to Nigella sativa Oil Concurrent with a Low-Calorie Diet in Obese Women: A Randomized, Double-Blind Controlled Clinical Trial. *Phytotherapy Research : PTR*, *29*(11), 1722–8.

Narisawa, A., Komatsuzaki, S., Kikuchi, A., Niihori, T., Aoki, Y., Fujiwara, K., … Kure, S. (2012). Mutations in genes encoding the glycine cleavage system predispose to neural tube defects in mice and humans. *Human Molecular Genetics*, *21*(7), 1496–1503.

Nau, H., Hauck, R. S., & Ehlers, K. (1991). Valproic acid-induced neural tube defects in mouse and human: aspects of chirality, alternative drug development, pharmacokinetics and possible mechanisms. *Pharmacology & Toxicology*, *69*(5), 310–321.

Neuman, M. G., Shear, N. H., Jacobson-Brown, P. M., Katz, G. G., Neilson, H. K., Malkiewicz, I. M., Abbott, F. (2001). CYP2E1-mediated modulation of valproic acid-induced hepatocytotoxicity. *Clinical Biochemistry*, *34*(3), 211–218.

Neural Tube Defects. (n.d.). National Library of Medicine. Retrieved from http://www.nlm.nih.gov/medlineplus/neuraltubedefects.html

Oi, S. (2003). Current status of prenatal management of fetal spina bifida in the world: worldwide cooperative survey on the medico-ethical issue. *Childs Nerv Syst*, *19*(7–8), 596–599.

Okada, A., Kushima, K., Aoki, Y., Bialer, M., & Fujiwara, M. (2005). Identification of early-responsive genes correlated to valproic acid-induced neural tube defects in mice. *Birth Defects Research. Part A, Clinical and Molecular Teratology*, *73*(4), 229–38.

Olney, J. W., Young, C., Wozniak, D. F., Jevtovic-Todorovic, V., & Ikonomidou, C. (2004). Do pediatric drugs cause developing neurons to commit suicide? *Trends in Pharmacological Sciences*, *25*(3) 135-139.

Ornoy, A. (2009). Valproic acid in pregnancy: How much are we endangering the embryo and fetus? *Reproductive Toxicology*, *28*(1), 1–10.

Parker, S. E., Mai, C. T., Canfield, M. A., Rickard, R., Wang, Y., Meyer, R. E., Correa, A. (2010). Updated national birth prevalence estimates for selected birth defects in the United States, 2004-2006. *Birth Defects Research Part A - Clinical and Molecular Teratology*, *88*(12), 1008–1016.

Parle-McDermott, A., Pangilinan, F., O'Brien, K. K., Mills, J. L., Magee, A. M., Troendle, J., Brody, L. C. (2009). A common variant in MTHFD1L is associated with neural tube defects and mRNA splicing efficiency. *Human Mutation*, *30*(12), 1650–6.

Pazin, M. J., & Kadonaga, J. T. (1997). What's up and down with histone deacetilation and transcription? *Cell*, *89*, 325–328.

Peixoto, L., & Abel, T. (2013). The role of histone acetylation in memory formation and cognitive impairments. *Neuropsychopharmacology : Official Publication of the American College of Neuropsychopharmacology*, *38*(1), 62–76.

Pelicano, H., Carney, D., & Huang, P. (2004). ROS stress in cancer cells and therapeutic implications. *Drug Resistance Updates : Reviews and Commentaries in Antimicrobial and Anticancer Chemotherapy*, *7*(2), 97–110.

Perveen, T., Haider, S., Kanwal, S., & Haleem, D. J. (2009). Repeated administration of Nigella sativa decreases 5-HT turnover and produces anxiolytic effects in rats. *Pakistan Journal of Pharmaceutical Sciences*, *22*(2), 139–144.

Perveen, T., Haider, S., Zuberi, N. A., Saleem, S., Sadaf, S., & Batool, Z. (2014). Increased 5-HT levels following repeated administration of Nigella sativa L. (black seed) oil produce antidepressant effects in rats. *Scientia Pharmaceutica*, *82*(1), 161–170.

Pezze, M. A., Marshall, H. J., Fone, K. C. F., & Cassaday, H. J. (2015). Dopamine D1 receptor stimulation modulates the formation and retrieval of novel object recognition memory: Role of the prelimbic cortex. *European Neuropsychopharmacology*, *25*(11), 2145–2156.

Phiel, C. J., Zhang, F., Huang, E. Y., Guenther, M. G., Lazar, M. A., & Klein, P. S. (2001). Histone Deacetylase is a Direct Target of Valproic Acid, a Potent Anticonvulsant, Mood Stabilizer, and Teratogen. *Journal of Biological Chemistry*, *276*(39), 36734–36741.

Pike, S. T., Rajendra, R., Artzt, K., & Appling, D. R. (2010). Mitochondrial C1-tetrahydrofolate synthase (*MTHFD1L*) supports the flow of mitochondrial one-

carbon units into the methyl cycle in embryos. *Journal of Biological Chemistry*, *285*(7), 4612–4620.

Podgorac, J., Pešić, V., Pavković, Ž., Martać, L., Kanazir, S., Filipović, L., & Sekulić, S. (2016). Early physical and motor development of mouse offspring exposed to valproic acid throughout intrauterine development. *Behavioural Brain Research*, *311*, 99–109.

Pritchett, J. C., Nanau, R. M., & Neuman, M. G. (2012). The Link between Hypersensitivity Syndrome Reaction Development and Human Herpes Virus-6 Reactivation. *International Journal of Hepatology*, *2012*, 1-20.

Putten, M. Van, Aartsma-Rus, A. & Louvain, L. (2012). The use of hanging wire tests to monitor muscle strength and condition over time. *Treat-Nmd.Eu*, 11, 1–12.

Racoma, I. O., Meisen, W. H., Wang, Q.-E., Kaur, B., & Wani, A. A. (2013). Thymoquinone inhibits autophagy and induces cathepsin-mediated, caspase-independent cell death in glioblastoma cells. *PloS One*, *8*(9), e72882.

Radad, K., Hassanein, K., Al-Shraim, M., Moldzio, R., & Rausch, W. D. (2014). Thymoquinone ameliorates lead-induced brain damage in Sprague Dawley rats. *Experimental and Toxicologic Pathology*, *66*(1), 13–17.

Radad, K., Moldzio, R., Taha, M., & Rausch, W.-D. (2009). Thymoquinone protects dopaminergic neurons against MPP+ and rotenone. *Phytotherapy Research : PTR*, *23*(5), 696–700.

Rai, K., Huggins, I. J., James, S. R., Karpf, A. R., Jones, D. A., & Cairns, B. R. (2008). DNA Demethylation in Zebrafish Involves the Coupling of a Deaminase, a Glycosylase, and Gadd45. *Cell*, *135*(7), 1201–1212.

Rapoport, J. L., Giedd, J. N., & Gogtay, N. (2012). Neurodevelopmental model of schizophrenia: update 2012. *Molecular Psychiatry*, *17*(12), 1228–38.

Ray, J. G., Meier, C., Vermeulen, M. J., Boss, S., Wyatt, P. R., & Cole, D. E. C. (2002). Association of neural tube defects and folic acid food fortification in Canada. *LANCET*, *360*(9350), 2047–2048.

Raza, M., Alghasham, A. A., Alorainy, M. S., & El-Hadiyah, T. M. (2008). Potentiation of Valproate-induced Anticonvulsant Response by Nigella sativa Seed Constituents: The Role of GABA Receptors. *International Journal of Health Sciences*, *2*(1), 15–25.

Raza, S., Harker, A., Richards, S., Kolb, B., & Gibb, R. (2015). Tactile stimulation improves neuroanatomical pathology but not behavior in rats prenatally exposed to valproic acid. *Behavioural Brain Research*, *282*, 25–36.

Redon, C., Pilch, D., Rogakou, E., Sedelnikova, O., Newrock, K., & Bonner, W. (2002). Histone H2A variants H2AX and H2AZ. *Current Opinion in Genetics*

and Development 12(2) 162-169.

Reece, E. A. (2012). Diabetes-induced birth defects: what do we know? What can we do? *Current Diabetes Reports*, *12*(1), 24–32.

Reece, E. A., Pinter, E., Leranth, C. Z., & M, H. G. M. J. (1985). Ultrastructural Analysis of Malformations of the Embryonic Neural Axis Induced by In Vitro Hyperglycemic Conditions, *32*(3) 363-373.

Reimers, A., Skogvoll, E., Sund, J. K., & Spigset, O. (2007). Lamotrigine in children and adolescents: The impact of age on its serum concentrations and on the extent of drug interactions. *European Journal of Clinical Pharmacology*, *63*(7), 687–692.

Rintoul, N. E., Sutton, L. N., Hubbard, A. M., Cohen, B., Melchionni, J., Pasquariello, P. S., & Adzick, N. S. (2002). A New Look at Myelomeningoceles: Functional Level, Vertebral Level, Shunting, and the Implications for Fetal Intervention. *Pediatrics*, *109*(3), 409–413.

Rodier, P. M., Ingram, J. L., Tisdale, B., Nelson, S., & Romano, J. (1996). Embryological origin for autism: Developmental anomalies of the cranial nerve motor nuclei. *Journal of Comparative Neurology*, *370*(2), 247–261.

Rosenberg, S. L., Chen, S., McLaughlin, N., & El-Dahr, S. S. (2011). Regulation of kidney development by histone deacetylases. In *Pediatric Nephrology 26*, 1445–1452.

Roste, L. S., Taubøll, E., Berner, A., Isojärvi, J. I., & Gjerstad, L. (2001). Valproate, but not lamotrigine, induces ovarian morphological changes in Wistar rats. *Experimental and Toxicologic Pathology*, *52*(6), 545–552.

Roullet, F. I., Lai, J. K. Y., & Foster, J. A. (2013). In utero exposure to valproic acid and autism - A current review of clinical and animal studies. *Neurotoxicology and Teratology*, *36*, 47-56.

Roullet, F. I., Wollaston, L., deCatanzaro, D., & Foster, J. A. (2010). Behavioral and molecular changes in the mouse in response to prenatal exposure to the anti-epileptic drug valproic acid. *Neuroscience*, *170*(2), 514–522.

Rousseaux, C. G., Nicholson, S., & Schiefer, H. B. (1985). Fatal placental hemorrhage in pregnant CD-1 mice following one oral dose of T-2 toxin. *Canadian Journal of Comparative Medicine. Revue Canadienne de Médecine Comparée*, *49*(1), 95–8.

Russell, A. J., Macpherson, H., Cairnie, V., & Brodie, M. J. (1996). The care of pregnant women with epilepsy--a survey of obstetricians in Scotland. *Seizure*, *5*(4), 271–7.

Sadler, T. W. (2010). The embryologic origin of ventral body wall defects. *Seminars in Pediatric Surgery*, *19*(3), 209–214.

Sahak, M. K. A., Mohamed, A. M., Hashim, N. H., & Hasan Adli, D. S. (2013). Nigella sativa oil enhances the spatial working memory performance of rats on a radial arm maze. *Evidence-Based Complementary and Alternative Medicine, 2013* 1-5.

Salem, M. L. (2005). Immunomodulatory and therapeutic properties of the Nigella sativa L. seed. *International Immunopharmacology*, *5*(13–14), 1749–1770.

Samson, A. L., Ju, L., Ah Kim, H., Zhang, S. R., Lee, J. A. A., Sturgeon, S. A., Xi, G. (2015). MouseMove: an open source program for semi-automated analysis of movement and cognitive testing in rodents. *Stroke*, *33*(Nov), 161-171.

Sander, J. W. (2003). The epidemiology of epilepsy revisited. [Curr Opin Neurol. 2003] - PubMed result. *Current Opinion in Neurology*, *16*(2), 165–70.

Sargar, K. M., Herman, T. E., & Siegel, M. J. (2015). Valproate fetopathy. *Journal of Perinatology : Official Journal of the California Perinatal Association*, *35*(1), 82–3.

Schneider, T., & Przewłocki, R. (2005). Behavioral alterations in rats prenatally exposed to valproic acid: animal model of autism. *Neuropsychopharmacology*, *30*(1), 80–9.

Schoenwolf, G. C. (1984). Histological and ultrastructural studies of secondary neurulation in mouse embryos. *American Journal of Anatomy*, *169*(4), 361–376.

Scott, W. J., Schreiner, C. M., Nau, H., Vorhees, C. V., Beliles, R. P., Colvin, J., & McCandless, D. (1997). Valproate-induced limb malformations in mice associated with reduction of intracellular pH. *Reproductive Toxicology*, *11*(4), 483–493.

Scott Adzick, N. (2013). Fetal surgery for spina bifida: Past, present, future. *Seminars in Pediatric Surgery*, *22*(1), 10–17.

Sedaghat, R., Roghani, M., & Khalili, M. (2014). Neuroprotective effect of thymoquinone, the Nigella sativa bioactive compound, in 6-hydroxydopamine-induced hemi-parkinsonian rat model. *Iranian Journal of Pharmaceutical Research*, *13*(1), 227–234.

Seghatoleslam, M., Alipour, F., Shafieian, R., Hassanzadeh, Z., Edalatmanesh, M. A., Sadeghnia, H. R., & Hosseini, M. (2015). The effects of Nigella sativa on neural damage after pentylenetetrazole induced seizures in rats. *Journal of Traditional and Complementary Medicine*, *6*(3), 262–268.

Semmler, A., Frisch, C., Bleul, C., Smith, D., Bigler, L., Prost, J.-C., Linnebank, M. (2017). Intrauterine valproate exposure is associated with alterations in hippocampal cell numbers and folate metabolism in a rat model of valproate teratogenicity. *Seizure*, *46*, 7–12.

Serrano, B. B., Sanchez, M. J. G., Otero, M. J., Buelga, D. S., Serrano, J., & Dom??nguez-Gil, A. (1999). Valproate population pharmacokinetics in children. *Journal of Clinical Pharmacy and Therapeutics*, *24*(1), 73–80.

Sha, K., & Winn, L. M. (2010). Characterization of valproic acid-initiated homologous recombination. *Birth Defects Research. Part B, Developmental and Reproductive Toxicology*, *89*(2), 124–32.

Shallcross, R., Bromley, R. L., Irwin, B., Bonnett, L. J., Morrow, J., & Baker, G. A. (2011). Child development following in utero exposure: Levetiracetam vs sodium valproate. *Neurology*, *76*(4), 383–389.

Sharp, L. K., & Lipsky, M. S. (2002). Screening for depression across the lifespan: A review of measures for use in primary care settings. *American Family Physician*, *66*(6) 1001-8.

Sheng, N., Xie, Z., Wang, C., Bai, G., Zhang, K., Zhu, Q., Jing, N. (2010). Retinoic acid regulates bone morphogenic protein signal duration by promoting the degradation of phosphorylated Smad1. *Proceedings of the National Academy of Sciences of the United States of America*, *107*(44), 18886–18891.

Shih, P., Halpin, R. J., Ganju, A., Liu, J. C., & Koski, T. R. (2010). Management of recurrent adult tethered cord syndrome. *Neurosurgical Focus*, *29*(1), 1-5.

Silva, M. F. B., Aires, C. C. P., Luis, P. B. M., Ruiter, J. P. N., IJlst, L., Duran, M., Tavares de Almeida, I. (2008). Valproic acid metabolism and its effects on mitochondrial fatty acid oxidation: A review. In *Journal of Inherited Metabolic Disease 31*, 205–216.

Smith, M. L., Chen, I. T., Zhan, Q., Bae, I., Chen, C. Y., Gilmer, T. M., Fornace, a J. (1994). Interaction of the p53-regulated protein Gadd45 with proliferating cell nuclear antigen. *Science (New York, N.Y.)*, *266*(5189), 1376–1380.

Smithells, R. W., Sheppard, S., & Schorah, C. J. (1976). Vitamin dificiencies and neural tube defects. *Archives of Disease in Childhood*, *51*(12), 944–50.

Smithells, R. W., Sheppard, S., Schorah, C. J., Seller, M. J., Nevin, N. C., Harris, R., Fielding, D. W. (1981). Apparent prevention of neural tube defects by periconceptional vitamin supplementation. *Archives of Disease in Childhood*, *56*(12), 911–918.

Spriet, I., Goyens, J., Meersseman, W., Wilmer, A., Willems, L., & Van Paesschen, W. (2007). Interaction between valproate and meropenem: A retrospective study. *Annals of Pharmacotherapy*, *41*(7–8), 1130–1136.

Stanford, S. C. (2007). The Open Field Test: reinventing the wheel. *Journal of Psychopharmacology (Oxford, England)*, *21*(2), 134–135.

Stays, H., & Charges, H. (2007). Hospital stays, hospital charges, and in-hospital deaths among infants with selected birth defects--United States, 2003. *MMWR.*

Morbidity and Mortality Weekly Report, *56*(2), 25–9.

Stiefel, D., Shibata, T., Meuli, M., Duffy, P. G., & Copp, A. J. (2003). Tethering of the spinal cord in mouse fetuses and neonates with spina bifida. *J Neurosurg*, *99*(2), 206–213.

Stodgell, C. J., Ingram, J. L., O'Bara, M., Tisdale, B. K., Nau, H., & Rodier, P. M. (2006). Induction of the homeotic gene Hoxa1 through valproic acid's teratogenic mechanism of action. *Neurotoxicology and Teratology*, *28*(5), 617–624.

Stromland, K., & Pinazo-Duran, M. D. (2002). Ophthalmic involvement in the fetal alcohol syndrome: clinical and animal model studies. *Alcohol Alcohol*, *37*(1), 2–8.

Sui, L., & Chen, M. (2012). Prenatal exposure to valproic acid enhances synaptic plasticity in the medial prefrontal cortex and fear memories. *Brain Research Bulletin*, *87*(6), 556–563.

Sveberg Roste, L., Tauboll, E., Isojärvi, J. I. T., Pakarinen, A. J., Huhtaniemi, I. T., Knip, M., & Gjerstad, L. (2002). Effects of chronic valproate treatment on reproductive endocrine hormones in female and male Wistar rats. *Reproductive Toxicology*, *16*(6), 767–773.

Svensson, K., Mattsson, R., James, T. C., Wentzel, P., Pilartz, M., MacLaughlin, J., Ohlsson, R. (1998). The paternal allele of the H19 gene is progressively silenced during early mouse development: the acetylation status of histones may be involved in the generation of variegated expression patterns. *Development (Cambridge, England)*, *125*(1), 61–9.

Takahashi, E., Niimi, K., & Itakura, C. (2010). Neonatal motor functions in Cacna1a-mutant rolling Nagoya mice. *Behavioural Brain Research*, *207*(2), 273–279.

Thomas, S. V., Tomson, T., & Harden, C. L. (2013). Management of Epilepsy and Pregnancy. In *Epilepsy in Women*, *8*(1), 196–199.

Tiboni, G. M., & Ponzano, A. (2015). Prevention of valproic acid-induced neural tube defects by sildenafil citrate. *Reproductive Toxicology*, *56*, 175–179.

Tilford, J. M., Grosse, S. D., Goodman, A. C., & Li, K. (2009). Labor market productivity costs for caregivers of children with spina bifida: a population-based analysis. *Medical Decision Making : An International Journal of the Society for Medical Decision Making*, *29*(1), 23–32.

Tomson, T., Battino, D., Bonizzoni, E., Craig, J., Lindhout, D., Perucca, E., EURAP Study Group. (2015). Dose-dependent teratogenicity of valproate in mono- and polytherapy. *Neurology*, *85*(10), 866–872.

Tomson, T., Battino, D., Bonizzoni, E., Craig, J., Lindhout, D., Sabers, A., group, E. study. (2011). Dose-dependent risk of malformations with antiepileptic drugs: an analysis of data from the EURAP epilepsy and pregnancy registry. *Lancet Neurol*, *10*(7), 609–617.

Tsurubuchi, T., Ichi, S., Shim, K.-W., Norkett, W., Allender, E., Mania-Farnell, B., Mayanil, C. S. (2013). Amniotic fluid and serum biomarkers from women with neural tube defect-affected pregnancies: a case study for myelomeningocele and anencephaly: clinical article. *Journal of Neurosurgery. Pediatrics*, *12*, 380–9.

Tung, E. W. Y., & Winn, L. M. (2011). Valproic acid increases formation of reactive oxygen species and induces apoptosis in postimplantation embryos: a role for oxidative stress in valproic acid-induced neural tube defects. *Molecular Pharmacology*, *80*(6), 979–87.

Tunnicliff, G. (1999). Actions of sodium valproate on the central nervous system. *Journal of Physiology and Pharmacology*, *1*(50), 347–365.

Utoguchi, N., & Audus, K. L. (2000). Carrier-mediated transport of valproic acid in BeWo cells, a human trophoblast cell line. *International Journal of Pharmaceutics*, *195*(1–2), 115–124.

Vairapandi, M., Balliet, A. G., Fornace, A. J., Hoffman, B., & Liebermann, D. A. (1996). The differentiation primary response gene MyD118, related to GADD45, encodes for a nuclear protein which interacts with PCNA and p21(WAF1/CIP1). *Oncogene*, *12*(12), 2579–2594.

Vajda, F. (2012). Dose issues in antiepileptic therapy. *Journal of Clinical Neuroscience*, *19*(11), 1475-1477.

Van Allen, M. I., Boyle, E., Thiessen, P., McFadden, D., Cochrane, D., Chambers, G. K., Uh, S.-H. (2006). The impact of prenatal diagnosis on neural tube defect (NTD) pregnancy versus birth incidence in British Columbia. *Journal of Applied Genetics*, *47*(2), 151–158.

van Gelder, M. M. H. J., van Rooij, I. A. L. M., Miller, R. K., Zielhuis, G. A., de Jong-van den Berg, L. T. W., & Roeleveld, N. (2010). Teratogenic mechanisms of medical drugs. *Human Reproduction Update*, *16*(4), 378-394.

Van Straaten, H. W. M., & Copp, A. J. (2001). Curly tail: A 50-year history of the mouse spina bifida model. *Anatomy and Embryology*, *203*(4), 225-237.

Veiby, G., Daltveit, A. K., Engelsen, B. A., & Gilhus, N. E. (2014). Fetal growth restriction and birth defects with newer and older antiepileptic drugs during pregnancy. *Journal of Neurology*, *261*(3), 579–588.

Verrotti, A., D'Egidio, C., Mohn, A., Coppola, G., Parisi, P., & Chiarelli, F. (2011). Antiepileptic drugs, sex hormones, and PCOS. *Epilepsia*, *52*(2), 199-211.

Verrotti, A., Loiacono, G., Laus, M., Coppola, G., Chiarelli, F., & Tiboni, G. M. (2011). Hormonal and reproductive disturbances in epileptic male patients: Emerging issues. *Reproductive Toxicology*, *31*(4), 519–527.

Verrotti, A., Scardapane, A., Franzoni, E., Manco, R., & Chiarelli, F. (2008). Increased oxidative stress in epileptic children treated with valproic acid. *Epilepsy Research*, *78*(2–3), 171–177.

Vorhees, C. V, & Williams, M. T. (2006). Forms of Learning and Memory. *Nat Protocols*, *1*(2), 848–858.

Vossel, K. A., Beagle, A. J., Rabinovici, G. D., Shu, H., Lee, S. E., Naasan, G., Mucke, L. (2013). Seizures and epileptiform activity in the early stages of Alzheimer disease. *JAMA Neurology*, *70*(9), 1158–66.

Vural, M., Camuzcuoglu, H., Toy, H., & Aksoy, N. (2010). Amniotic Fluid Prolidase Activity and Oxidative Status in Neural Tube Defects. *Fetal Diagnosis and Therapy*, *28*(1), 34–39.

Wadaan, M. A. M. (2009). Long-term effects of black seed and garlic oil on the offspring of two consecutive pregnancies in rats. *Journal of King Saud University - Science*, *21*(3), 155–161.

Wagner, G. C., Reuhl, K. R., Cheh, M., McRae, P., & Halladay, A. K. (2006). A new neurobehavioral model of autism in mice: Pre- and postnatal exposure to sodium valproate. *Journal of Autism and Developmental Disorders*, *36*(6), 779–793.

Walf, A. A., & Frye, C. A. (2007). The use of the elevated plus maze as an assay of anxiety-related behavior in rodents. *Nature Protocols*, *2*(2), 322–8.

Wallingford, J. B., A., N. L., Shaw, G. M., & Finnell, R. H. (2013). The Continuing Challenge of Understanding, Preventing, and Treating Neural Tube Defects. *Science*, *339*, 1047–1055.

Wang, B., Yang, R., Wu, Y., Li, H., Hu, Z., Chen, Y., & Zou, S. (2013). Sodium valproate inhibits the growth of human cholangiocarcinoma in vitro and in vivo. *Gastroenterology Research and Practice*, *2013*.

Wang, C.C., Lin, H.C., Chan, Y.H., Gean, P.W., Yang, Y. K., & Chen, P. S. (2013). 5-HT1A-receptor agonist modified amygdala activity and amygdala-associated social behavior in a valproate-induced rat autism model. *The International Journal of Neuropsychopharmacology*, *16*(9), 2027–39.

Wang, Z., Zang, C., Cui, K., Schones, D. E., Barski, A., Peng, W., & Zhao, K. (2009). Genome-wide Mapping of HATs and HDACs Reveals Distinct Functions in Active and Inactive Genes. *Cell*, *138*(5), 1019–1031.

Watanabe, M., Jo, J., Radu, A., Kaneko, M., Tabata, Y., & Flake, A. W. (2010). A tissue engineering approach for prenatal closure of myelomeningocele with

gelatin sponges incorporating basic fibroblast growth factor. *Tissue Engineering. Part A*, *16*(5), 1645–1655.

Watanabe, M., Li, H., Kim, A. G., Weilerstein, A., Radu, A., Davey, M., Flake, A. W. (2015). Complete tissue coverage achieved by scaffold-based tissue engineering in the fetal sheep model of Myelomeningocele. *Biomaterials*, *76*, 133–143.

Watson, E., & Cross, J. (2005). Development of Structures and Transport Functions in the Mouse Placenta. *Physiology*, *20*, 180–193.

Wells, P. G., Lee, C. J. J., McCallum, G. P., Perstin, J., & Harper, P. A. (2010). Receptor- and reactive intermediate-mediated mechanisms of teratogenesis. *Handbook of Experimental Pharmacology*, *196*, 131-162.

Wells, P. G., Mccallum, G. P., Chen, C. S., Henderson, J. T., Lee, C. J. J., Perstin, J., Wong, A. W. (2009). Oxidative stress in developmental origins of disease: Teratogenesis, neurodevelopmental deficits, and cancer. *Toxicological Sciences*, *108*(1), 4–18.

Wilffert, B., Altena, J., Tijink, L., van Gelder, M. M. H. J., & de Jong-van den Berg, L. T. W. (2011). Pharmacogenetics of drug-induced birth defects: what is known so far? *Pharmacogenomics*, *12*(4), 547–558.

Wisner, K. L., Leckman-Westin, E., Finnerty, M., & Essock, S. M. (2011). Valproate prescription prevalence among women of childbearing age. *Psychiatric Services (Washington, D.C.)*, *62*(2), 218–20.

Wong, L. Y., & Paulozzi, L. J. (2001). Survival of infants with spina bifida: a population study, 1979-94. *Paediatric and Perinatal Epidemiology*, *15*(4), 374–378.

Wu, G., Nan, C., Rollo, J. C., Huang, X., & Tian, J. (2010). Sodium valproate-induced congenital cardiac abnormalities in mice are associated with the inhibition of histone deacetylase. *Journal of Biomedical Science*, *17*(1), 16-22.

Xu, W., Chi, L., Xu, R., Ke, Y., Luo, C., Cai, J., Liu, R. (2005). Increased production of reactive oxygen species contributes to motor neuron death in a compression mouse model of spinal cord injury. *Spinal Cord*, *43*, 204–213.

Yanaguita, M. Y., Gutierrez, C. M., Ribeiro, C. N. M., Lima, G. a, Machado, H. R., & Peres, L. C. (2008). Pregnancy outcome in ethanol-treated mice with folic acid supplementation in saccharose. *Child's Nervous System : ChNS*, *24*(1), 99–104.

Ybot-Gonzalez, P., Savery, D., Gerrelli, D., Signore, M., Mitchell, C. E., Faux, C. H., Copp, A. J. (2007). Convergent extension, planar-cell-polarity signalling and initiation of mouse neural tube closure. *Development (Cambridge, England)*, *134*(4), 789–799.

Yu, H. Y., Shen, Y. Z., Sugiyama, Y., & Hanano, M. (1990). Drug interaction. Effects of salicylate on pharmacokinetics of valproic acid in rats. *Drug Metab Dispos*, *18*(1), 121–126.

Zhan, Q., Bae, I., Kastan, M. B., & Fornace, A. J. (1994). The p53-dependent gamma-ray response of GADD45. *Cancer Research*, *54*(10), 2755–60.

Zhang, W., Bae, I., Krishnaraju, K., Azam, N., Fan, W., Smith, K., Liebermann, D. a. (1999). CR6: A third member in the MyD118 and Gadd45 gene family which functions in negative growth control. *Oncogene*, *18*(35), 4899–4907.

Zhang, W., Hoffman, B., & Liebermann, D. A. (2001). Ectopic expression of MyD118/Gadd45/CR6 (Gadd45beta/alpha/gamma) sensitizes neoplastic cells to genotoxic stress-induced apoptosis. *International Journal of Oncology*, *18*(4), 749–757.

Zhao, L., Chen, Y. H., Wang, H., Ji, Y. L., Ning, H., Wang, S. F., Xu, D. X. (2008). Reactive oxygen species contribute to lipopolysaccharide-induced teratogenesis in mice. *Toxicological Sciences*, *103*(1), 149–157.

Zhao, W., Mosley, B. S., Cleves, M. A., Melnyk, S., James, S. J., & Hobbs, C. A. (2006). Neural tube defects and maternal biomarkers of folate, homocysteine, and glutathione metabolism. *Birth Defects Research Part A - Clinical and Molecular Teratology*, *76*(4), 230–236.

Zhou, Z., Wang, L., Song, Z., Lambert, J. C., McClain, C. J., & Kang, Y. J. (2003). A critical involvement of oxidative stress in acute alcohol-induced hepatic TNF-alpha production. *The American Journal of Pathology*, *163*(3), 1137–1146.