EFFECTS OF SODIUM ARSENITE IN THE MODULATION OF TESTOSTERONE SYNTHESIS BY THE HYPOTHALAMO-PITUITARY TESTICULAR AXIS AND ITS EFFECT ON MEMORY AND LEARNING IN THE RAT

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LIST OF ABBREVIATIONS

ACTH	Adrenocorticotrophic hormone
AD	Alzheimer's disease
ANOVA	Analysis of variance
AP-1	Activator protein-1
ARASC	Animal Research and service centre, Universiti Sains Malaysia
AS3MT	Arsenic 3 methyltransferase enzyme
ATP	Adenosine triphosphate
BBB	Blood brain barrier
BD	Becton Dickinson
BDMA	Benzyl dimetylamine
BDNF	Brain-derived neurotrophic factor
BSA	Bovine serum albumin
Ca^{2+}	Calcium
CA1	Cornu Ammonis-1
CA2	Cornu Ammonis-2
CA3	Cornu Ammonis-3
Ca^{2+}	Calcium
Camp	Cyclic adenosine monophosphate CBP CR binding protein
CC	Central Canal
CNS	Central nervous system
CO	Corticosterone

DEPC	diethylpyrocarbonate
DG	Dentate gyrus
DNA	Deoxyribonucleic acid
EDTA	Ethylenediamine-tetraacetic acid
ELIZA	Enzyme linked immunosorbent assay
EMS	Early maternal separation
ER	Endoplasmic Reticulum
FSH	Follicle stimulating hormone
GA	Golgi apparatus
GABA	Gamma-aminobutyric- acid
GCL	Granular cell layer
GIT	Gastrointestinal tract
GR	Glucocorticoid receptor
HWS	Hazardous waste sites
LSD	Least Significant Difference
LH	Luteinizing hormone
LTP	Long term potentiation
LTM	Long term memory
ML	Molecular layer
MCI	Mild cognitive impairment
Mg^{2+}	Magnesium
M W	Molecular weight
MWM	Morris water maze
NA	Noradrenaline
NA^{+}	Natrium ion

NaCl	Natrium Chloride
NCS	Neuronal calcium sensor
NGF	Nerve growth factor
NGS	Normal goat serum
NMDA	N-methyl-d-aspartate
NMDAR	NMDA receptor
Na_2HPO_4	Disodium hydrogen phosphate
PB	Phosphate buffer
PBS	Phosphate buffer saline
PC12	Pheochromocytoma cell line 12
PND	Postnatal day
PKC	Protein kinase C
PLC	Phospholipase C
PM	Plasma membrane
PT	Probe test
RNA	Reactive oxygen species
RT	Reverse transcription
RT	Room temperature
SEM	standard error mean
SE	Standard error
SHBG	sex-hormone-binding globulin
SPSS	Statistical package of social sciences software
SS	Swimming speed
TBS	Tris base saline
TEM	Transmission electron microscopy

TrKB	Tropomyosin receptor kinase B
TS	Transverse section
UNDG	Upper blade of the dentate gyrus
WHO	World health organization
3β-HSD	17-betahydroxysteroid dehydrogenase

KESAN NATRIUM ARSENIT DI DALAM MODULASI SINTESIS TESTOSTERON OLEH PAKSI HIPOTALMUS-PITUITARI TESTOSTERON DI TIKUS DAN KESANNYA PADA INGATAN DAN PEMBELAJARAN

ABSTRAK

Natrium arsenit merupai bahan logam yang sentiasa wujud di alam semulajadi. Namun, dari sudut kesihatan awam, pendedahan kepada sebatian arsenit, terutamanya melalui air minuman, amat membimbangkan kerana dikaitkan dengan peningkatan risiko untuk pelbagai penyakit. Bukti terkini menunjukkan bahawa ianya boleh membawa kesan negatif kepada sistem pembiakan dan sistem saraf dimana ianya mampu merencat kognisi. Matlamat kajian ini ialah untuk meneliti perubahan morfologi struktur testis, ketidakseimbangan hormon dan kesan keatas kognisi pada tikus yang telah diberi pelbagai dos natrium arsenit. Tikus jantan matang jenis Sprague Dawley yang berumur 90 hari dibahagikan kepada 4 kumpulan yang mengandungi 8 tikus setiap satu kumpulan. Kumpulan I di beri salina manakala yang tiga lagi menerima natrium arsenit sebanyak 3, 5 atau 8mg/kg berat badan selama 60 hari melalui suntikan intraperitonium. Seteruanya, sampel darah dianalisis untuk hormon perangsang folikel (FSH), hormon perlutinan (LH), testosteron dan faktor neurotropik pemerolehan otak (BDNF). Seterusnya daya ingatan spatial diuji dengan "Morris water maze" (MWM) pada hari ke 60. Tikus dibedah dan testis diperolehi untuk kajian histologi manakala hipokampus diperolehi untuk kajian mikroskop transmisi elektron. Terbukti bahawa pendedahan natrium arsenit secara kronik membawa kesan yang berkaitan dos. Pengurangan signifikan ($p \leq 0.05$) turut dilihat pada tahap plasma testosteron, LH, FSH dan BDNF pada kumpulan tikus yang diberi dos arsenit yang tinggi (5mg/kg and 8mg/kg berat

badan) berbanding dengan kumpulan tikus kawalan. Ujian MWM menunjukkan rencatan daya ingatan spatial dan pembelajaran selepas pendedahan kepada natrium arsenit. Seterusnya tikus dibedah dan hipokampus diperolehi untuk kajian histologi. Terdapat rencatan signifikan ($p < 0.05$) pada kelakuan kognisi semasa ujian MWM pada kumpulan tikus berdos tinggi (5mg/kg and 8mg/kg berat badan) berbanding dengan kumpulan kawalan. Penilaian histologi menunjukkan kerosakan pada ultrastruktur hipokampus yang berkaitan dengan dos yang diberikan manakala tiada perubahan signifikan yang diperolehi daripada kumpulan dos rendah (3mg/ kg) berbanding dengan kumpulan kawalan. Kajian ini telah menunjukkan bahawa pendedahan kronik kepada natrium arsenit menyebabkan perubahan degeneratif di hipokampus, rencatan rembesan BDNF yang seterusnya merencat daya ingatan spatial pada tikus jantan Sprague Dawley. Begitu juga, uktrastruktur testis turut mengalami kerosakan dimana pendedahan kronik kepada natrium arsenit merencat proses spermatogenesis akibat kerosakan kepada vesikel seminal pada kumpulan tikus yang diberi dos natrium arsenit yang tinggi (5 mg/kg dan 8 mg/kg). Begitu juga, tiada perubahan signifikan yang diperolehi daripada kumpulan dos rendah (3 mg/ kg) berbanding dengan kumpulan kawalan, Maka, pendedahan kronik kepada natrium arsenit telah mengakibatkan toksisiti di testis tikus jantan Sprague Dawley. Tahap testosteron menurun setelah pendedahan kepada natrium arsenit dan ini menunjukkan hubungan terus di antara testosteron dan prestasi kognisi. Bagi lelaki yang mempunyai kedua-dua gangguan kognitif dan paras testosterone rendah, penggantian testosteron boleh dipertimbangkan. Kajian jangka panjang dan panjang yang menilai kesan penggantian testosterone pada fungsi kognitif untuk lelaki yang terdedah kepada natrium arsenit adalah dibenarkan.

EFFECTS OF SODIUM ARSENITE IN THE MODULATION OF TESTOSTERONE SYNTHESIS BY THE HYPOTHALAMO-PITUITARY TESTICULAR AXIS AND ITS EFFECT ON MEMORY AND LEARNING IN THE RAT

ABSTRACT

Sodium arsenite is a toxic metalloid that exists ubiquitously in the environment. Human exposure to arsenic compounds is a significant public health concern. In most populations, the main source of arsenic exposure is the drinking water. Chronic exposure to arsenic is associated with increased risks of various diseases. Recent emerging evidences suggest that arsenic exposure affects the reproductive and developmental toxicity that leads to the decrease of cognition by affecting on nervous system. The aim of this current research was to investigate the morphological changes in structure of testis, hormonal imbalance and effect on cognition in rats treated with different doses of sodium arsenite. Mature male Sprague Dawley rats at the age of 90 days were divided into 4 groups of 8 animals each. Group I received saline water whereas the other three groups received sodium arsenite at doses of 3, 5 and 8mg/kg of body weight of rats respectively, for 60 days by intraperitoneal injection. The blood samples were collected for follicle stimulating hormone (FSH), Luteinizing hormone (LH), testosterone and Brain-derived neurotrophic factor (BDNF) measurements at day 60. The Morris water maze (MWM) test was performed for spatial memory at day 60. Following this, the animals were then dissected and their testes were collected for histological studies. It was found that the effect of chronic exposure was dose dependent. A significant decrease ($p \leq 0.05$) was observed in plasma levels of testosterone, LH, FSH and

BDNF in the higher dose groups (5mg/kg and 8mg/kg of body weight) in comparison to the control group. To characterize behavioural alterations induced by arsenic exposure, Morris water maze test was used. The Morris Water Maze test was performed for spatial memory at day 60. Following this, the animals were then dissected and their hippocampus was collected for histological studies. A significant decrease ($p < 0.05$) was observed in cognitive behaviour, during Morris water maze test, in the higher dose groups 5mg/kg and 8mg/kg) in comparison to the control group. Histological evaluation revealed dose-dependent, gradual damage in histoarchitecture of hippocampus. Moreover no significant change was observed in any experimental parameter in the low dose group (3mg/kg) in comparison to the control group. The results revealed that sub chronic exposure to sodium arsenite caused degenerative changes in hippocampus; decrease the level of BDNF and effects spatial memory in a dose dependent manner. Sodium arsenite exposure caused complete arrest of spermatogenesis with disrupted seminiferous tubules in the testes in high dose groups (5 and 8mg/kg). Moreover, no significant change was observed in any experimental parameter in the low dose group (3mg/kg of body weight) in comparison to the control group. Testosterone level was decreased by the exposure of sodium arsenite. Low levels of endogenous testosterone can be associated with poor performance on cognitive tests. For men with both cognitive impairment and low testosterone, testosterone substitution may be considered. Large, long- term studies evaluating the effects of testosterone substitution on cognitive function in older men and testosterone substitution on cognitive function in affected men exposed by sodium arsenite are warranted.

CHAPTER 1

INTRODUCTION

1.1 Sodium arsenite

Arsenic (MW=75) is an element that is ubiquitously distributed in the earth's crust. It is a naturally occurring element that is present in both organic and inorganic form in the environment. It is an ordinarily steel grey metal-like material that can exist in biological systems in different forms. There are very rare chances that arsenic is found in a pure state; instead it occurs in both trivalent and pentavalent oxidation states in the form of chemically unstable sulphide or oxide, or as a salt of calcium , potassium, and sodium. Sodium arsenite is a compound of arsenic, a group of 5th A (VA) elements of the periodic table of chemical elements and its chemical formula is $NaAso_2$ with a molecular weight of 129.91. It is white or greyish white in colour having no odour and occurs solid at room temperature with a density of 1.87 at 25°C. It forms a basic solution in water and it is freely soluble in water and ethanol. Sodium arsenite decomposes when it is heated (Considine , 2005).

Human exposure to arsenic is a significant public health concern. Arsenical compounds are environmental toxins with multiple effects in animal and human populations (Waalkes *et al*., 2003). The ubiquitous distribution of arsenic (a metalloid) in the soil, air and water makes it an environmental contaminant of global concern. Arsenic compounds represent a concern to environmental and occupational

health when their presence in the environment increases due to natural or anthropogenic sources. Anthropogenic sources of sodium arsenite include nonferrous metal mining and smelting, pesticides application, coal combustion and waste incineration. Most anthropogenic release of arsenic is to land or soil, primarily in the form of pesticides or solid wastes. The environmental levels of arsenic and its derivatives keep on changing on account of a number of dynamic processes by natural and human activities (Rodriguez *et al*., 2003).

1.1.1 Sources of sodium arsenite in environment

The main sources of the sodium arsenite in the environment are burning of fossil fuels, weathering of rocks, smelting of ores and burning of coal. The level of sodium arsenite keeps increasing in the environment as many industries also continue to contribute to the dispersion of arsenic in the environment (Waalkes *et al*., 2003). Over 80% of arsenic and its compounds are used to manufacture products with agricultural applications such as insecticides, fungicides, herbicides, algaecides, sheep dips, dye-stuffs and wood preservatives. Sodium arsenite is also used to prepare the medicines for the elimination of tapeworms in sheep and cattle (Tchounwou *et al*., 2003). It has also been noted that humans are often exposed to sodium arsenite by consuming polluted food material particularly sea food (WHO, 1999).

Inorganic arsenic (iAs) is a very important metalloid and it has application in many industries. Sodium arsenite is widely used in agriculture industry to manufacture pesticides. It has also been used to manufacture electronic devices due

to its semiconductor capacities. In furniture industry it has been used in wood preservation. Arsenic compounds are also used as a chemotherapeutic agent (ATSDR, 2007). It can be found in arsenic-containing minerals, ores and groundwater. This epidemiologically significant natural pollutant has been recognized as a global health problem because according to Tyler & Allan, 2014, an estimated 100 million people around the world are exposed to high concentrations of arsenic via drinking water. It has been reported in a survey, globally, more than 200 million of individuals drink water with levels of sodium arsenite that are above the World Health Organization reference value of 10 μg/L. It has been documented that the concentration of iAs is very high in the groundwaters of Argentina, Chile, China, India, Mexico, Taiwan and USA. In these countries people are chronically exposed to inorganic arsenic by drinking water from polluted wells. These wells get polluted not only from environmental pollutants but also as a consequence of geothermal activities due to naturally occurring mineral dissolution or deposition in water (Rodriguez *et al*., 2002).

Around the globe a large population in different countries are exposed to sodium arsenite. There are three main routes through which the exposure of sodium arsenite takes place. These are the oral routes of ingestion, inhalation and dermal contact. The most involved and permanent route of exposure is through oral dietary intake, with an average intake of 50 μg /day in many countries. The other sources are air, soil and water to a much lesser extent (Tchounwou *et al*., 2003).

Individuals who are at higher risk than normal are those working in the manufacture of products in which the main component is arsenic. This includes individuals working in pharmaceuticals, semiconductor manufacturing, pesticides manufacturing, glass making refining of metallic ores, ceramics and smelting. Another possible source of arsenic contamination is hazardous waste sites. From these waste sites the sodium arsenite cause contamination of water, air and soil. It is incorporated into the food chain. Sodium arsenite concentration in environment is increasing day by day and now it is considered as a major pollutant. It is also considered as an active toxic mutagenic xenobiotic metalloid (Flora *et al*., 2009).

1.1.2 Diseases caused by sodium arsenite

Various health organizations around the globe consider that water contamination by sodium arsenite is a major public health concern as the exposure by sodium arsenite can cause very severe health problems. It causes many diseases and disorders that destroy the body systems that leads to diabetic mellitus, haematological disorders, nephrotoxicity, cardiovascular abnormalities, neurotoxicity and hearing loss. Sodium arsenite can cause cancers in lung, skin, bladder, kidney, colon and uterus and it has been proved that sodium arsenite is a carcinogen (Hansen ,1990).

Sodium arsenite has a tendency to accumulate in liver, kidney heart and lungs. Sodium arsenite is also able to accumulate in the neuronal tissues as it can cross the blood brain barrier. The metabolites of sodium arsenite cause cellular toxicity by inhibiting the action of over 200 enzymes. Sodium arsenite also interferes with

deoxyribonucleic acid (DNA) synthesis and repair. When individuals drink the contaminated water, it accumulates in the tissues of vital organs and can lead to tumour formation in these organs (WHO,1990).

Sodium arsenite also leads to an increased prevalence of ischemic heart diseases, diabetes, atherosclerosis, hypertension, hepatotoxicity and cancer of the skin, bladder, and lungs (Hansen, 1990). Bangladesh, Taiwan, India, Mexico, China, Chile and Argentina are all major countries that are at high risk of sodium arsenite contamination. In Bangladesh and West Bengal, approximately 150 million people are at risk of sodium arsenite contamination. In 1980's it was first reported that in West Bengal, when the water of tube wells of 18 districts were screened, it was found that out of 18 wells, 9 were contaminated with sodium arsenite. Pesticides used in agriculture are the major cause of food contamination with sodium arsenite (Armstrong *et al.,* 1984). According to the World Health Organization (1999), it was documented that water contaminated by sodium arsenite exposure caused severe health issues and preventive and treatment measures should be immediately undertaken (WHO, 1999).

There are several factors that determine the toxicity of sodium arsenite such as dose, frequency and duration. The other factors that may be involved to determine the toxicity of sodium arsenite are age , gender, the biological species and genetic profile. The possible mechanisms of action of arsenic toxicity include apoptosis, necrosis of cell, disruption of signal transduction pathways and cell proliferation

(Vahidnia *et al*., 2007). These different pathways prove that sodium arsenite has extremely broad affects and acts on specific targets. Although epidemiological studies have shown that there are various sources of exposure with global impact on public health, nevertheless the mechanisms by which arsenic affects different systems, is not yet well established. There is a need to conduct further research in order to understand the underlying mechanism of arsenic toxicity (Rodriguez *et al*., 2003).

1.2 General mechanism of action of sodium arsenite

A variety of mechanisms have been proposed for sodium arsenite toxicity. According to these postulations sodium arsenite affects numerous signal transduction pathways and can cause severe damage to the cell by stimulating apoptosis, necrosis and growth inhibition. It also interferes with cell differentiation and angiogenesis. However, the final and accurate mechanism underlying sodium arsenite toxicity is not clear although it has recently been hypothesized that sodium arsenite causes modification of deoxyribonucleic acid (DNA) repair and methylation by producing reactive oxygen species and oxidation stress (Qian *et al.,* 2003). According to one study, sodium arsenite binds directly to thiol protein and this binding can modify many protein functions. It can act on regulatory enzymes at the cellular level and stimulates the activation and inactivation of several transduction pathways (Qian *et al*., 2003).

In another study, it has been found that high concentration of sodium arsenite in the cell decreases the rates of cell migration (Ishrath *et al*., 2002). Cell migration is a complex process that depends upon focal adhesions. These focal adhesion mediate

the connection between the actin cytoskeleton and the extracellular matrix and sodium arsenite affects cell migration by affecting the assembly and disassembly of these focal adhesions. There are several other regulators that are involved in cell migration such as different protein kinases (Zamir & Gieger, 2001) and sodium arsenite can cause severe damage to these kinases which are also involved in cell signalling events (Ishrath *et al*., 2002).

According to the above findings it has been proposed that sodium arsenite can cause blockage of protein kinases that regulate focal adhesion in cell signalling events and directly reduces cell migration. It has been shown that sub-lethal concentration of sodium arsenite modifies focal adhesion by decreasing protein phosphorylation of focal adhesion proteins and also affects cell adhesion to substrate (Trouba *et al*., 2000).

Overall the changes in the structure of focal adhesion and reduced cell migration are the main cause of increased necrosis, apoptosis and arrest of cell cycle that leads to damage of body systems and cause diseases. These findings helped researchers to further understand the resulting health affects caused by exposure to sodium arsenite (Zamir & Gieger, 2001).

1.3 Effects of sodium arsenite exposure on the male reproductive system

Exposure to sodium arsenite disrupts testicular androgenesis by decreasing the levels of gonadotrophins with a resultant decrease in testosterone levels (Aman,1982). It also directly affects the testis and causes elimination of germ cells, decreased spermatogenesis and loss in testicular mass (Chapin & Lamb, 1984). Sodium arsenite also disrupts the essential enzymes which are important for testosterone production such as Δ5, 3β-HSD and 17β-HSD (Jana *et al*., 2006). Thus, decreased spermatogenesis, low levels of sex hormones and low levels of androgenesis are the direct evidences of the reproductive toxicity of sodium arsenite (Sarkar *et al*., 2003).

1.3.1 Effect on reproductive hormones

Spermatogenesis in the testis depends upon the release of two specific reproductive hormones; Luteinizing Hormone (LH) and Follicle Stimulating Hormone (FSH) secondary to stimulation from Gonadotrophin-Releasing Hormone (GnRH) as shown in Figure 1. These hormones are required for normal spermatogenesis in pubertal rats. Sodium arsenite exposure causes a decrease in the level of LH and FSH that directly decreases spermatogenesis in the testis (Jana *et al*., 2006). Many studies have been conducted to show the relationship between plasma and intratesticular concentrations of testosterone in rats following exposure to sodium arsenite. These studies observed that sodium arsenite inhibited the activity of testicular enzymes which regulate the process of testosterone synthesis in the testis.

Consequently it caused a corresponding decrease in the testosterone synthesis in the affected rats (Jana *et al*., 2006).

Moreover, the low levels of GnRH also cause decrease in LH and FSH hormone, both of which are prime regulators of testicular androgenic activities. This decrease in levels of LH and FSH ultimately cause decrease in the testosterone synthesis. On the other hand LH not only regulates testicular androgenesis, but also low levels of LH leads to decrease in testicular enzyme activity which further affect testicular androgenesis (Sarkar *et al*., 2003).The reduction in testosterone production may therefore be held responsible for the sodium arsenite induced changes in spermatogenesis.

The cascade of actions decrease GnRH in the hypothalamus and this leads to decrease in LH and FSH which subsequently decrease testosterone synthesis. Normally, a high level of testosterone in testis is important for normal spermatogenesis as well as for the preservation of structural morphology and normal physiology of the seminiferous tubules of the testis. However, the presence of sodium arsenite not only leads to low intratesticular testosterone secondary to inhibition of spermatogenesis, but arsenic treatment can also lead to germ cell degeneration and altered morphology of the testis (Chowdhury, 1979).

1.3.2 Dose dependent effect of sodium arsenite

The results of many experiments suggest that the critical factors that are responsible for its detrimental affect on testicular activities are duration and route of sodium arsenite treatment. A large number of adverse health outcomes are associated with sodium arsenite exposure. Sodium arsenite is a potent carcinogen and it can cross the placenta and affects fetal development (Vahter, 2008). However, relatively little research has been conducted on the effect of sodium arsenite on the human reproductive system.

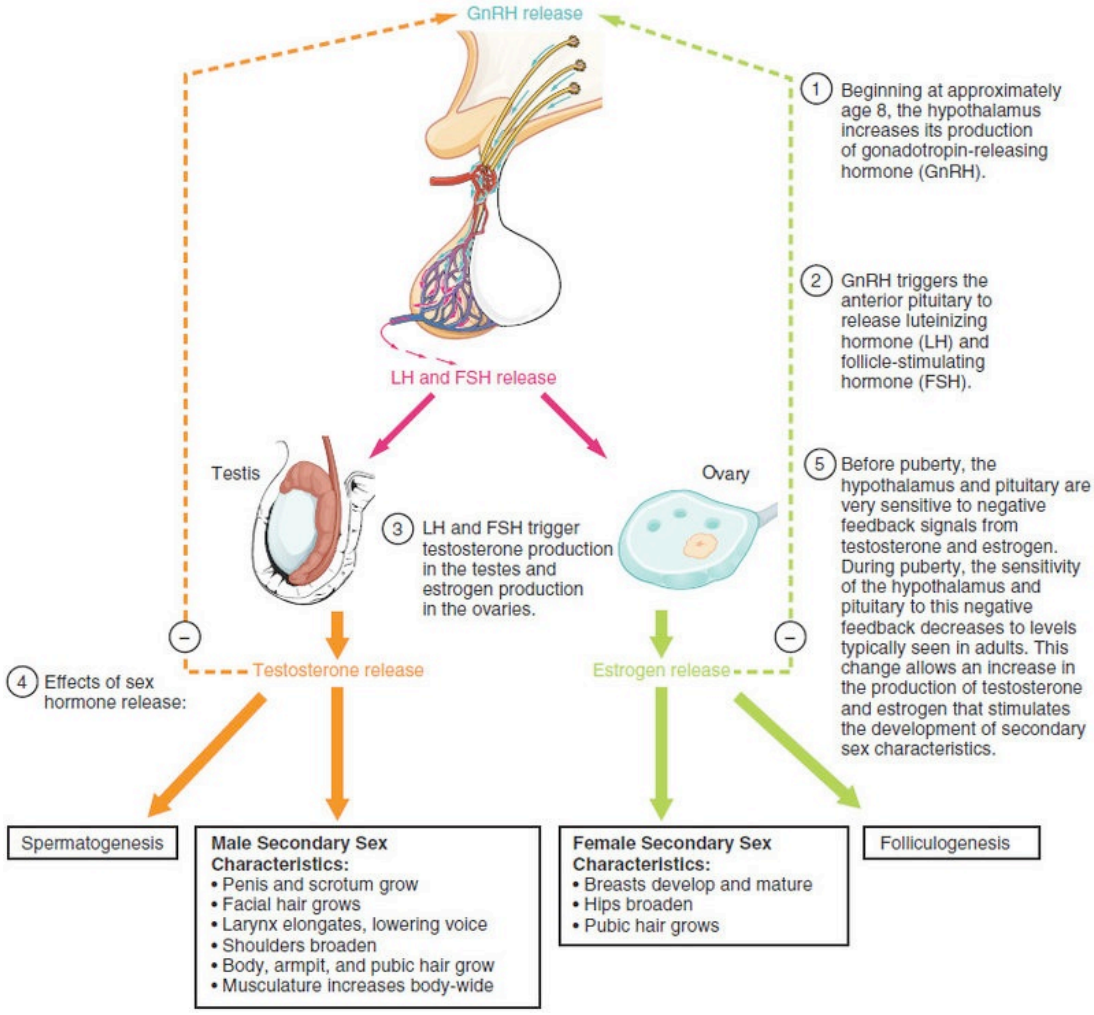

Figure 1.1: The role of hormones in reproduction. (Adapted from Brown, 2004).

It was documented in numerous studies that children of workers of smelters and nearby residents suffer severe abnormalities because of high concentration of sodium arsenite in these areas. In smelter areas the sodium arsenite accumulates in the dust and air. Nordstrom and colleagues (1979) observed adverse reproductive impacts among the offspring of employees and nearby residents of smelting area.

It has been shown that occupational and residential exposure to sodium arsenite can cause spontaneous abortion. Moreover, if pregnant mothers were employed in jobs with high exposure to sodium arsenite, their infants had a greater chance of developing congenital malformations(Vahter, 2008). It was found that the rate of the mortality from congenital malformations were notably higher in smelter areas that were contaminated from various metals in Bulgaria (Zaldivar , 1980). In Hungary, many studies were conducted on the populations exposed to sodium arsenite and it was observed that there were increased rates of spontaneous abortions and stillbirths. Similarly, the high rate of mortality from congenital cardiovascular anomalies (Zierler *et al*., 1988) and spontaneous abortions (Aschengrau *et al*., 1989) were reported in three studies conducted in contaminated area of the United States.

1.4 Effect on nervous system

Sodium arsenite affects the central nervous system (CNS) and causes seizures, encephalopathy, peripheral neuropathies and behavioural changes. The high concentration of sodium arsenite has neurotoxic affects on the central and as well as on the peripheral components of the mature nervous system. The exposure of sodium

arsenite is more lethal in childhood. It affects cell division of neurons and cause increased apoptosis and necrosis in cultured developing neurons. Although the exact mechanism involved in sodium arsenite neurotoxicity has yet to be established (Vahidnia *et al*., 2007) nevertheless, it has been reported in many studies that sodium arsenite exposure triggers peripheral neuropathy (Schoolmaster & White, 1980) and decreases the conduction velocity in peripheral nerves. There is also a direct relationship between sodium arsenite exposure and increased risk of microvascular diseases (Vahidnia *et al*., 2007).

1.4.1 Sodium arsenite and the blood brain barrier

Sodium arsenite accumulates in the brain by crossing the blood brain barrier and leads to neurobehavioral abnormalities (Tripathi *et al.,* 1997; Itoh *et al*., 1990). It has also been shown that basal ganglia in brain are also vulnerable to arsenite toxicity (Ghafghazi *et al*., 1980; Rodriguez *et al*.,2001). To understand the mechanism of arsenic induced neurotoxicity (Shila *et al*., 2005) , studies have been carried out in whole brain (Flora *et al*., 2009) and brain regions. It has been observed that sodium arsenite exposure has a marked effect on corpus striatum, cortex and hippocampus (Shila *et al*., 2005). The delayed maturation of Purkinje cells and their defective migration during fetal development leads to nervous system abnormalities that are stimulated by sodium arsenite exposure from postnatal days 4 to 10 (Dhar *et al*., 2007). Sodium arsenite exposure impaired learning and memory in adults and children in highly arsenic contaminated areas (Calderon *et al*., 2001). It is also observed during one study on rats that sodium arsenite exposure alters motor behaviour (Rodriguez *et al.*, 2003).

Environment and genetics both are essential components in the development of neuropathology. It has been observed in many experimental and epidemiological studies, that two factors that play a vital role in these neural abnormalities are diet components and chronic exposure to heavy metals. Sodium arsenite exposure and neurological deterioration have a very strong link with each other, particularly during the development and maturation of the nervous system. Sodium arsenite causes impairments in learning and memory (Rosado, 2007; Asadullah and Chaudhury, 2008) low intelligence as well as decreased verbal coefficients (Calderon *et al.,* 2001). The most important factors in the neurological and cognitive dysfunction are the concentration, timing and duration of sodium arsenite exposure (Rodriguez, 2013).

1.4.2 Possible molecular mechanism of arsenate toxicity in the nervous system

Kumagai and Sumi (2007) reported that in human and mammalian species sodium arsenite is methylated into trivalent and pentavalent components and conjugated with glutathione (GSH, L-γ-glutamyl-L-cysteinyl- glycine). Oxidative stress in the cell is generated due to this methylation (Kumagai & Sumi, 2007). It has been reported that the presence of these methylated metabolites have been detected in the blood of population who is residing near smellters (Parajuli , 2015). In the developing fetus, these metabolites can cross the placenta and cause severe neural tube defects. Thus it can be concluded that sodium arsenite exposure has damaging effects on brain function (Vahter, 2008; Parajuli *,* 2015; Tyler & Allan, 2014).

Several studies have reported that sodium arsenite causes neurotoxic effects during gestation. The different areas of the brain that have enzyme the arsenic 3

methyltransferase (AS3MT), these areas undergo methylation during gestation because sodium arsenite can cross the blood brain barrier (Rodriguez, 2013; Sanchez-Pena, 2010). It has been also observed that during gestation the transfer of arsenic metalloids occurred from pregnant mice to fetus , these metabolites can cross the transplacental barrier. The arsenic could be methylated in fetal tissues because AS3MT mRNA has been detected in fetal blood. During metabolism of sodium arsenite, the presence of S- adenosyl methionine as cellular methyl donor and like thioredoxin as methyl reductants and glutathione (GSH) are required (Thomas *et al.,* 2001).

In the central nervous system, GSH is the main antioxidant. It is consumed during this methylation process and the chances of disease susceptibility increases with this resultant deficiency of GSH in the nervous system. Overall it shows sodium arsenite affects the protective system of the nervous system by damaging the oxidants such as GSH during the process of methylation in the metabolism of sodium arsenite (Singh, *et al*., 2011).

The most widely available excitatory neurotransmitter in the CNS is glutamate. Its effects are mediated by ionotropic and metabotropic receptors. The abundantly expressed ionotropic receptors (named after the agonists that activate them) in the central nervous system (CNS) are α-amino-3-hydroxy-5-methyl- 4-isoxazolepropionic acid receptor (AMPAR) and N-methyl-D-aspartate receptor (NMDAR). These receptors are very important and mediate the entry of ions. An exact property of NMDAR is that it facilitates the entry of calcium Ca^{2+}, as well as it

mediates the passage of potassium $K^+$and sodium Na^+ ions . So it helps in initiating the signalling cascades.

Moreover, Ca^{2+} levels in the postsynaptic neuron are raised by excitatory postsynaptic potentials that can possibly act as a second messenger commencing signalling cascades. The NMDAR activation is also voltage-dependent due to the extracellular blockage by magnesium Mg^{2+} or zinc Zn^{2+}. The passage of cations (mostly Ca^{2+}) take place when the blockage is removed by a huge number of excitatory inputs or the simultaneous firing of the presynaptic cell. The bases of synaptic plasticity, learning and memory storage processes are mediated by these properties (Luscher *et al*., 2012)

During development the expression of NMDAR subunits is managed in response to synaptic activity. Many protein subunits combine to produce receptor isoforms of NMDAR. The NR1/NR2A isoforms of NMDAR dominate at synaptic sites in the adult nervous system whereas NR1/NR2B receptors predominate during development and concentrated at extra synaptic sites. The pharmacological and functional properties of the NMDA receptor are modulated by NR2B subunits. Overall, in the process of learning and memory, NR2B has been implicated in regulating the synaptic function. Moreover, it has been found in previously conducted research that during brain development, the exposure to xenobiotics such as sodium arsenite might interfere with the expression of NMDAR subunits NR2A and NR2B (Nicoll, 2015).

During the study on differentiating PC12 cells it was found that the sodium arsenite cause most severe damage on the early stages of neurite production and growth. It was observed *in vitro* that sodium arsenite affects initial neurite outgrowth and these effects are concentration and time dependent. It was observed after exposure to micromolar levels of sodium arsenite for five days that there is decreased neurite production, outgrowth and complexity in newly differentiating PC12 cells. These results shows that exposure to sodium arsenite affect morphological development at early stages of neural differentiation and could potentially be involved in the long term disruption of neurons (Rodriguez, *et al*., 2002).

The effect of sodium arsenite in neurotoxicity has been explored in rodents. It was observed during one study that in rats treated with 20 mg/kg body weight of sodium arsenite for 28 days, there was a significant decrease in locomotor activity, grip strength (26%) and rota-rod performance (82%). High arsenic levels were observed in brain areas such as in corpus striatum (6.5 fold), frontal cortex (6.3 fold) and hippocampus (7.0 fold). This high level is the major cause of oxidative stress in corpus striatum, frontal cortex and hippocampus The sodium arsenite also cause decrease in the binding of striatal dopamine receptors (32%) in striatum. On the other hand the enhanced oxidative stress in these brain regions, affects processes such as lipid peroxidation, low levels of glutathione and activity of superoxide dismutase (Shila *et al*., 2005).

Several studies have been conducted in order to clarify the underlying biochemical mechanisms involved in neurotoxicity caused by sodium arsenite exposure. Sodium arsenite exposure modifies the levels of neurotransmitters such as

dopamine, norepinephrine and serotonin. It has been observed in several experimental studies that the biogenic amines plays an important role in the neurotoxicity of sodium arsenite (Tripathi *et al*., 1997). Indeed, other studies have shown that sodium arsenite neurotoxicity affects the catecholaminergic system, while the increased oxidative stress in the different areas of the brain is associated with decreased antioxidant defence (Shila *et al*., 2005; Flora, 2009). Sodium arsenite thus creates a situation inside the neural cell which increases vulnerability towards oxidative stress and thus leading to severe damage inside the brain (Wang *et al*., 2012; Chen *et al*., 1998; Shila *et al*., 2005).

Sodium arsenite exposure increases the release of reactive oxygen species inside the brain by decreasing the synthesis of brain nitric oxide. In many studies it has been reported that sodium arsenite exposure modifies acetylcholine and glutamate and many other neurotransmitters. It has also been shown that chronic sodium arsenite exposure can cause a significant reduction in monoamines such as adrenaline, dopamine and noradrenaline within the corpus striatum, frontal cortex and hippocampus. It stimulates p38 mitogen-activated protein kinase (p38MAPK) to induce increased apoptosis in the cerebral neurons (Zarazua *et al*., 2010).

One of the possible mechanisms involved in neurotoxicity caused by sodium arsenite exposure is initiated by destabilization and interruption of the cytoskeletal framework that ultimately leads to axonal degeneration (Vahter, 2009). The neuronal complications are also broadly affected by the deficiency of thiamine

(vitamin B1) secondary to sodium arsenite exposure where it has been shown that sodium arsenite can cause thiamine deficiency and blockage of pyruvate decarboxylase, which directly elevates blood pyruvate and hence causes encephalopathy (Rodriguez *et al*., 2003). Moreover, the oxidative DNA damage and subsequent brain cell death secondary to sodium arsenite toxicity can cause the degeneration of dopaminergic neurons resulting in Parkinson-like symptoms (Felix *et al*., 2005).

Sodium arsenite exposure also causes various neuropsychiatric abnormalities, peripheral neuropathy and extrapyramidal disorders as it causes a decrease in cholinesterase activity and hence generates cholinergic crisis-like situations with altered mental status and weakness (Patlolla & Tchounwou, 2005). The damage to neuroskeletal integrity lowers the nerve conduction velocity in the peripheral nerves to cause peripheral neuropathy and severely affects the peripheral nervous system (Bardullas *et al*., 2009). It has also been shown that sodium arsenite exposure is able to suppress the NMDA receptors in hippocampus thus leading to impairment of synaptic plasticity, memory and learning which subsequently leads to cognitive dysfunction and neurobehavioral disorders (Kruger *et al*., 2009).

The morphological changes in axons and nerve fibres of the striatum which disrupts central structural organization seems to play an important role in neurotoxicity caused by chronic sodium arsenite exposure (Rios *et al*., 2009). In summary, as mentioned above, sodium arsenite neurotoxicity can lead to oxidative

stress, induction of thiamine deficiency, inhibition of acetyl cholinesterase and inhibition of pyruvate decarboxylase, as well as reduction in biogenic monoamines. Figure 2 summarizes the interrelationships between these effects in terms of arsenic-induced neurotoxicity. The findings of many previously conducted studies mentioned above are in relation to animal studies. They may show some inconsistencies in neurotoxicity because of varying doses, duration, and routes of administration of sodium arsenite. Nevertheless, these variations in outcomes of studies help us to further understand the pathophysiological mechanisms involved in arsenic induced neurotoxicity.

Sodium arsenite-induced neurotoxicity has also been studied in humans. In a cross-sectional study in Mexico, the impact of arsenic, lead and undernutrition on neuropsychological performance was evaluated in school children aged 6 to 9 years. Subjects included 41 children living within 1.5 km of a smelter complex (Morales Zone) with increasing arsenic and lead concentrations, 39 children living 7 km upwind from the smelter (Martinez Zone) .The arsenic level in urine was higher in the Morales children than in the Martinez children. Neuropsychological performance was analysed using the Weschler Intelligence Scale for children, revised version for Mexico (WISC-RM) The Morales children scored significantly lower than the Martinez children on the full-scale Intelligent Quotient (IQ) test and other neuropsychological sub scores (Rocha-Amador, 2007).

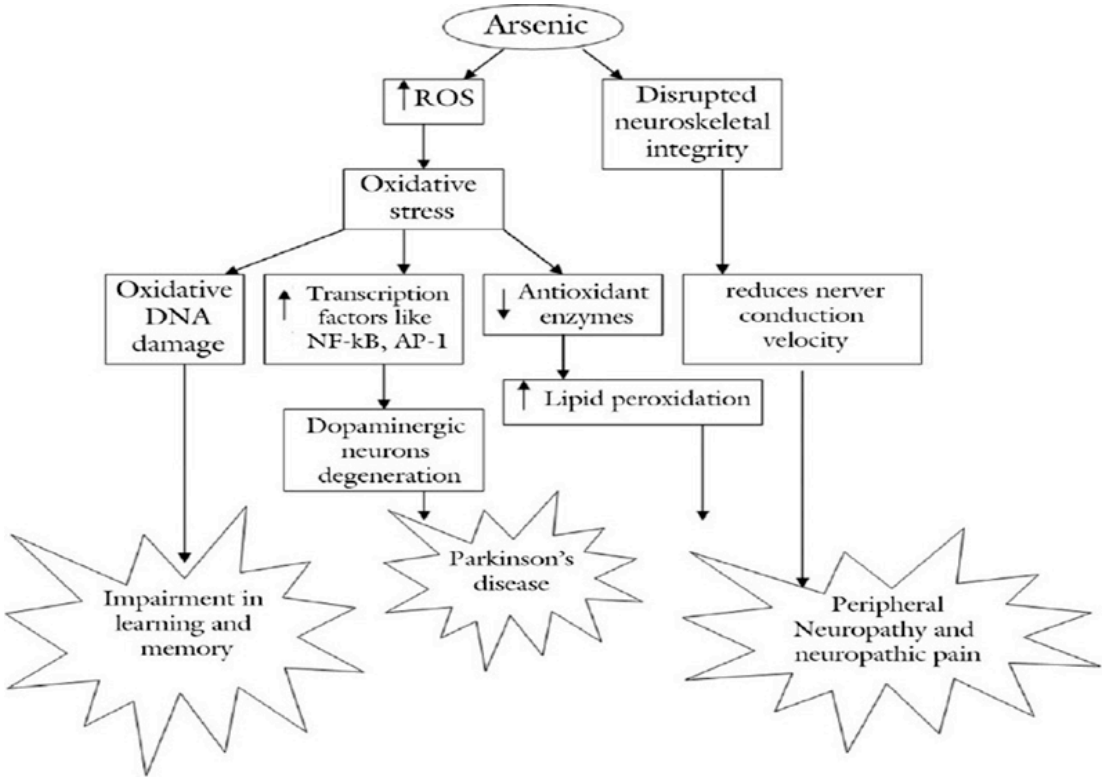

Figure 1.2: Pathophysiological mechanisms involved in sodium arsenite toxicity within the nervous system (Adopted from Rodriguez *et al*.,2003).

1.5 Role of the hippocampus in cognition

1.5.1 Location

The hippocampus is a brain structure that is in paired form, one half is located in the left brain hemisphere while the other half is located in the right hemisphere within the temporal lobe, adjacent to the amygdala. It is sometimes grouped with the dentate gyrus and several other structures to form the hippocampus which means "seahorse" in Latin (for its unique shape in coronal slices). It is a part of the limbic system of the brain which is involved in the formation of new memories and connecting emotions with senses, such as smell and sound of memories. The formation of autobiographical and fact memories are the particular function of the hippocampus. The hippocampus is known as a memory "gateway" because all memories pass through it before entering the brain. It is also known as a memory indexer because the hippocampus sorts out memories prior to storage and recall (Arisi, 2014).

The Basic hippocampal circuit Starts at the dentate gyrus and working inward along the S-curve of the hippocampus means traversing a series of narrow zones. The first of these, the dentate gyrus (DG), is actually a separate structure, a tightly packed layer of small granule cells wrapped around the end of the hippocampus proper, forming a pointed wedge in some cross-sections, a semicircle in others. Next come a series of *Cornu Ammonis* areas: first CA4 (which underlies the dentate gyrus), then CA3, then a very small zone called CA2, then CA1, The CA areas are all filled with densely packed Pyramidal cells similar to those found in the neocortex. After CA1 comes an area called the subiculum. After this comes a pair of ill-defined areas

called the presubiculum and parasubiculum, then a transition to the cortex proper (mostly the entorhinal area of the cortex). Most anatomists use the term "hippocampus proper" to refer to the four CA fields, and hippocampal formation to refer to the hippocampus proper plus dentate gyrus and subiculum (The major pathways of signal flow through the hippocampus combine to form a loop. Most external input comes from the adjoining entorhinal cortex, via the axons of the so-called perforant path.These axons arise from layer 2 of the entorhinal cortex (EC), and terminate in the dentate gyrus and CA3. There is also a distinct pathway from layer 3 of the EC directly to CA1, often referred to as the temporoammonic or TA-CA1 pathway. Granule cells of the DG send their axons (called "mossy fibers") to CA3. Pyramidal cells of CA3 send their axons to CA1. Pyramidal cells of CA1 send their axons to the subiculum and deep layers of the EC. Subicular neurons send their axons mainly to the EC.

The perforant path-to-dentate gyrus-to CA3 to CA1 was called the trisynaptic circuit by Per Andersen, who noted that thin slices could be cut out of the hippocampus perpendicular to its long axis, in a way that preserves all of these connections. This observation was the basis of his *lamellar hypothesis*, which proposed that the hippocampus can be thought of as a series of parallel strips, operating in a functionally independent way (Anderson et al., 1971). The lamellar concept is still sometimes considered to be a useful organizing principle, but more recent data, showing extensive longitudinal connections within the hippocampal system, have required it to be substantially modified (Anderson et al., 2000).

Perforant path input from EC layer II enters the dentate gyrus and is relayed to region CA3 (and to mossy cells, located in the hilus of the dentate gyrus, which then send information to distant portions of the dentate gyrus where the cycle is repeated). Region CA3 combines this input with signals from EC layer II and sends extensive connections within the region and also sends connections to strata radiatum and oriens of ipsilateral and contralateral CA1 regions through a set of fibers called the Schaffer collaterals, and commissural pathway, respectively (Hjorth-Simonsen ,1973; swanson et al ., 1978).

Region CA1 receives input from the CA3 subfield, EC layer III and the nucleus reuniens of the thalamus (which project only to the terminal apical dendritic tufts in the stratum lacunosum-moleculare). In turn, CA1 projects to the subiculum as well as sending information along the aforementioned output paths of the hippocampus. The subiculum is the final stage in the pathway, combining information from the CA1 projection and EC layer III to also send information along the output pathways of the hippocampus (Laurberg, 1979).

The hippocampus plays several roles and regulates many processes including emotional responses, the formation of new memories and spatial orientation. The main function of the hippocampus is learning and memory retention. Hippocampal damage can proceed to loss of ability to form new memories, whereas older memories may be safe. Thus, someone who gets an injury to the hippocampus may have a good memory of his childhood and the years before the injury, but comparatively little memory for anything that happened since. However, memory for