A Study on Aluminum Toxicity with Female Rats: Can Vitamin E Help to Reduce its Effect on Biological Systems?

A project submitted

by

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BRAC
UNIVERSITY

Inspiring Excellence

Dhaka, Bangladesh

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Dedicated to:

My beloved parents, my brother and my husband, who always support and inspire me to face every challenge of life.

Certification Statement

This is to certify that the project titled "**A Study on Aluminum Toxicity with Female Rats: Can Vitamin E help to Reduce its Effect on Biological Systems?"** submitted in order to fulfill the partial requirements for the degree of Bachelor of Pharmacy from the Department of Pharmacy, BRAC University under the supervision of Dr. Sharmind Neelotpol, Assistant Professor, Department of Pharmacy, BRAC University and proper referencing have been made where the language, concept or writings of others are used.

Signed,

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Countersigned by the supervisor,

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Acknowledgement

Abstract

Increasing complaints about metal toxicity have been reported with the modernization of medical science. However, in this field, aluminum has been a renewed matter of concern. Therefore, more impotence is given to find out natural as well as synthetic substances that can alleviate the toxic effect of aluminum in the body. That is why; this study has aimed to identify the effect of vitamin E in reducing the toxic effect on biological systems caused by Aluminum. The study was conducted for 28 days and with total 18 female albino Wistar rats weighing 138-150g. 18 rats were divided into three groups- control, aluminum treated and aluminum plus vitamin E treated. The control group was receiving normal drinking water along with two drops of Tween 80 whereas aluminum treated group was getting aluminum nitrate solution 1ml/rat containing half 'x' of body weight and two drops of Tween 80. Moreover, aluminum plus vitamin E treated group was receiving aluminum nitrate solution 1ml/rat containing half 'x' of body weight along with 1ml/rat vitamin containing 22.5 mg of vitamin E per kg and also two drops of Tween 80. After 28 days, the rats were sacrificed, and blood was collected from the heart using ventricular puncture method. The collected blood was sent for hematological and clinical bio-chemistry examinations. At the same time, the liver, skin, brain, kidney and ovary of the rats were collected in the formalin and sent for histopathological study. The clinical biochemistry examination showed significant results for all the parameters except basophils, while the histopathology showed that vitamin E has the potential to eliminate the toxic effects caused by aluminum.

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List of Abbreviations

ALT: Alanine aminotransferase

AST: Aspartate aminotransferase

DFO: Deferoxamine

EFSA: European Food Safety Authority

ESR: Erythrocyte Sedimentation Rate

HCT: Hematocrit

JECFA: Joint FAO/WHO Expert Committee on Food Additives

MCH: Mean Corpuscular Hemoglobin

MCHC: Mean Corpuscular Hemoglobin Concentration

MCV: Mean Corpuscular Volume

MRL: Minimum Risk Level

RBC: Red blood cell

SGOT: Serum glutamic oxaloacetic transaminase

SGPT: Serum glutamic pyruvic transaminase

USA: United State of America

WBC: White blood cell

WHO: World Health Organization

CHAPTER 1
INTRODUCTION

1.1.   Introduction

Throughout the years of evolution of humankind metal has been the blessing as well as the hoariest toxic element. There are almost 105 elements in the periodic table among which 80 elements are metal and 30 have been stated as toxic elements. From ancient period, human started to use metals for several purposes, for instance- in 200 B.C., utilization of lead begun when their supply was plenty and was derived from ores as a byproduct of silver. Furthermore, scientist cited arsenic and mercury in 370-287 B.C. and 23-79 A.D. respectively. Similarly the exploitation of zinc, aluminum, cadmium, etc. has been observed from a long time (Goyer & Clarkson, 2001). Unlike the other toxic substances, metals can neither be propagated nor be obliterated by human (Beijer & Jernelov, 1986) and they are normally redistributed in the environment by both geological and biological phases. (Goyer & Clarkson, 2001). The pathway of this transport process of metal elements is shown in figure 1.1.
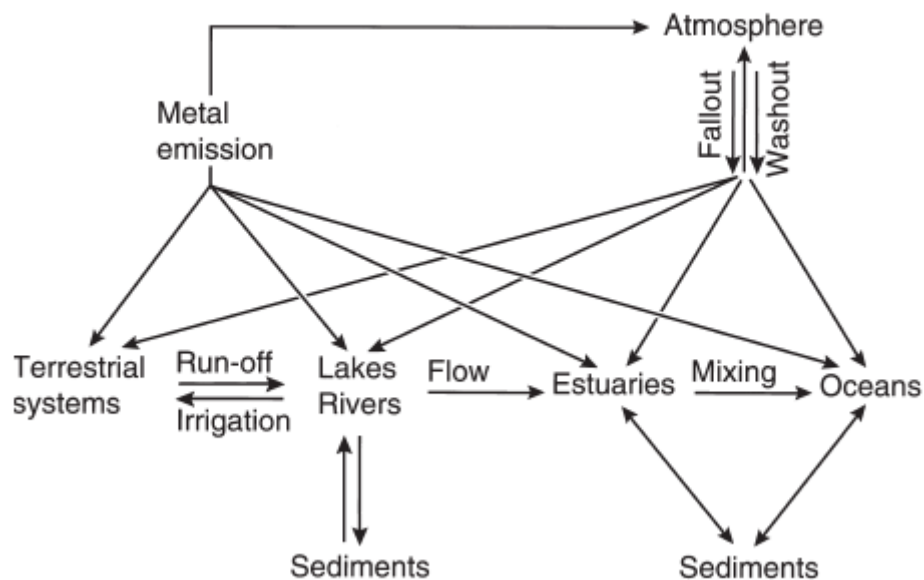
Figure 1.1: Pathway of transport of metals in environment (Goyer & Clarkson, 2001)

The potential health impacts of metals are influenced by the human in two ways. The first way is by environmental transport which is caused by human or anthropogenic contribution to food, air, soil, etc. and the last way is by modifying the definite or biochemical form of the elements (Beijer & Jernelov, 1986). It has been reported that

most of the metals are highly toxic to living organism along with those who are quoted as essential (Mudgal, Madaan, Mudgal, Singh, & Mishra, 2010). The flow of metals on human body has been showed below in figure 1.2. Within these toxic metals, cadmium is found to cause various morphological changes in the kidney especially when used for a longer period of time (Wu, et al. 2001). Even if it is used in pregnancy, it can reduce birth weight as well as can cause premature death of the fetus (Henson & Chedrese, 2004). Moreover, arsenic is cited as harmful for cardiovascular system and also reported as one of the significant reasons behind hypertension (Mudgal, et al. 2010).
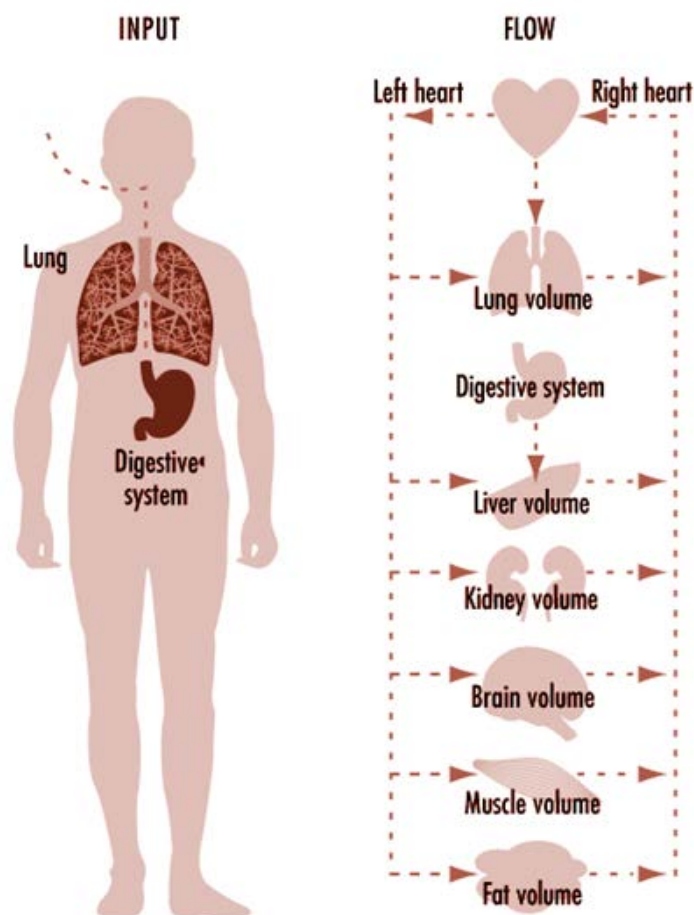
Figure 1.2: Flow of metals within human body (Silbergeld, 2012)

Aluminum is one of the most significant metals (Shaw & Tomljenovic, 2013) and considered as the third profuse element of the crust (Rebai & Djebli, 2008). In daily human life, aluminum is consumed through food, beverage, aluminum containing antacids, etc. Moreover, it is also found in air, antiperspirants, drying agents,

hemodialysis, etc (Analytical Research Labs, INC., 2012). Water, food additives and contamination by aluminum utensils and containers are some of the sources of aluminum in the food supply (Greger J. , 1992). Aluminum is absorbed both orally and by inhalation and also eliminate readily from the body, along with the concentrated presence in human tissues, bone, brain, kidney, liver, etc. (Bassioni, Mohammed, Zubaidy, & Kobrsi, 2012). Furthermore, due to the insoluble nature, the bioavailability of aluminum is restricted despite of being a ubiquitous element and low content of it is found in bio-system (Vasudevaraju, Govindaraju, Palanisamy, Sambamurti, & Rao, 2008)

A low dose of aluminum usually does not affect human health. Moreover, it has been established by the World Health Organization (WHO) that daily tolerable amount of aluminum intake is 1mg/kg body weight. However, now-a-days, for an excessive availability of aluminum in daily life, the risk of getting exposed to and ingest more than what we can manage has increased (Bassioni, et at. 2012). Several studies have found that aluminum causes different types of toxicity in the human body. The target organs for aluminum toxicity are the lungs, bone, skin or central nervous system (Goyer & Clarkson, 2001). It is a debatable issue whether chronic aluminum exposure to the animal may cause neurotoxicity and contribute to diseases like Alzheimer (Abubakar, Taylor, & Ferns, 2003). However, upon aluminum exposure brain tissue calcium rises which also exert a toxic effect. Furthermore, during occupational exposure to aluminum dust, excessive deposition of aluminum dust on lung can cause lung fibrosis. In case of aluminum toxicity on the bones, if aluminum, especially aluminum-containing antacid is taken excessively, osteomalacia can occur due to its interference with intestinal phosphate absorption (Goyer & Clarkson, 2001). Besides, studies of aluminum on the animal model have also shown nephrotoxicity (Geyikoqlu, Turkez, Bakir, & Cicek, 2013), hepatotoxicity (Abubakar, et al. 2003), toxicity in reproductive system (Llobet, Colomina, Sirvent, Domingo, & Corbella, 1995) etc.

In everyday life, vitamin E is also consumed by human through different natural sources along with synthetic vitamin E. A study revealed that 100-400 mg/d (30 IU/d) is consumed from both natural as well as synthetic sources by a large group of people in the United States. Vitamin E is nothing but a fat soluble vitamin which is found in liver, nuts, milk, egg, green plants, vegetables, etc. (Bendich & Machlin, 1987). Along with this, it is considered as one of the most important components found in the human diet. By counteracting lipid peroxidation, vitamin E helps to body's healthy biological system

(Abdul-Hamid, 2013). Furthermore, about the effect of vitamin E on aluminum toxicity, the researchers revealed that vitamin E along with selenium may minimize the harmful effect of nephrotoxicity in pregnant rats developed by aluminum (Abdul-Hamid, 2013). Additionally, vitamin E also shows positive effect on hepatotoxicity (Bendich & Machlin, 1987). However, sufficient works have not been found which revealed the effect of vitamin E on aluminum toxicity especially on other biological system such as brain, skin, bone, reproductive system etc.

1.2.   Aim:

The overarching aim of this study was to evaluate the *in vivo* effect of vitamin E on aluminum toxicity.

1.3.   Objectives:

In this study the main focus was on aluminum toxicity in skin, reproductive system, liver and kidney. Therefore, the objectives of this study were-

1. to fix a specific dose for the induction of aluminum toxicity and to fix a therapeutic dose for vitamin E on rats.

2. to observe the effect of vitamin E on aluminum toxicity in kidney, liver, skin, reproductive system, and brain.

1.4.   Rationales of the study

Initially, the reason for conducting this study was the enormous availability, use, and toxicity of aluminum. Every day people are being exposed to aluminum through several sources as it is present almost everywhere around us with a large number of applications. It is most extensively found in the environment and about 8% of the earth crust contains aluminum (Rebai & Djebli, 2008). Air, water, and dietary sources are cited as primary sources of aluminum. It is used for the purpose of packaging of almost 95% beverage cans (Greger J. , 1992). Other possible sources are housing materials (such as cookware utensils, serving utensils, etc.), cookware, aluminum foils, cosmetics, etc.

Secondly, vitamin E is one of the most important fat soluble vitamins which are required for several functions of the body. Like aluminum, vitamin E is also consumed by most of the people, almost daily from both natural as well as synthetic sources. Furthermore, in different reports, it has been stated that vitamin E may minimize the adverse effects of aluminum on biological system (Abubakar, et al. 2003).

Lastly, in Bangladesh no significant work has been found to be documented on the toxic effect of Aluminum on biological systems. The reason behind less focus on aluminum toxicity might be due to lack of awareness. People in Bangladesh are using aluminum extensively without knowing its side effect and in the long run they may face irreversible adverse impact of using aluminum. Therefore, an animal model has been designed to evaluate the toxic effect of aluminum and see the effect of vitamin E whether it can reduce the toxicity of aluminum.

CHAPTER 2
BACKGROUND OF THE STUDY

2.1. History

In accordance to a leading chemistry researcher, we are now in the age of aluminum (Shaw & Tomljenovic, 2013). Aluminum is found in large amount in the environment and due to this reason, it is declared as the third most enormous metal (Rebai & Djebli, 2008). Besides that, it is also included in the group of metals that are started to use by the human from the pristine period. Though Hans Christian Ørsted discovered aluminum in 1825 (Analytical Research Labs, INC., 2012), L.B.G de Moveau proposed the name alumina for the base of alum in 1761and in 1807, the name aluminum was proposed by Sir Humphery Davy. The name "Aluminum", derived from alumen which is a Latin name for alum (Aluminum: History). This alum (Aluminum potassium sulfate) was first started to use in Egypt over 500 years ago. Furthermore, the ancient Greek also used anhydrous aluminum sulfate as an astringent. In 1800, Humphry Davy started to experiment with electrolysis which is a central process in the history of aluminum. This remarkable chemist tried several times to isolate aluminum, but he did not achieve success till his death. Finally, Hans Christian Ørsted succeeds to isolate aluminum in small amount by rapidly heating aluminum chloride along with potassium amalgam and after that by distilling out the mercury (Geller, 2007).
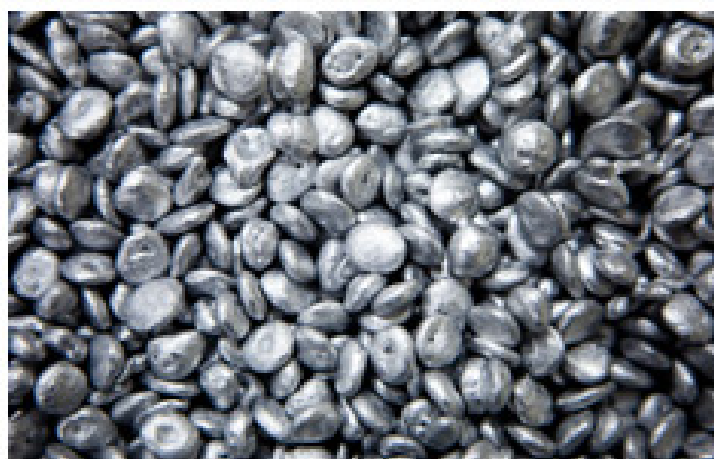
Figure 2.1: Aluminum (CKOE, 2011)

2.2. Properties of Aluminum

Aluminum indicates to Al, is a silver white powdered substance with a melting point of 660.37°C. It is readily inflammable and on new aluminum surfaces in air or water, a hard transparent few molecules thick oxide layer forms due to its high affinity for oxygen. Additionally aluminum is a light metal with a density of 2.7 g/cm^3 (Willey, 2012).

2.3. Fate of Aluminum in Environment

It is the natural process by which aluminum is released into the environment. Factors that manipulate the release and subsequent transport of aluminum in the environment are chemical specification, hydrological flow paths, interaction between soil and water and the geological materials (WHO, 1997). Moreover, for dissolving and transporting aluminum in an alpine soil, organic acids have been considered as an important agent (Litaor, 1987). Beside that in water, it can produce different forms for instance monomeric and polymeric hydroxide species, colloidal polymeric solution, etc. Aluminum has a minimum solubility in pure water with the pH range of 5.5-6 (WHO, 2003).

2.4. Sources of Aluminum

There are thousands of sources through which we can expose to aluminum such as drinking water, food, vaccines (for more details, see Appendix 3), air, use of some consumer as well as pharmaceutical products or even in the workplace where the workers deal with the production or handling of aluminum products. Table 2.1. and figure 2.2. are showing some sources of aluminum (for more details about the aluminum source, see Appendix 1).

Table 2.1: Sources of Aluminum (Shaw & Tomljenovic, 2013)

Sources of Al	Daily intake (mg)
Food (Natural)	1-10
Food (with al additives)	1-20
Water	0.08-0.224
Pharmaceutical products	126-5,000
Vaccines	0.51-4.56
Consumer products	70
Cooking Utensils	0-2

THE EARTH'S CRUST   ROCKS AND SOILS   VEGETATION   WATER   DUST PARTICLES IN THE AIR   CLAYS
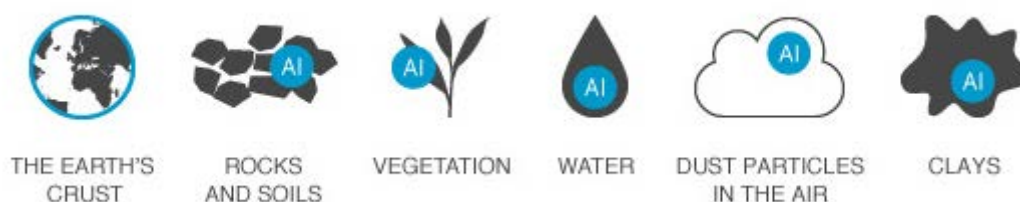
Figure 2.2: Source of Aluminum (HYDRO, 2012)

2.5. General population exposure

In the environmental air, the concentration of aluminum in areas without any emission is 0.05 to 0.5µg/m^3 whereas in an urban area it is 0.5 to 4µg/m^3 and near aluminum emission source the concentration is 4 to 15µg/m^3 (Wilhelm, 1994). Earlier it has been said that aluminum has a strong affinity to oxygen for which it form a protective oxide layer which is considerably insoluble in the pH range of 4.5 and 8.5. Due to the formation of this protective layer at the workplace, workers don't experience any primary contact with metallic aluminum.

Furthermore, aluminum is also found in foods naturally as well as the use of aluminum-containing food additives. Potatoes, spinach, tea, etc. are natural foods that contain a high amount of aluminum. The aluminum concentrator in food can also increase through the use of aluminum cookware, utensils, and wrappings (WHO, 1997). Besides that, the total aluminum exposure through the air is insignificant; an average aluminum intake from food of 5 mg/ day and aluminum concentration in drinking water is 0.1 mg/liter (WHO, 2003). A study in German on the concentration of aluminum in food had found that plant origin has high aluminum content than animal food (Wilhelm, 1994) . If we consider aluminum content in drinking water, the geology and pH of the water are important factors (DFG, 2013).

In addition to this, aluminum is also present in many pharmaceutical products like vaccines, antacids (Aluminum Hydroxide), buffered aspirins and also in consumer products like cosmetics. In multiple daily doses for an extended period, antacids and buffered aspirins are taken containing 5-562 mg/kg of aluminum (Lione, 1983).

2.6. Occupational exposure

People works in or live near industries where aluminum products are manufactured, are at high risk of increased level of aluminum exposure and in the metal industries, foundries and plant processing or treating the corresponding materials it is expected to expose to dust containing aluminum or other forms of aluminum (DFG, 2013). The exposure level has been found to be higher in inert gas and active gas welding compare to other working areas. Depending on the situations of the welding procedure, the welding fumes containing aluminum ozone is produced which is also accelerated by UV radiation as well as the highly reflective surface of the work pieces (Spiegel-Ciobanu, 2009) .

2.7. Global production of aluminum

Day by day the production and use of aluminum in the global market are increasing. Primarily aluminum is manufactured in 42 countries with USA (United State of America), China, Canada, etc. For the production of aluminum, one of the most essential raw materials is Bauxite. In 2006, with a view to produce aluminum, USA converted 90% of the bauxite to alumina (Al_2O_3) (USGS, 2007d). An estimated 2.3 million metric tons of aluminum metal were produced in five USA companies in 2006 whereas 58% of the total world bauxite was manufactured by Australia, Brazil and China (DFG, 2013).

2.8. Absorption, Distribution, Metabolism, and Excretion

It has been reported that oral bioavailability of aluminum is 0.1 to 0.4%, and it is augmented by citrate, acidic pH, and uremia and may be decreased by a silicon containing compounds. Aluminum in the acidic condition such as in stomach primarily occurs as monomolecular hexahydrate which is referred as "free" aluminum. However in stomach, this acidic condition as well as residence time ensures the solubilization of consumed aluminum to free aluminum species and this solubilized form either can re-complex with the anion to the initial form or can form a new complex with dietary ligands which play an important role in the complexation process. Additionally aluminum hydroxide complexes form in the duodenum where the pH increases which is quickly precipitated as insoluble salt and finally excretes in the feces (Reiber, Kukull, & Standish-Lee, 1995). Aluminum concentration in normal plasma level is 1 to 2µg/L, and concentration is higher in lung tissue compare to bone (Krewski, et al. 2009). Since there are no suitable aluminum radioisotopes present in the environment, aluminum absorption has been tough to quantify. There is only one isotope of aluminum with a half-life of 7.2×10^5 years which is [26]Al whereas others have half-lives of less than 10 minutes. But using [26]Al is too costly for radiochemical detection (Day, Barker, Evans, Perks, & Seabright, 1991). Another reason for which aluminum quantification is found to be difficult is that the gathering and examination of the fecal sample do not provide significant data for detecting aluminum absorption especially when it is less than 1% (Alfery, 1989).

By inhalation, aluminum absorption is less among the general population than occupational exposure. Generally, 0.01 and 0.2 mg aluminum is inhaled per day depending on the individual exposure condition. It has been calculated that 1.9% of a quantity of aluminum firstly deposited in the lung was available in the blood (Priest, 2004).

Though the exact mechanism of aluminum absorption is unknown but it has been expected that aluminum absorption in intestine follows both paracellular passage routes and transcellular passage routes. It has been found that 23% aluminum uptake is done by nonsaturable processes, and the rest is due to active or saturable process when it is perfused as $AlCl_3$ at pH 2 (Adler & Berlyne, 1985). Nevertheless, it has been assumed through different studies that the absorption of aluminum is relatively very low and is sensitive to aluminum intake (Greger J. L., 1993). Urine is the main route for aluminum excretion although a small amount can be detected on fecal sample (WHO, 2003). Moreover, it is the bone and liver concentrations which are used as indices of aluminum exposure studies since aluminum concentration in these tissues are susceptible to oral and parenteral aluminum content (Greger J. L., 1993). Albumin and transferring are the most important plasma proteins among the all with which aluminum binds.

2.9. Toxicity:

Several studies have been revealed about aluminum toxicity on exposure to human as well as animals via inhalation, oral or dermal exposure. It has been suggested after occupational exposure studies and animal studies that lungs, as well as the nervous system may be the most susceptible part for aluminum toxicity (ATSRD, 2008). Aluminosis is a disease of the lung caused by aluminum which is characterized by diffuse interstitial fibrosis and in which aluminum compounds can accumulate in lung along with disturbance in lung clearance. Disease of the central nervous system is also observed on long-term exposure to aluminum (DFG, 2013).

2.10. Minimum Risk Level

An estimate of daily human exposure to a perilous substance without a considerable risk of adverse effects over a specific duration of exposure is called minimal risk to human or

MRLs and it can be determined by short, intermediate or long-term exposure for inhalation as well as oral route (DFG, 2013).

From acute, intermediate and chronic duration inhalation, no MRLs were derived for aluminum but exposed respiratory effects include an increase in alveolar macrophages, granulomatous lesions in the lungs and peribronchial lymph nodes, and increases in lung weight (Drew, Gupta, & Bend, 1974). For oral exposure of aluminum, MRLs were obtained. But for an acute duration oral MRLs those data were not adequate where as the MRLs for intermediate and long-duration oral MRLs for aluminum were enough (DFG, 2013).

2.11. Effect on Human

There are few studies indicating aluminum toxicity on acute exposure to aluminum via different sources. In 1988, aluminum was accidently distributed to the population of Camelford, England through water supply which was treated with aluminum sulfate. However, this aluminum exposure was indicated by nausea, vomiting, mouth ulcer, skin ulcer, etc (WHO, 2003). As these indications were mild and short-lived, so no long lasting effect was experienced (Clayton, 1989). After occupational exposure to nano-particles of aluminum, quite a few deaths have been reported for example, a 19-year male worked in a place widely contaminated with aluminum particles found to have dyspnea after two and half years. Nevertheless, after sometimes, the symptoms became worse for which finally he died after eight months. From that factory where that male used to work, 27 workers were examined among of which two dies and four others had radiological changes on chest X-ray (WHO, 2003). Besides that in a study, metabolism of aluminum along with real failure caused by this metal were observed in 36 control subjects, 30 non-dialyzed uremic patients, 57 dialyzed uremic patients died due to different causes and 38 dialyzed uremic patients died of dialysis encephalopathy. From this study, it was revealed that aluminum is readily excluded from the body which is altered with renal failure. It was found that bone and liver aluminum level increased in non-dialyzed uremic patient and patients dying of dialysis encephalopathy, tissue aluminum level were highest (Alfery, 1989). Aluminum is also widely used as a vaccine adjuvant and due to this reason experiment was done on aluminum toxicity when used as vaccine. The result clearly revealed that sever immunological disorder can develop with

aluminum vaccine adjuvant (Tomljenovic & Shaw, 2011). Regarding the cardiovascular effect of aluminum toxicity on both acute and intermediated-duration inhalation exposure in human, no significant studies were established. Whereas upon chronic exposure to aluminum, dilation and hypertrophy of the right side of the heart were found (McLaughlin, Kazantzis, & King, 1962) (Mitchell, Manning, & Molyneux, 1961). Moreover, it had been assumed that aluminum exposure might be a risk factor for Alzheimer disease in human and to identify this, WHO (World Health Organization) had evaluated 20 epidemiological studies which have been carried out in this matter. Out of six studies showing the relationship between aluminum and Alzheimer diseases, three indicated positive results where the other three did not (WHO, 2003). Aluminum is also considered as a carcinogenic agent for human since some bladder cancer cases were found to people where aluminum exposure is high (DFG, 2013).

Furthermore, in a study leaching of aluminum from aluminum foil was carried out using three analytical techniques and indicated that use of aluminum foil for cooking contributes significantly to the daily exposure of aluminum. This leaching of aluminum was affected more in acidic solution and has health risk on human (Bassionil, Mohammed, Zubaidy, & Kobrsi, 2012). On human, no reproductive or dermal aluminum toxicity was established.

2.12. Effect on animals

Many studies have been done on animals for aluminum toxicity, and most of them showed significant effects. For 100 days, aluminum nitrate in drinking water was given to groups of 10 Sprague-Dawler rats at the dose of 0, 26, 52, and 260 mg/body weight and their organs, as well as body weight, histopathology of the brains, hearts, lungs, kidneys, livers and spleens were examined. From this study, no significant result was observed except increase in body weight (Domingo, Llobet, Gomez, Tomas, & Corbella, 1987). Another study was done by aluminum nitrate on male Sprague-Dawler rats at 0, 13, 26 or 52 mg/body weight for 60 days prior to mating and to virgin females for 14days before mating along with the treatment continuing throughout mating, gestation, parturition and weaning off the litters. This study reported no fertility, litter size or intrauterine or postnatal offspring mortality (Domingo, Paternain, Llobet, & Corbella, 1987). To evaluate and characterized the *in vitro* cellular effect of aluminum oxide nano-

particle a study was done on rat lung macrophage. The result of this study revealed none to negligible toxicological effects that occurred with exposure of macrophages as much as 500µg/ml for 24 hours (Wagner, et al. 2001).

In another study on Sprague-Dawler rats, sodium aluminum phosphate or aluminum hydroxide was given for 28days at 0,5,76,141 or 288/302 mg of aluminum/kg of body weight. In this study, no deposition of aluminum on bone or any effect on body were observed (Hicks, Hackett, & Sprague, 1987).

2.13.   Methods of reducing toxic effect

Despite the fact that the exact mechanism of aluminum absorption has not identified yet, aluminum studies that elucidated these mechanisms would be beneficial for establishing method or treatment of reducing the absorption followed by distribution. There are some chelating agents which are used against aluminum toxicit,y but some of them have limited use for their toxic effect, for instance, DFO (deferoxamine) which have been found to use for reducing the aluminum body burden. Another chelating agent which have been used for aluminum toxicity is 1,2-dimethyl-3-hydroxypyrid-4-one (DFG, 2013). Similar to the mechanism of aluminum absorption the mechanism of toxicity has not been recognized for most of the toxic end point.

2.14.   Studies on Aluminum and Vitamin E

Only few researches have been conducted to evaluate the effect of vitamin E on aluminium toxicity. Since kidney is thought to be one of the most effected organs by aluminum toxicity, a study was aimed to find out the adverse effect of aluminum chloride on the kidney of pregnant rats at the same time in this study the effect of vitamin E and selenium in aluminum toxicity was also observed. This study was conducted for 3 months and the result indicated histopathological changes in pregnant rats which were minimized by vitamin E and selenium (Abdel-Hamid, 2013).

Another study was done to evaluate aluminum toxicity on lung and effect of vitamin E on this toxicity. For developing aluminum toxicity a group of male albino rats were treated with aluminum toxicity only and another group was treated with aluminum along

with vitamin E. After 4 weeks the animals were sacrificed and found that the plasma and liver aluminum level of first group was increased markedly whereas plasma and liver aluminum level were controlled in the group treated with vitamin E (Abubakar, et al. 2003).

CHAPTER 3
MATERIALS & METHODS

3.1. Reagents

The reagents used in this study were aluminum nitrate, E-Cap, Tween 80 and ketamine. Aluminum nitrate was collected from BRAC University Department of Pharmacy. Vitamin E was purchased as capsule called E-Cap manufactured of Drug International. Tween 80 was used to dissolve vitamin E in water which was collected from Jahangirnagar University, Dhaka, Bangladesh. Moreover, ketamine was brought from Lag Pharma Bangladesh.

3.2. Experimental animal

Female albino Wistar rats weighing 138-150g were kept in the pharmacology laboratory of Jahangirnagar University. Throughout the whole study, the rats were maintained in $22\pm1°C$ temperature, and were allowed drinking water and foods for 28 days. Besides that, they were divided into three groups, with six animals per group:

1. Normal drinking water with two drops of Tween 80 (Control group).

2. Aluminum nitrate solution 1ml/rat containing half x of body weight aluminum nitrate (Al treated group) along with two drops of Tween 80.

3. Aluminum nitrate 1ml/rat along with 1ml/rat vitamin E solution containing 22.5mg of vitamin E per kg and two drops of Tween 80 (Rats treated with both Al and Vitamin E).

Both aluminum nitrate solutions and vitamin E solution were administrated orally.

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┌─────────────────┐
│  Total 18 Rats  │
└────────┬────────┘
         │
    ┌────┴────┬──────────────┐
    ▼         ▼              ▼
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Control Group (6 rats)	Treated with Aluminum Nitrate (6 rats)	Treated with Aluminum Nitrate and Vitamin E (6 rats)
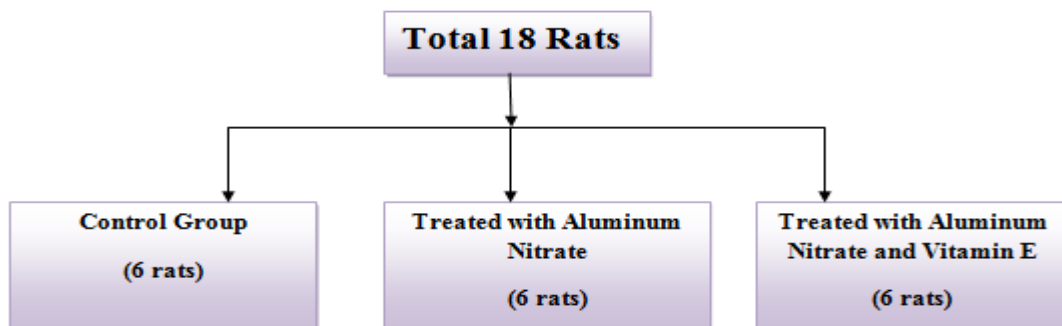
Figure 3.1: Grouping of experimental rats

3.3. Experimental procedure

It has already been said that the experimental rats were grouped into three small groups with 3X6=18 rats. These rats were receiving the treatments orally on a daily basis for 4 weeks and within these 4 weeks the rats were weighed in order to keep the records. At the same time, a small portion of rat's skin were shaved with a view to apply aluminum nitrate solution and aluminum nitrate plus vitamin E solution respectively to the rats from both aluminum treated and aluminum plus vitamin E treated groups. After 4 weeks these rats were sacrificed. Before scarifying, ketamine were administrated intra-peritoneally to the rats. After that, blood samples were collected from post vena cava and then transferred into 10 ml heparinised glass tubes. The collected samples were taken to the laboratory, where these samples were tested using waite chemistry by auto biochemistry analyzer of Olympus Company in order to determine bilirubin, SGOT, SGPT, Urea and creatinine level and with the help of automated hematology analyzer, hematology examination was done.

3.4. Preparation of plasma

In order to remove the red blood cells and to separate plasma, the collected bloods were centrifuged in a centrifugation machine at 4000 rpm for 10 minutes. The plasma samples were then collected using dry Pasteur pipette and stored for analysis in a refrigerator.

3.5. Histopathology

At first, the livers, kidneys, ovaries and skins of two rats and brain of a rat from each group were collected. After that the fat of these organs was removed and kept in a container containing 10% neutral buffered formalin and with the help of graduated ethanol (50-100%) the organs were dehydrated. Then the organs were cleared in xylene and embedded in paraffin. After staining with hematoxylin and eosin dye, the organ tissues were sectioned and examined under the light microscope (Arsad, Esa, & Hamzah, 2014) (Ikawa, et al. 2002).

3.6. Statistical Analysis

Statistical analyses were carried out using the Portable IBM SPSS Statistics version 19. For continuous metric variables, mean and standard deviation, standard error were used as descriptive measures, while the independent t-test was used for comparisons when the data could be shown to be normally distributed

CHAPTER 4
RESULTS

4.1. Results

From the collected blood samples, two types of tests were performed. The first test is hematology test, and another test is clinical biochemistry examination. The liver, kidney, skin, ovary and brains of the rats were collected in order to conduct the histopathological test.

4.2. Body weight
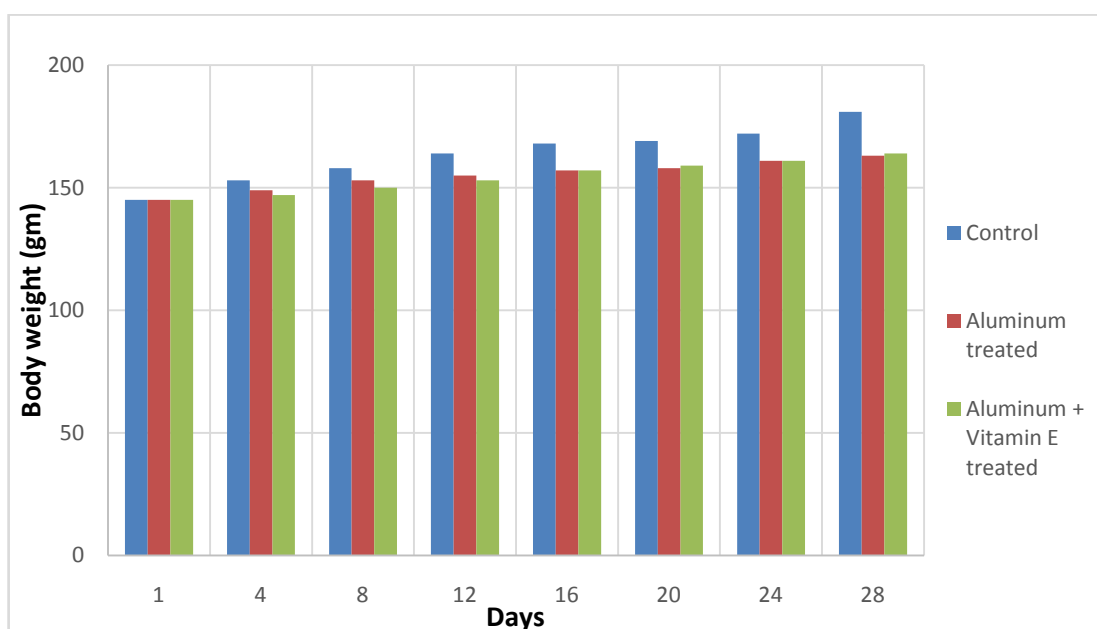
Figure 4.1: Comparison of body weight among control, Al-treated and Al+Vit E treated rats

In this study body weight variation, among the rats of three groups has been observed.

4.3. Liver

For liver Bilirubin, AST (Aspartate aminotransferase)/SGOT (Serum glutamic oxaloacetic transaminase) and ALT (Alanine aminotransferase)/SGPT (Serum glutamic pyruvic transaminase) level of control, aluminum treated and aluminum plus vitamin E treated groups were determined.

Table 4.1: Statistical output for liver

Parameters	Control	Treated with Al	Treated with Al+Vit E	p-value
	Mean (±SE)			
Bilirubin	0.22 (±0.02)	0.24 (±0.02)	0.25 (±0.2)	0.001
SGOT	156.83 (±10.85)	144.40 (±6.95)	169.20 (±6.67)	0.000
SGPT	67.67 (±3.96)	57.20 (±2.63)	47.20 (±2.46)	0.000

In case of liver toxicity, vitamin E showed reduction capacity of aluminium toxicity significantly ($p < 0.001$ and 0.000).

4.3.1. Histopathology output of liver
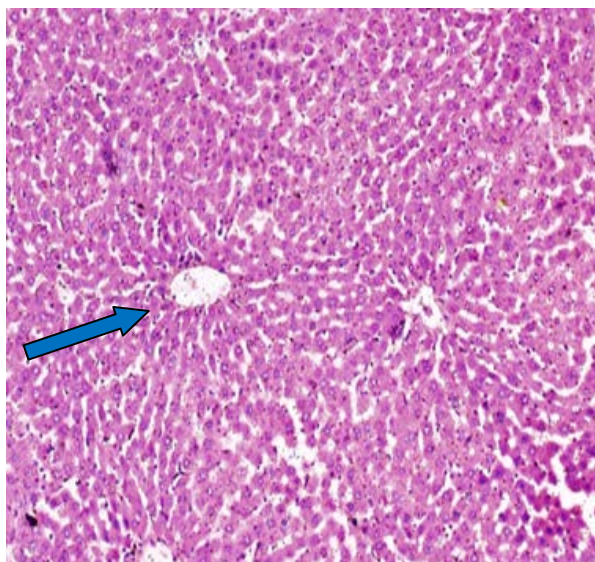
Figure 4.2: Histopathological Study of rat liver from control group
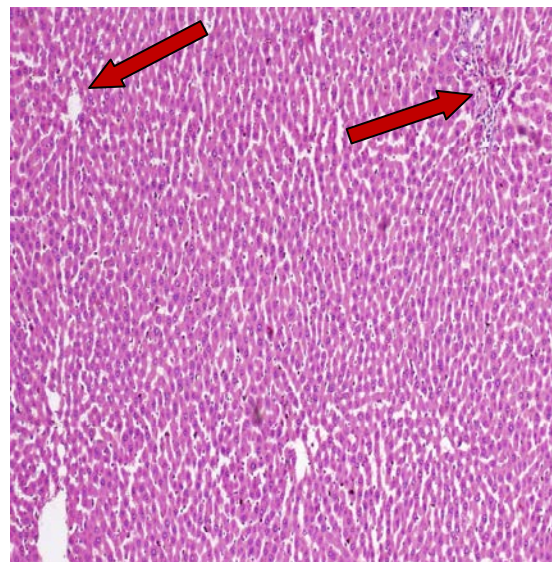
Figure 4.3: Histopathological Study of rat liver from Al - treated group

*Blue arrow = Normal
*Red arrow = Inflamed
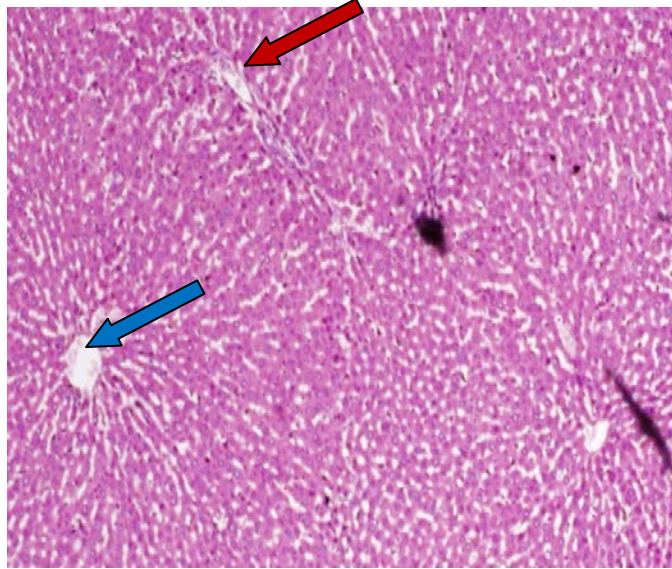
**Figure 4.4: Histopathological Study of
rat liver from Al + Vit E treated group**

The histopathology tests of liver also proved that vitamin E has the capacity to reduce the aluminum toxicity in liver.

4.4. Kidney

Since from theory and also from different studies it is found that the two important parameters for kidney test are the determination of urea and creatinine level, in this study these to parameters were also observed.

Table 4.2: Statistical output for kidney

Parameters	Control	Treated with Al	Treated with Al+Vit E	p-value
	Mean (±SE)			
Urea	30.18 (±0.59)	29.94 (±1.85)	28.64 (±0.50)	0.000
Creatinine	0.36 (±0.03)	0.36 (±0.03)	0.35 (±0.02)	0.000

After analyzing the data, it has been shown that vitamin E can reduce aluminium toxicity significantly ($p<0.000$). Here the two utilized parameters were urea and creatinine.

4.4.1. Histopathology output of kidney
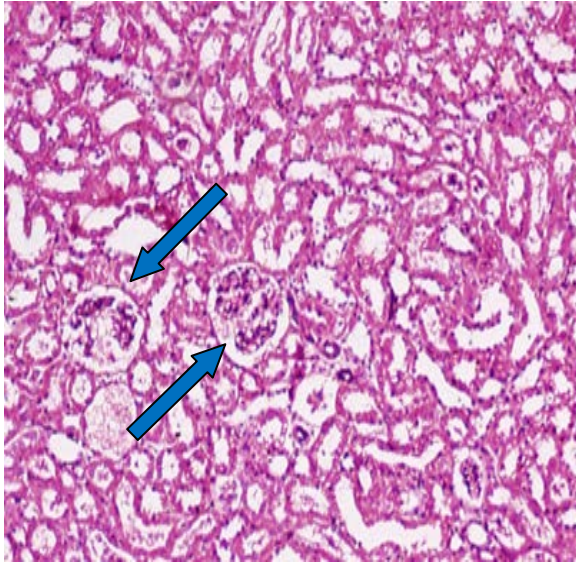
Figure 4.5: Histopathological Study of rat kidney from control group
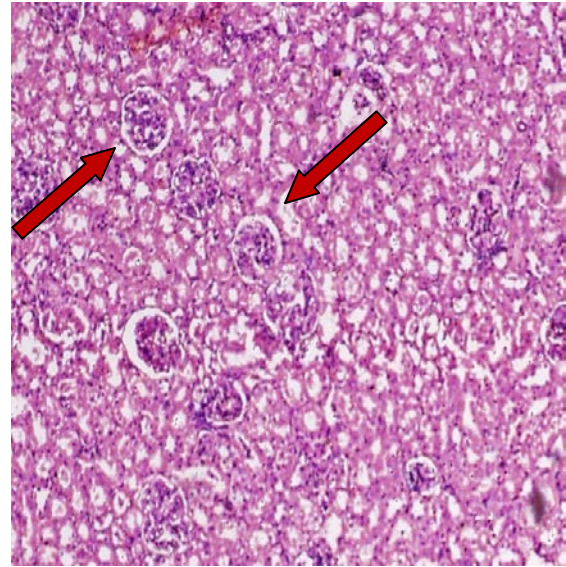
Figure 4.6: Histopathological Study of rat kidney from Al - treated group
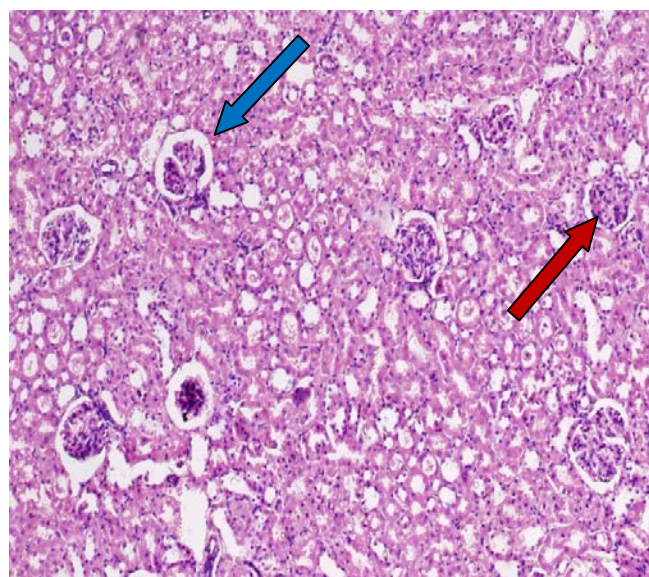
Figure 4.7: Histopathological Study of rat kidney from Al + Vit E group

Similar to liver, histopathlogy study of kidney indicated that vitamin E has the capacity to reduce the aluminium toxicity in kidney.

4.4. Histopathology output of skin, ovary and brain

Figure 4.8: Histopathological Study of rat skin from control group

Figure 4.9: Histopathological Study of rat skin from Al - treated group
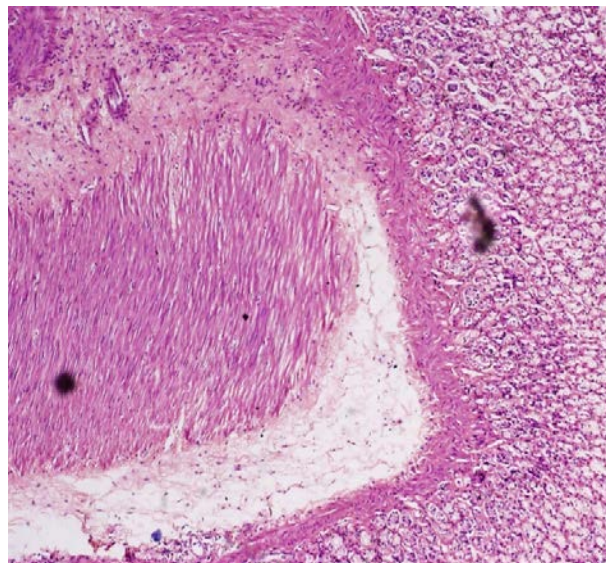
Figure 4.10: Histopathological Study of rat skin from Al + Vit E treated group

Figure 4.11: Histopathological Study of rat ovary from control group

Figure 4.12: Histopathological Study of rat ovary from Al - treated group

Figure 4.13: Histopathological Study of rat ovary from Al + Vit E treated group
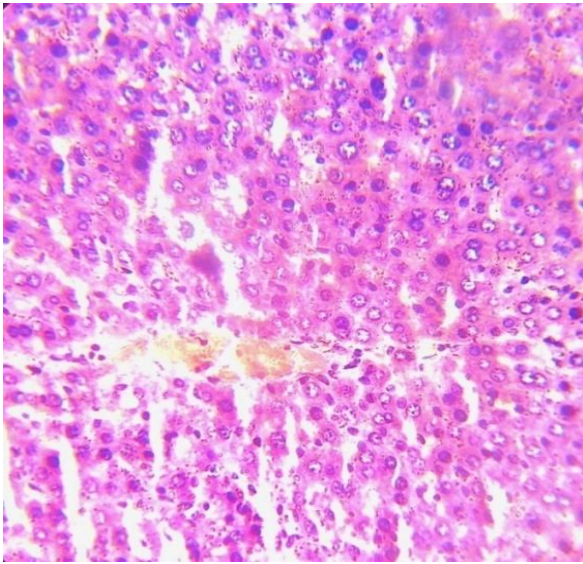
Figure 4.14: Histopathological study of rat brain from control group
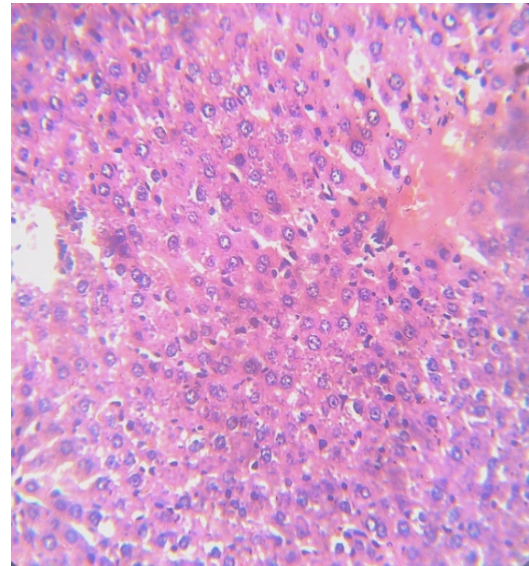
Figure 4.15: Histopathological study of rat brain from Al-treated group

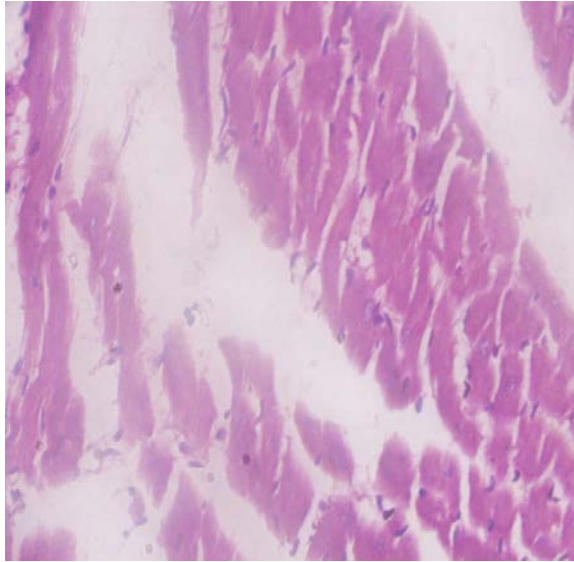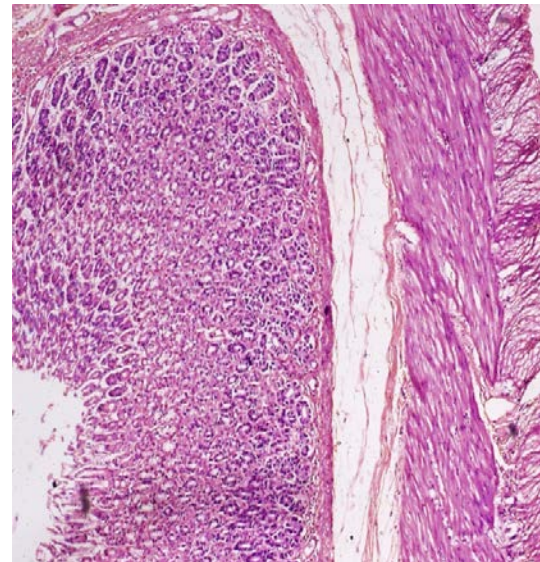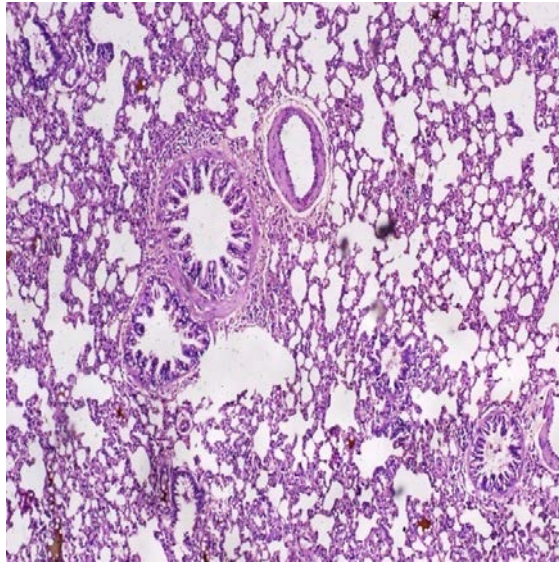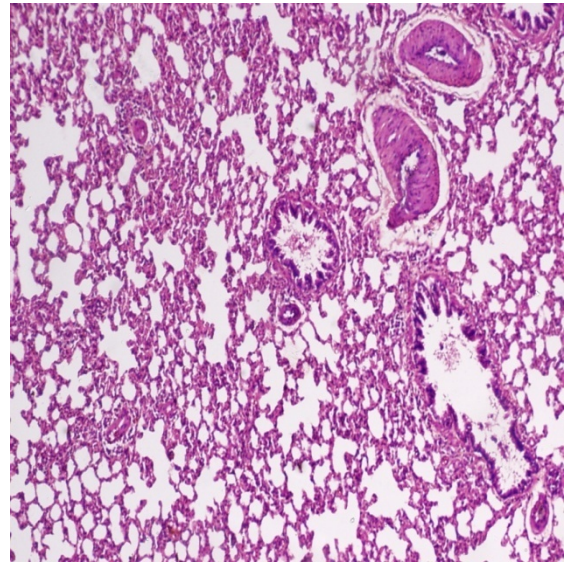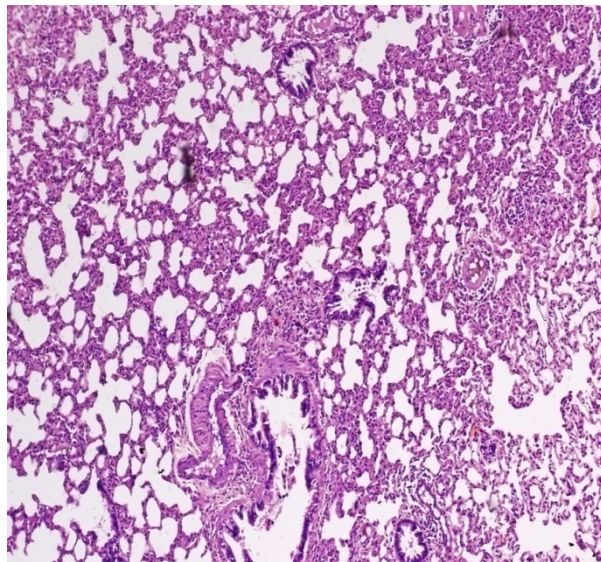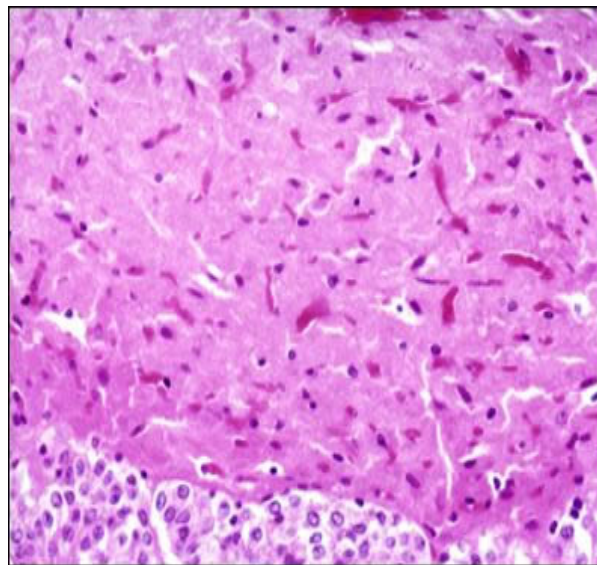**Figure 4.16: Histopathological study of rat brain from Al+Vit E treated group**

Histopathlogy study of skin, ovary and brains of the rat have not showed any visual changes.

4.5. Hematology test

In this study, development of toxicity treated with aluminum was compared with control group. On the other hand, the group treated with aluminum and vitamin E was treated with both control group and the group treated with aluminum. In this test, hemoglobin, HCT (hematocrit), MCV (mean corpuscular volume), MCH (mean corpuscular hemoglobin), MCHC (mean corpuscular hemoglobin concentration), ESR (erythrocyte sedimentation rate), WBC (white blood cell), RBC (red blood cell), Platelet, Neutrophil, Lymphocytes, Monocytes, Eusinophiles and basophil were observed. Statistical input showed significant results for all the parameters except for basophil.

Table 4.3: Statistical output for hematology test

Parameters	Control	Treated with Al	Treated with Al+Vit E	p-value
	Mean ($\pm$SE)			
Hemoglobin	12.80 ($\pm$0.45)	12.44 ($\pm$0.11)	12.34 ($\pm$0.24)	0.000
HCT	0.41 ($\pm$0.01)	0.39 ($\pm$0.01)	0.40 ($\pm$0.01)	0.000
MCV	61.47 ($\pm$1.15)	59.94 ($\pm$1.53)	56.82 ($\pm$0.90)	0.000
MCH	18.75 ($\pm$0.22)	18.62 ($\pm$0.39)	18.20 ($\pm$0.54)	0.000
MCHC	30.48 ($\pm$0.36)	31.80 ($\pm$0.65)	30.78 ($\pm$0.24)	0.000
ESR	8.00 ($\pm$0.58)	10.80 ($\pm$1.24)	8.20 ($\pm$0.97)	0.001
WBC	5783.33 ($\pm$300.46)	7020.00 ($\pm$726.22)	6800.00 ($\pm$856.74)	0.001
RBC	6841.67 ($\pm$285.74)	6700.00 ($\pm$151.67)	6660.00 ($\pm$180.55)	0.000
Platelet	476000.00 ($\pm$36834.77)	648200.00 ($\pm$40631.76)	597600.00 ($\pm$35176.13)	0.000
Neutrophil	16.00 ($\pm$0.73)	12.00 ($\pm$0.84)	21.80 ($\pm$0.80)	0.000
Lymphocytes	79.33 ($\pm$1.36)	83.00 ($\pm$1.22)	71.60 ($\pm$0.68)	0.000
Monocytes	2.00 ($\pm$0.37)	3.20 ($\pm$0.73)	4.60 ($\pm$0.68)	0.002
Eusinophiles	2.00 ($\pm$0.37)	1.80 ($\pm$0.20)	2.00 ($\pm$0.32)	0.012
Basophiels	0.00 ($\pm$0.00)	0.00 ($\pm$0.00)	0.00 ($\pm$0.00)	

CHAPTER 5

DISCUSSION

5.1. Discussion

Among the total mineral components of earth, about 8% contains aluminum (Jiang, Chen, Han, Tang, & Smith, 2008) and this trivalent cation is found in plant tissues as well as in most of the animals. Moreover, aluminum is mostly found in combination with other elements as it is highly reactive in nature (Bernardo, 2015). Since it is abundantly found in the environment, for different purposes aluminum is being used. However, universally aluminum has acknowledged as a poison (Adekunle, 2008) and reported to exert toxic effect particularly when excess amount of aluminum is accumulated in the body through different sources for instance, water, dietary source, environment etc (WHO, 2003). The main mechanisms of this toxicity are inhibition of the activity of enzyme and alternation in nuclic acid function through protein synthesis which leads to change in cell membrane permeability. As aluminum toxicity has recently become a matter of concern, several researches have been started to conduct with a view to find natural dietary sources that can reduce this toxic effect of aluminum on health (Bendich & Machlin, 1987) (Hodaman, Steer, & Arsenault, 1988)

This present study illustrated that the growth of the adult female Wistar rats of control group is normal but compare to control, the growth of aluminum treated rats is less. The same result was also shown with aluminum nitrate conducted by Domingo, et al. (1987). An interesting finding of this study is that, the average weight of the rats from aluminum plus vitamin E treated group was less initially. However, the trend of increasing the body weight has started with time. This finding indicated that if the study could be carried out for long time, it would give similar effect with control group. Furthermore, the resist of body weights of the rats was focusing on the fact that the food consumption of the rats might be affected by aluminum or it might cause any genetically changes responsible for body weight gain (Domingo, et al. 1987).

Moreover, a previous study was performed to observe the effect of vitamin E in reducing aluminum hepatotoxicity which was showed positive results (Abubakar, et al. 2003) and an analogues result has been found from this study. The clinical biochemistry test for bilirubin (p <0.001), SGOT and SGPT have showed a significant results (p <0.000) indicating that bilirubin, SGOT and SGPT level were increased in the rat from aluminum treated groups. Whereas, aluminum plus vitamin E treated group has showed the decreased level of bilirubin, SGOT and SGPT significantly. These findings focused on

the fact that vitamin E has the potentiality to reduce aluminum hepatotoxicity as bilirubin, SGOT and SGPT are considered as parameters for identifying hepatotoxicity. Furthermore, liver histopathology has showed that although there were no gross changes occurred in hepatic cells of control group, some changes have been observed in aluminum treated group and aluminum plus vitamin E treated group. However, in aluminum treated rat's liver it is observed that most of the central veins are injured due to aluminum toxicity, whereas some of the central veins in aluminum plus vitamin E treated rat's liver are present in normal architecture like control group indicating that the inflammation caused by aluminum toxicity is reduced by the effect of vitamin E.

Besides that, urea and creatinine level of the experimental rats were determined and compared as these two are considered as important parameters for determining diseases related to kidney. Like bilirubin, SGOT and SGPT, urea and creatinine level in aluminum plus vitamin E treated group has exhibited significant with aluminum treated group (p <0.000). From this study, it can be said that in aluminum treated group, oral administration of aluminum nitrate have developed nephrotoxicity which is indicated by the accelerated level of urea. Beside that the result for aluminum plus vitamin E treated group revealed that the nephrotoxicity developed by aluminum nitrate can be reduced by vitamin E and similar result was found from a previous study (Abdel-Hamid, 2013). Moreover, in histopathology study, remarkable changes have been observed for kidneys of aluminum treated group which were absent in control group and aluminum plus vitamin E treated group.

Moreover, for analyzing the changes in skin, ovary and brain, histopathology test was performed. Although aluminum is considered as a reason for Alzheimer disease (Foster, 2000) and even is known as a neurotoxic metal, (Martac, Podgorac, & Sekulic, 2010) no noticeable changes have been found from histopathology study of the brain. Since different studies have indicated that aluminum has toxic effect on brain (Krishnan, McLachlan, Krishnan, Fenton, & Harrison, 1988) (Martac, et al. 2010), the present study might also show the similar effect if it could be performed for more time. Besides that, as vitamin E has showed definite result on eliminating aluminum toxicity for liver and kidney, it can be expected that vitamin E would also show similar result for neurotoxicity. However, no visual changes have been observed for aluminum treated and aluminum plus vitamin E treated group in case of skin and ovary.

Finally, the hematology test reports of aluminum plus vitamin E treated group have also showed significant results compare to aluminum treated group especially for hemoglobin ($p < 0.000$), HCT ($p < 0.000$), MCV ($p < 0.000$), MCH ($p < 0.000$), MCHC ($p < 0.000$), ESR ($p < 0.000$), WBC ($p < 0.001$), RBC ($p < 0.000$), Platelet ($p < 0.000$), Neutrophil ($p < 0.000$) and, Lymphocytes ($p < 0.000$) but in case of monocytes ($p < 0.002$), eusinophiles ($p < 0.012$) and basophiles ($p < 0.003$) it did not show any specific changes. After analyzing the hematology report of aluminum treated group, negative changes were observed in most of the parameters. These changes are the indications for several diseases, for example, the increased ESR rate indicates inflammation, anemia, infection, kidney diseases etc. (Holm, 2015). Moreover the significant changes observed in aluminum plus vitamin E treated group is an evidence that vitamin E is capable of reducing the blood effects that are developed from aluminum toxicity.

CHAPTER 6
CONCLUSION

6.1. Limitations of the study

  - This study was performed for 28days only. However, if the study could be carried out for at least three months, it might give better results.
  - It would be better if the study could be conducted with few more groups having different doses of aluminum and aluminum with vitamin E.

6.2. Recommendations

  - In case of food habit, intake of vitamin E should be increased since it may reduce aluminum toxicity.
  - Aluminum cookware utensils can be replaced with other cookware that is enamel coated.
  - Aluminum foil should not be used to cook or grill the foods especially acidic foods since it has been estimated that 4 mg aluminum can be deposited to acidic foods during cooking with aluminum foil or in aluminum cookware (Rae, 2013).
  - Foods, medicine, cosmetics etc. containing aluminum can be avoided.
  - Habit of taking natural foods containing potassium, iron etc. (Moon, Davison, & Bandy, 1992) can be developed in order to diminish the toxic effect of aluminum.
  - Awareness program can be conducted more in order to prevent aluminum toxicity.
  - More study should be performed on aluminum toxicity in Bangladesh.

(For details about aluminum toxicity treatment, see appendix 2)

6.3. Conclusion

Due to gradual increase of the use of metals in several industries, agriculture, domestic and technological fields, the chance of human exposure to these metals have risen significantly. Nevertheless, excess metals exposure can be a severe threat for both human and plant, as they are easily distributed to the biochemical process (Mudgal. et al. 2010). Aluminum is also extensively present in nature and several reviews on the effect of aluminum upon human health have been reported. Still according to EFSA and JECFA there are lacks of specific toxicological data for aluminum (SCCS, 2014). Therefore, this study was performed to identify whether vitamin E can mitigate the venomous effect of

aluminum on biological systems or not. However from this study it is found that, vitamin E which is best known for its antioxidant effect, has potential to reduce the toxic effect on biological systems induced by aluminum. In addition to this the findings of this study regarding aluminum toxicity have led to concern about effect of aluminum in different organs but the satisfactory fact is that there are some substances like vitamin E that can reduce this outcome. Although global science has made a huge progression in the area of health sector, complains about the lacking of initiatives and specification of toxicity are coming on constant basis. In order to dispel this complain more study need to perform to identify the prevention of these toxicity.

CHAPTER 7
APPENDIX

7.1. Appendix 1: Sources of aluminum in details (Shaw & Tomljenovic, 2013)

Major sources of Al exposure in humans	Daily Al intake (mg/day)	Weekly Al intake (mg/day)	÷ PTWI * (1 mg/kg body weight; for an average 70 kg human, PTWI = 70 mg)	Amount delivered daily into systemic circulation (at 0.25 % absorption rate*)
Natural food	1–10	7–70	0.1–1	2.5–25 µ g
Food with Al additives	1–20 (individual intake can exceed 100)	7–140 (700)	0.1–2	2.5–50 µ g (250 µ g)
Water	0.08–0.224	0.56–1.56	0.1–2	0.2–0.56 µ g
Pharmaceuticals (antacids, buffered analgesics, anti-ulceratives, anti-diarrheal drugs)	126–5000	882–35,000	12.6–500	315–12,500 µ g
Vaccines (HepB, Hib, Td, DTP)	0.51–4.56	NA	NA	510–4560 µ g**
Cosmetics, skin-care products and antiperspirants***	70	490	NA	8.4 µ g (at 0.012 % absorption rate)
Cooking utensils and food packaging	0–2	0–14	0–0.2	0–5 µ g

* PTWI (provisional tolerable weekly intake) is based on orally ingested Al; generally, only 0.1-0.4 % of Al is absorbed from the gastrointestinal tract; however, Al may form

complexes with citrate, fluoride, carbohydrates, phosphates and dietary acids (malic, oxalic, tartaric, succinic, aspartic and glutamic), which may increase its gastrointestinal absorption (0.5-5 %). Co-exposure with acidic beverages (lemon juice, tomato juice, coffee) also increases Al absorption as well as conditions of Ca^{2+}, Mg^{2+}, Cu^{2+} and Zn^{2+} deficiency

** A single dose of vaccine delivers the equivalent of 204-1284 mg orally ingested Al (0.51-4.56 mg), all of which is absorbed into systemic circulation

*** The risk of antiperspirants is both from dermal exposure and inhalation of aerosols. Inhaled Al is absorbed from the nasal epithelia into olfactory nerves and distributed directly into the brain

7.2. Appendix 2: Treatment of aluminum toxicity

Recognition of aluminum toxicity is considered as important part of immediate medical treatment on the basis of risks for instance, exposure to aluminum, renal insufficiency etc. and symptoms like anemia, osteoporosis. It includes elimination of aluminum from the diet, TPN, dialysate, medications, antiperspirants, and an attempt at the elimination and chelation of the element from the body's stores which can be attained through the administration of deferoxamine through any of various routes.

- Serum aluminum level greater than 50-60 µg/L (mcg/dL) suggests aluminum overload, may correlate with toxicity, and can be used as an indication to start chelation therapy in symptomatic patients.

- Symptomatic patients with lower serum aluminum levels (eg, greater than 20 mcg/dL) may require chelation therapy.

- It is suggested by Kan et al that a low dose of deferoxamine therapy (2.5 mg/kg/wk) is therapeutically effective as standard dose (5 mg/kg/wk) for the treatment of aluminum overload.

Chelation therapy with deferoxamine should be initiated in consultation with a nephrologist and a medical toxicologist, and this can be performed upon admission.

- Deferoxamine, the metal-free ligand of the iron-chelate isolated from the bacterium *Streptomyces pilosus,* is used for acute and chronic iron toxicity and aluminum toxicity.

- It has a high affinity for ferric iron and does not affect iron in hemoglobin or cytochromes.

No surgical care is applicable to this disorder. Hemodialysis is performed in conjunction with deferoxamine as therapy for whole-body chelation (Bernardo, 2015).

7.3. Appendix 3: Aluminum neurotoxicity in various species

(Tomljenovic & Shaw, 2011).

Aluminum source/compound	Dose & duration	Route	Species	Neurodevelopmental adverse effects
Standard infant feeding solution	~20 μg/kg/day, >10 days	Intravenous (parenteral)	Human, premature infants	Reduced developmental attainment at the corrected post-term age of 18 months, as evidenced by significantly lower Bayley Mental Development Index (BMDI) scores (mean loss of one point on the BMDI/day of full intravenous feeding, after adjustment for potentially confounding factors) compared to infants fed with Al-depleted solutions
Al-containing antacids	Chronic	Oral	Human infants	Craniosynostosis (premature ossification of the skull and obliteration of the sutures
Al-containing dialysis fluid (derived from Al-sulphate treated tap water)	1 ppm, chronic (2-5 years)	Intravenous	Human, kidney failure patients (15-61 years old at the start of the dialysis treatment)	Speech impairments (stuttering, dysarthria, dyspraxia, motor aphasia), movement disorders (twitches, tremors, myoclonic jerks, seizures, motor apraxia), cognitive impairments and behavioural changes (progressive dementia, paranoia, confusion,

				psychosis), death
Al-sulphate (present as flocculant in potable water supplies, accidentally released in high amounts)	500-3000 x the acceptable limit under European Union legislation (0.200 mg/L), chronic (15 years)	Oral	Human adult (female, 44 years old)	Sporadic early-onset amyloid angiopathy (Alzheimer's-related disease), difficulty in finding words, progressive dementia, visual hallucinations headache, anxiety, cerebral ischaemia, death
Various dietary	Chronic	Oral	Elderly human subjects	Impaired visuo-motor coordination, poor long-term memory, and increased sensitivity to flicker (correlated with high Al-serum levels
Al-oxide fumes, occupational exposure	0.13-1.95 mg/m3, chronic	Inhalation	Human, adults (mean age 39 years)	Headache, emotional irritability, concentration difficulty, insomnia, mood liability
Various: Al-chloride, Alphosphate, Al-powder slurry	Single sub-lethal dose	Intracerebral injection	Cats, rabbits	Decline in memory, impaired learning responses, deterioration in psychomotor control, epileptic seizures and death, neurofibrillary degeneration (resembling Alzheimer's disease neurofibrillary Tangles
Al-hydroxide	2 injections, 2 weeks apart	Subcutaneous injection (behind the neck)	Mice, 3-month old	Motor neuron degeneration and apoptosis, motor function deficits, decrease in strength, cognitive deficits and decreased performance in

				learning tasks, decrements in spatial memory, activation of microglia
Al-containing food pellets	0.5-1.7 mg/kg/day (typical human), chronic (22-32 months)	Oral	Rats, 6-month old at the start of treatment	Cognitive deterioration and impaired performance in learning tasks, impaired concentration, behavioral changes including confusion and repetitive behavior
Al-lactate	500-1000 ppm, chronic (during gestation and lactation)	Oral	Mice dams	Hind limb paralysis, seizures and death (dams), lower neurobehavioral development and altered performance on a neurobehavioral test battery in pups (foot splay, forelimb and hind limb grip strengths

CHAPTER 8
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