

Toxicopathological Effects of Aluminum Chloride (AlCl₃) in Reproductive System of Female Albino Mice

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Abstract:

The present study was designed to investigate the toxic effects of aluminum chloride AlCl₃ injected intraperitoneally of albino female to study the clinical signs and histopathological changes in reproductive system. (159) of Female mice in the age of puberty and weights ranged (30-35) g were used in this study which included three experiments: **first experiment:** - This experiment was conducted on 87 female mice, divided into 2 groups by intraperitoneal injection, the first group contained (15) female mice for the purpose of knowing the lowest value and the highest value in the pilot study, while (72) female mice were used for the purpose of determining the dose toxin medium lethal (LD₅₀), and by the way probit method for the purpose of examining the symptoms of acute toxicity, severity, time of appearance and disappearance clinical signs, which estimated 1100 mg / kg of body weight, **second experiment:** This experiment was conducted on (36) female mice for a period of (14) days, mice were divided into two groups, as follows: **1- sub acute high toxic exposure group:** -Injected with I / P high dose(221.83)mg/kg of body weight included 18 females mice **2-Control group:** - Injected I / P solution Physiologic solution included (18) female mice. **3rd experiment:** -This experiment was conducted on (36) female mice for a period of (60) day of the experiment, they were divided into two groups, as follows: - **1 – Sub chronic low toxic exposure group:** injected I / P low dose (55.45) mg/kg of body weight included (18) females mice. **2– Control group:** -injected I / P solution Physiologic and included (18) females mice. The study were conducted at days (3, 7, 14) for the sub acute toxic value exposure and at (15, 30, 60) exposure to toxic sub chronic. The study showed that the effects increased with increasing AlCl₃dose and duration of exposure. Where results showed clinical signs that were depression of animal, neurological signs, diarrhea, with paralysis and suffocation and ended up comatose and death within half an hour, pathological changes characterized by degeneration and distraction of ovary with oviduct and uterus, necrosis, papillary hyperplasia of endometrial epithelial lining mostly in sub chronic toxic effects at day 60 .

Key word: AlCl₃ toxicity, Female Reproductive system, Histopathological changes

التأثير السمي المرضي لكلوريد الالمنيوم على الجهاز التناسلي في اناث الفئران البيض

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المستخلص:

اجريت الدراسة لمعرفة تأثير سمية كلوريد الالمنيوم AICI₃ المحقون في غشاء الخلب للفئران البيض من خلال دراسة العلامات السريرية والتأثيرات المرضية النسيجية للجهاز التناسلي الانثوي ، استخدمت في الدراسة (159) من اناث الفئران البيض بعمر البلوغ وبأوزان تراوحت (35.30) غم وشملت الدراسة ثلاث تجارب: التجربة الاولى :- أجريت التجربة على (87) من اناث الفئران البيض ، قسمت الى مجموعتين ، احتوت المجموعة الاولى على (15) فأرانثى لغرض معرفه أقل قيمه وأعلى قيمه في Pilot study بينما احتوت المجموعة الثانية على (72) فأرانثى لغرض تحديد الجرعه السمي المميته النصفيه (LD₅₀) وحسب طريقه Probit method ولغرض دراسه الاعراض السمي الحاده وشدتها ووقت ظهورها واختفاءها والتي قدرت (1100) ملغم / كغم من وزن الجسم . التجربة الثانيه :- أجريت التجربة على (36) فأر اناث ولمدة (14) يوم حيث قسمت الى مجموعتين ، 1- مجموعة التعرض السمي شبه الحاد : حقنت وجرعه (221.83) ملغم / كغم من وزن الجسم شملت (18) فأر اناث ، 2- مجموعة السيطرة : حقنت I/p المحلول الملحي الفسلجي وشملت (18) فأر اناث ، التجربة الثالثة : اجريت على (36) فار اناث ولمدة (60) يوم وحقنت باستمرار من التجربة حيث قسمت الى مجموعتين 1- مجموعة التعرض السمي شبه المزمّن : حقنت I/p وجرعة (55.45) ملغم / كغم من وزن الجسم شملت (18) فار اناث 2- مجموعة السيطرة: حقنت I/p المحلول الملحي الفسلجي وشملت (18) فار اناث . اجريت معايير الدراسة في اليوم (3,7,14) للتعرض السمي شبه الحاد وفي اليوم (15 ، 60، 30) للتعرض السمي شبه المزمّن وتضمنت الدراسه العلامات السريره كما تم رفع الاعضاء التناسليه الانثويه (المبايض و قناة البيض والرحم) . اوضحت الدراسة ان تأثير AICI₃ يزداد بزيادة الجرعة وفترة التعرض حيث اظهرت نتائج العلامات السريره حمول الحيوان وعلامات عصبية واسهال ، مع الشلل والاختناق وانتهت بالغيوبه والموت خلال نصف ساعة. تضمنت التغيرات المرضية التنكس وتنخر نسيج المبيض وقناة البيض والرحم ، مع فرط التنسج الغدي في بطانة الرحم وخاصة في اليوم 60 من التعرض السمي المزمّن.

الكلمات المفتاحية: سمية كلوريد الالمنيوم ، الجهاز التناسلي الانثوي، التغيرات النسيجية المرضية

Introduction:

Aluminum(Al), the most abundant metal, makes up about 8% of the Earth's crust and is found in combination with oxygen, silicon, fluorine and other elements in the soil, rocks, clay and gems. It has no known biological function (12). Aluminum enters into the body from the environment and from diet, Al-containing diet is mainly corn, yellow cheese, salts, herbs, spices, tea, and cosmetics such as antiperspirant and deodorant preparation (46). It is incorporated in some medications such as antacids, buffered aspirin, anti diarrheal products, vaccine and allergen injection, and it is used as a component of veterinary medicine, glues and disinfectants (22). There is little indication that aluminum is acutely toxic for the general population, Prolonged exposure to aluminum, however, can cause systemic toxicity, mainly affecting the gastrointestinal tract and causing neurological and skeletal effects and bone disease (5). Chronic toxicity occurs almost exclusively in persons undergoing dialysis for renal failure, who are likely to develop osteomalacia or aplastic bone disease, respiratory diseases (e.g., pulmonary fibrosis, occupational or potroom asthma, and chronic bronchitis), neurological effects, including impairment of cognitive function and motor dysfunction, Alzheimer's disease, amyotrophic lateral sclerosis (ALS) and Parkinson's disease, (17). AlCl₃ induced reproductive toxicity and adverse effect on the steroidogenesis (47). Exposure rats to Al (200) mg/kg.bw determined sever structural changes in ovary, vacuolar epithelial cells, follicles with large oocytes, very evident odema of the parenchymatous zone, follicles destruction, destruction of parachyma (10;33). (39) noticed that exposure to AL in drinking water (200,400,1000) mg/kg.bw increase level proteins in ovary, fullpoin tubes and uterus that sever congestive and degeneration changes in ovary and uterus. (1) indicated that I/P injection of AlCl₃ to adult non pregnant female mice caused highly congested blood vessels all over the ovary with large number of atractic follicles at different stage of development. (2) showed that exposure rat to Al (400-1000)mg/kg.bw orally causes necrosis of uterine gland partial destruction of the lining uterine epithelium, passive vascular congestion necrosis of the connective tissue rarefaction of connective tissue almost complete detachment uterine epithelium. (1) noticed that short exposure of AlCl₃ show toxic effects on mouse embryos fetuses and even short exposure lead to fetal death, decrease and skeletal anomalies.

Material and method:

1. Experimental animal: Female (159) mice with ages about three months and body weight ranged between (30 – 35g). The albino mice were obtained from animals house colony of Embryo Research and Infertility Treatment Institute / Al-Nahrain University. Animals were housed in plastic cages 30*10*10 c.m³ placing in the room for two weeks for adaptation. Standard rodent diet (Commercial feed pellets) and drinking water were given. Housing conditions were maintained at 22± 4C°, the air of the room was changed continuously by using ventilating vacuum and light/dark cycle

(14/10) hr/per/day. The experiments of this study were conducted in the animal house of the pathology department at college of veterinary medicine, Baghdad University.

2. Experimental study:

1. Half lethal dose (LD₅₀) :- (87) female mice for determination the (LD₅₀) and divided as following:

A. Pilot study:-(15) female mice were used for determination the ranges of the lethal doses of AlCl₃ (14) female with (1) control, this method was repeated daily for seven days by dividing into two groups each group contain (2) mice

B. probit method (72) female mice were divided into nine groups ,each groups consists of eight animals , They were injected intraperitoneal with the following doses of AlCl₃ mg /kg.bw :-

Group 1 : 800 mg/kg.bw.

Group 2 : 900 mg/kg.bw.

Group 3 :1000 mg/kg.bw.

Group 4 :1100 mg/kg.bw.

Group 5: 1200 mg/kg.bw.

Group 6 : 1300 mg/kg.bw.

Group 7 : 1400 mg/kg.bw.

Group 8 : 1500 mg/kg.bw.

Group 9: control injected with physiologic solution.

The animals were watched for 24 hr. to record development of toxicity symptoms and lethality.

The parameter of LD₅₀ study were done by:-

* Drawing the log dose–probit response curve from which LD₅₀ was determined according to probit method (23).

3. Experimental design: -

A- First experiment- Sub acute high toxic effects

(36) Female mice were treated with AlCl₃ by I/P injection for (14) days as following:-

1st group(18) Female mice were treated with AlCl₃ at high dose I/P injection of (221.83)mg\Kg.bw.

2nd group(18) female mice were treated with I/P injection of Physiologic solution (control).

The parameters of acute toxicity study were done at the days (3\7\14) mice were scarified for histopathological changes.

B- Second experiment- Sub chronic low toxic effect.

(36) mice male & were injected I/P for (60) days with AlCl₃ as following:-**1st group (18)** Female mice were injected I/P with low dose of ALCL3 (55.45) mg \Kg.bw.

2nd group (18) female mice were injected I/P Physiologic solution (control).

The parameter of chronic toxic study were done at the day (15\30\60)-eight female mice were scarified at each injected& control groups.

4. The following parameters were studied:-

1. Clinical sign: - Clinical signs were checked continuously after treatment with ALCL3 along the period of experiments (60days), also any changes in activity or behavior of the animals were recorded.

2. Doses and Concentration:- Different concentration were prepared according to the dose used in sub acute and sub chronic study of ALCL3 toxic effect by dissolving the suitable amount of pure ALCL3 in distilled water in order to be given to mice at dose volume of (0.1/10 g .B.W).

3. Pathological study: - All animals were sacrificed by inhalation of chloroform and post mortem were done for all animals .ovary, oviduct, macroscopically observed to record any abnormal changes. Fix were taken from these organs, specimens kept in 10 % formaldehyde solution, for fixation, then processed done by using the histokinette (26).

4. Statistical analysis:-

Factorial experiment applied in completely randomized design (CRD) was used to study the effect of treatment and month in different trails. Least significant difference (L.S.D) was considered as guide of significant difference between means of treated groups .Data were analyzed using statistical analysis system (SAS, 2002) program.

Result and discussion:

1. Acute toxicity:

A. Pilot study:

The range of ALCL3 LD50 were mainly (800 mg/kg to 1500 mg/kg.bw) which caused the death of four animals from eight animals.

B. probit method: The mortality percent and conversion to probit No. according to acute toxic effect of ALCL3 in G1,G2,G3,G4,G5,G6,G7,G8,G9 are listed in (table:1).

Table 1: LD₅₀ of ALCL3 (Acute toxic effect with log. concentration)

Group	Dose	Log dose	N	Live	Dead	Dead %	Corrected %	Probit unit
G1	800	2.90	8	8	0	0	3	3.12
G2	900	2.95	8	7	1	13	13	3.87
G3	1000	3.00	8	6	2	25	25	4.33
G4	1100	3.04	8	4	4	50	50	5.00
G5	1200	3.08	8	3	5	63	63	5.33
G6	1300	3.11	8	2	6	75	75	5.67
G7	1400	3.14	8	1	7	88	88	6.18
G8	1500	3.17	8	0	8	100	97	6.88
G9	control		8	8				

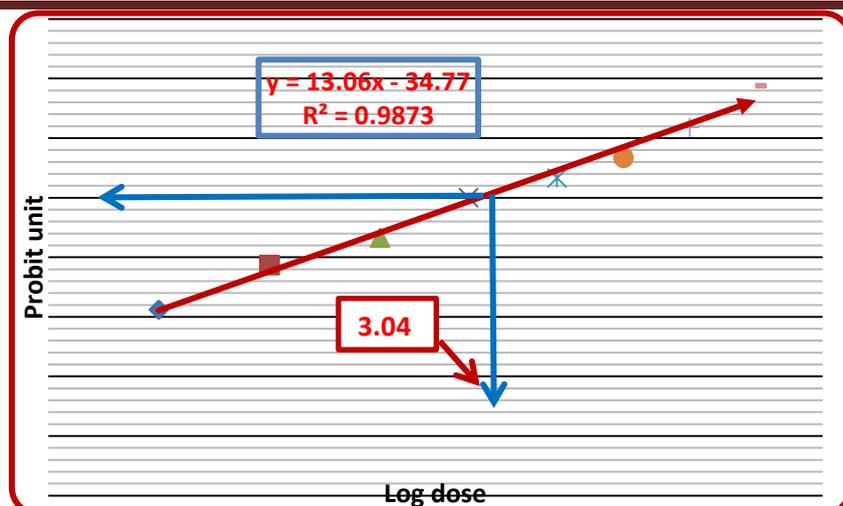


Figure 1: Photograph of LD50

$y = 13.06x - 34.77$, $5 = 13.06x - 34.77$, $13.06x = 5 + 34.77$, $x = 39.77/13.06 = 3.045$ Antilog₍₁₀₎ 3.045 = 1109.17, (LD₅₀) Corrected formula*: For the 0% dead: $100(0.25/n) = 100(0.25/8) = 3$, For the 100% dead: $[(n-0.25)/n] = 100; [(8-0.25)/8] = 97$, n is the number of animals in the group.

LD₅₀ was measured after we used plot the logarithm of doses was used against probit response from which LD₅₀ was determined by vertical cross link from the 5 probit response to the log no. dose (Figure:1) LD₅₀ was calculated as antilog of the Log no.(2.90) which equal to (800) mg/kg.bw depend on (7). According to toxicity rate the LD₅₀ of AlCl₃ which was given to mice by indirect intake through I/P injection was fall under toxicity rate which since LD₅₀ was nearly (3.045) mg/kg. bw in mice and considered as highly toxic compound. Our results are disagreed with other researches, (44) were showed that the range of AlCl₃ LD₅₀ was (25-82) mg/kg.bw in mice while (3) showed that the LD₅₀ of AlCl₃ in white mice was (54) mg/kg .bw.

2. Clinical Signs :

The severity of clinical sings is gradually depended on the severity of doses toxicity so at dose (800) mg/kg. bw clinical sings record depression, convulsion, diarrhea ,while at dose (1500) mg/kg .bw paralysis, asphyxia ,coma, with death at (1/2-6) hrs. Were the most important clinical signs as shown in table (2)

Table: 2. Clinical sings of AlCl₃ (Acute toxic effect in mice)

It was noticed that more acute toxicity signs were developed according to increasing dosing groups with more severity and less appearance time that delayed when disappeared No. of dead animals were increased in high doses groups .The present clinical observation were (24 ; 37 ; 36) AlCl₃ has neurotoxic effect characterized by neurodegeneration by causing changes in brain cells membrane . Al is acholin toxin agent which induce oxidative stress (30) by increasing the production of (AChE) which caused a reduction in brain (Na , Ka) ATPase activity

(28) induced membrane lipid peroxidation impair the function of membrane ion – motive ATPase (Na,K,Ca) ATPase result in membrane depolarization a decreased in cellular ATP levels. ALCL3 decrease brain vitB12 brain homocysteine(bcy) level which decrease methoine synthesis in brain caused neuronal degeneration and brain necrosis with the appearance of neurofiberbrillares tangle neuron necrosis (27; 34 , 29) . AlCl3 indicated that AlCl3 caused necrosis of neuron by injury to hippocampal , region . In addationALCL3 induce an increase in free radicals in brain which caused change in physical activity of neuron membrane (40;35) indicated that Al caused neurodegeneration disease like Alzheimers , parakinsons disease, myotrophic lateral sclerosis &Encephalopathy. While (43) recorded that AlCl3 caused depression, ataxia, slugsh motor movement with generalized . convulsion , incoordination & dead during (5-10) years .

3. Pathological study

3.1 Macroscopic findings

Control group:

There was normal macroscopic findings

Sub acute toxicity study:

There was no significant gross findings lesion

Sub chronic toxicity study:

The main gross features characterized by congested of oviduct mainly at 60 day post treatment that appear darked with rounded border Fig (2) with evidence of uterus enlargement (3).



Fig.2: Macroscopic appearance of(A) oviduct (B)uterus mice low sub chronic toxic effect dose (55.45)mg/kg.bw I/P at day 60 (A) showing congestion ,enlargement of oviduct marking (B) showing Enlarged in size, severe congestion , dark red in color of uterus marking.

The present gross pathological lesions showed that the animals exposed to toxic doses according to the dose exposure to AlCl3 depends on the toxic effect dose and duration of injection& mainly seen at day 60 that macroscopic appearance of organ in chronic toxicity was characterized by severe congestion of uterus and oviduct and that reflect the severe vascular effects of AlCl3 as similar disrupt blood brain barrier permertability(21)Similar results have been reported by(4) .

3.2. Microscopical findings

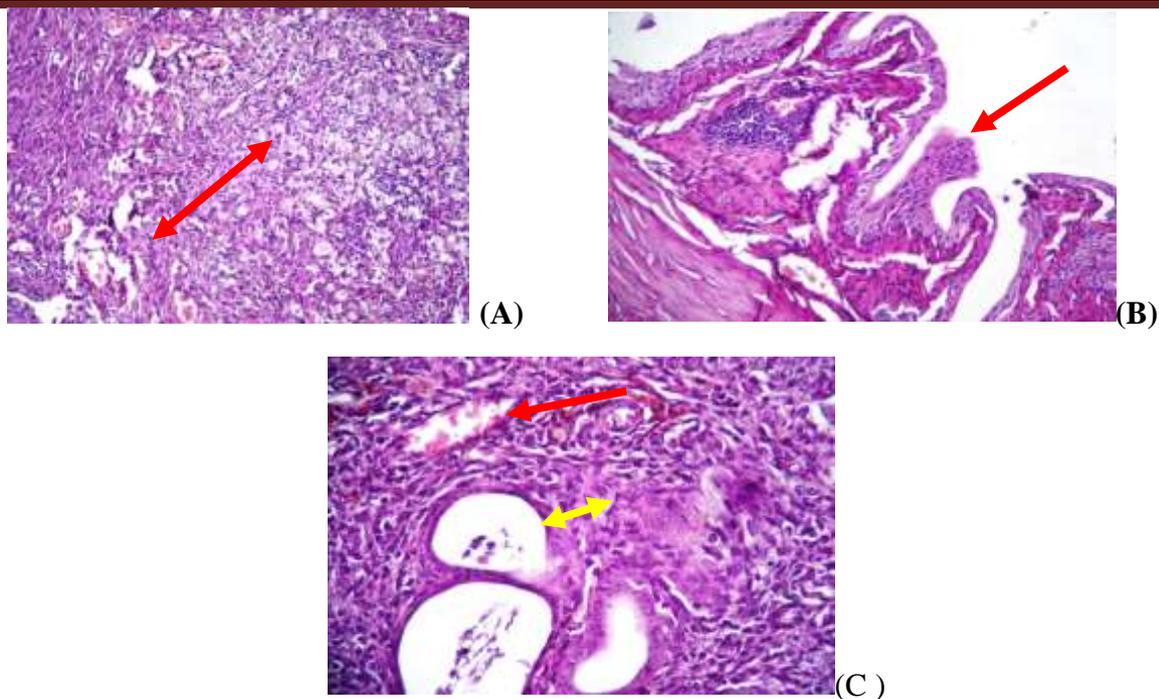
3.2.1. Sub acute high toxic effect

Aluminum chloride (221.83 mg/kg. bwI/P injection)

Ovary :There was focal & diffuse degenerative changes of ovarian follicle associated with intense PMNCs infiltration ,together with sever b.v congestion in the ovarian stroma Fig:29,30,The ovarian lesion at day 14 revealed follicular cyst with ,Presence of variable number of degenerated primordial follicles accompanied with appearance of cytic follicles pyknosis of follicle together with slight congestion, Moderate to sever degeneration of ovarian follicle associated with b.v congestion & focal stromal destruction together with pyknosis of follicular with distraction of stromal tissue There was evidence of b.v congestion & destruction in the stromal tissue with vacuolization & necrosis of antral follicle. Also ovary showed extensive vacuolization with b.v congestion in the stromal tissue with vacuolization & necrosis , Also ovary showed number of atretic follicle with destruction lesion associated with b.v congestion in ovarian stromal tissue Fig : **3 , 4(A)** **Oviduct** : The characteristic feature showed focal cellular aggregate consist mainly of neutrophil in (L.P) with b.v congestion together with slight epithelial hyperplasia ,While other section showed variable sloughing with evidence of (L.P) congestion mainly at day 7,oviduct reveal mucosa epithelial hyperplasia with papillary growth associated with proliferation of glandular tissue cell (PMNCs) as well as tissue infiltrate &b.v congestion with evidence of slight fibrosis, Sever mucosal epithelial hyperplasia with papillary growth that fill the lumen with evidencete of sub mucosal congestion,(Figure:**3,4 (B)**). **Uterus** :The main histopathological lesion characterized by cystic distension of endometrial glands (cystic hyperplasia of uterine glands with slight debris together with diffuse PMNCs infiltration in the endometrial stroma associated with b.v congestion together with hemosiderosis Fig: (28),Other section .Showed sever vaculation& necrotic changes of uterine glands with marked PMNCs infiltration with uterine stroma that give suppurative endometritis, Other section showed sever vacular changes in epithelial uterine mucosa with stromal destruction & PMNCs infiltrationAlso results showed endometrial hyperplasia with slight tissue debris mixed with mucin & PMNCs infiltration also with desquamation in some areas with cellular infiltration sub epithelial layer together with evidence of mucopurulant endometrial Fig:**3,4(C)** .

Day 3

No important histopathological changes were found



"Fig.3: Histopathological section of (A) ovary (B) oviduct (c) uterus of high acute toxic dose at 7 day shown (A) Diffuse degenerative changes of ovarian follicle & C.L with b.v congestion in ovarian stroma (↔) (H&EstainX40), (B) focal cellular aggregate in L.P consist mainly of neutrophile together with slight epithelial hyperplasia (→) (H&EstainX10), (C) Cystic distension of endometrial gland with slight debris together with diffuse PMNCs infiltration ,b.v congestion ,hemosidrein deposition in the endometrial stroma (↔) (H&EstainX40).

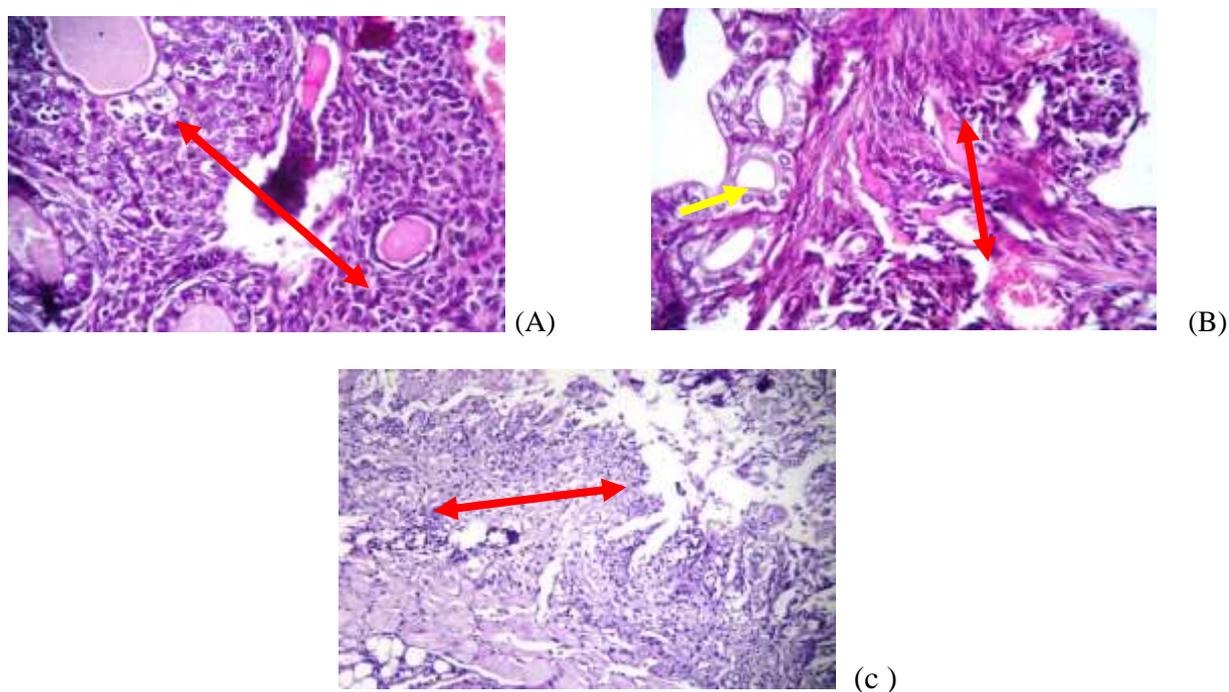


Fig.4: Histopathological section of (A) ovary (B) oviduct (c) uterus of high acute toxic dose at 14 day shown (A) Moderate to sever pyknosis of ovarian follicles (Secondary follicles and intera follicles) with destruction of stromal tissues (↔) (H&E stain X 40), (B) Mucosa epithelial hyperplasia with proliferation of glandular tissue (→) as well as PMNCs infiltration&b.v congestion with evidence of slight fibrosis (↔) (H& Estain X10) ,(C) Endometrial epithelial vacuolation& sloughing with tissue debris & cellular infiltrate observed in uterine lumen (↔) (H&EstainX40) .

3.2.2. Sub chronic low toxic effect

Aluminum chloride (55.45 mg/kg. bwI/P injection)

Ovary: The ovarian tissue showed prominence of corpus luteum together with moderate mononuclear cells infiltration in ovarian stroma together with blood vessels congestion .as well as the ovarian follicles observed with degeneration changes of its antral area of lining in addition to antrum. ,Increase in diameter of ovarian follicle as well as increase no. of ovarian follicle with evidence of ciliated epithelia while other showed atresia ,increase in no. of atretic follicle& stromal congestion, other section showed increase in total no. of ovarian follicle with increase in diameter of mature follicle with evidence of stromal congestion associated with atrophy of corpus luteum clumping of follicular all in same follicle, The characteristic change was moderate stromal fibrosis together with number of developed ovarian follicle ,Presence number of development ovarian follicle associated with slight degenerative changes as well as blood vessels congestion in stromal tissue (**Fig:5,6,7,(A)**)**Oviduct:** Mucosa epithelial hyperplasia with papillary growth fill the lumen of tube with evidence of sub mucosa at day 15 while at day 30 the characterized feature was sever vaculation of oviduct epithelial together with papillary hyperplasia & cellular infiltration in the sub mucosa moderate to sever mononuclear cells infiltration consist mainly of lymphocyte accompanied with slight fibrosis seen mainly at day 60 Fig:(59) prominence of cilia with hyperplasia (**Fig:5,6,7,(B)**)

Uterus :The uterine lesion revealed massive destruction in endometrial layer with necrosis of endometrial glands together with tissue debris mixed with mucopurulent exudates ,Sever necrosis of endometrial glands with PMNC infiltration, endometrial sever necrosis , vacuolization with polymorphnuclear cells infiltration, The characteristic feature of reveals polymorphnuclear cells aggregate in the uterine lumen associated with epithelial sloughing of necrotic debris Uterus showed sever cystic endometrial gland dilation(**Fig:5,6,7, (C)**).

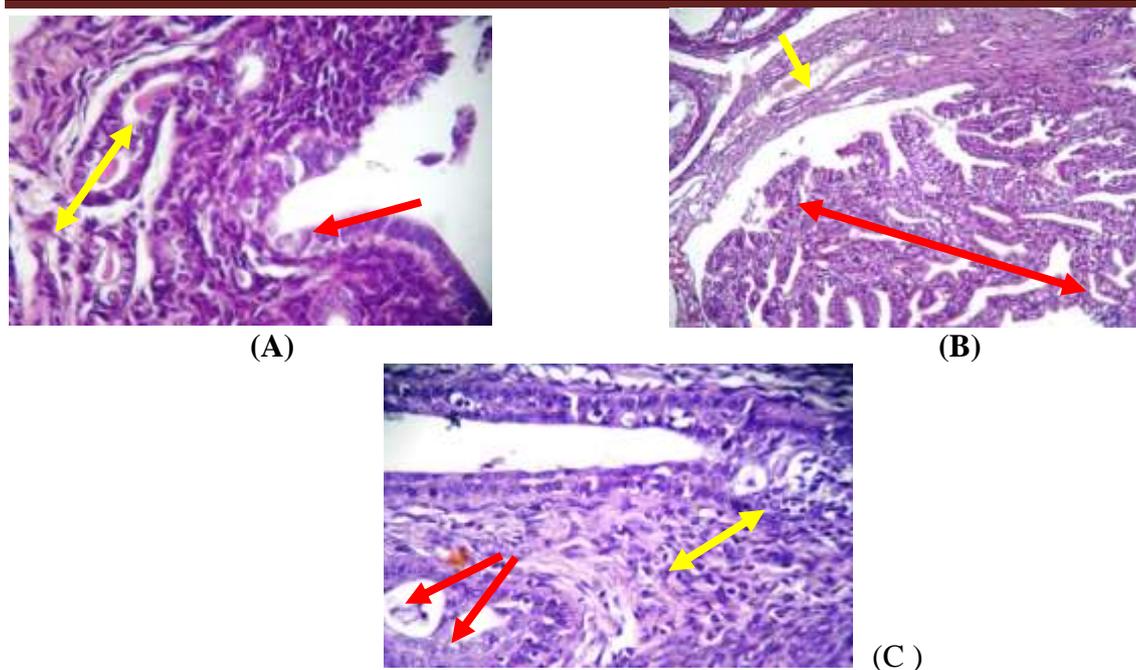


Fig.5: Histopathological section of (A) ovary (B) oviduct (c) uterus of low chronic toxic dose at 15 day shown (A) Desquamation of ovarian epithelial lining with vacuolation (red arrow) together with stromal vascular changes & atretic follicle (red double arrow) (H&E stain X40), (B) Mucosal epithelial hyperplasia with papillary growth (red double arrow) at fill the lumen with evidence of sub mucosal congestion (yellow arrow) (H&E stain X40), (C) Moderate MNCs aggregation in sub mucosal layer with slight fibrosis (red double arrow) together with uterine gland hyperplasia (yellow double arrow) (H&E stain X40).

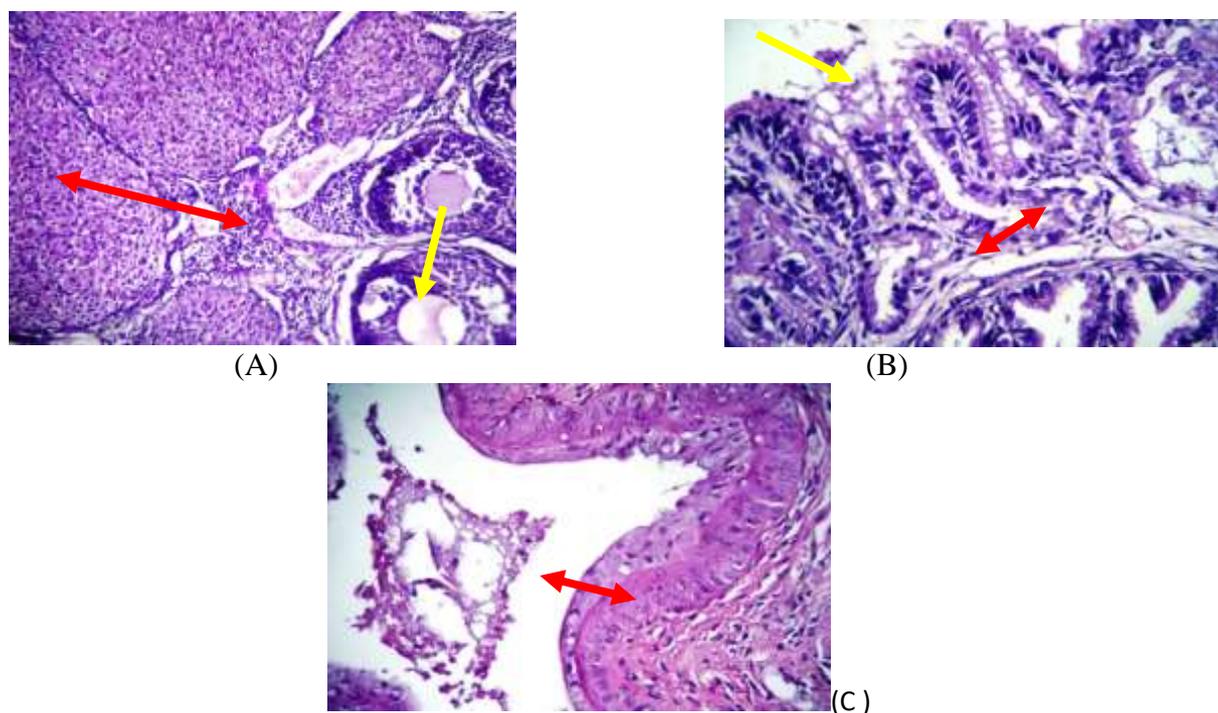


Fig.6: Histopathological section of (A) ovary (B) oviduct (c) uterus of low chronic toxic dose at 30 day shown (A) Prominence of C.L with moderate MNCs infiltration in ovarian stroma (red double arrow) with in variable degenerative changes in antral ovarian follicle (yellow double arrow) (H&E)stain (X40), (B) Severe vacuolation of mucosal epithelium together with papillary hyperplasia (yellow arrow) & cellular infiltration & b.v congestion in L.P (red double arrow) (H&E stain X40), (C) Higher magnification of uterine endometrial part showed hyperplasia with slight tissue debris mixed with mucin & PMNCs infiltration in the lumen (red double arrow) (H&E stain X40).

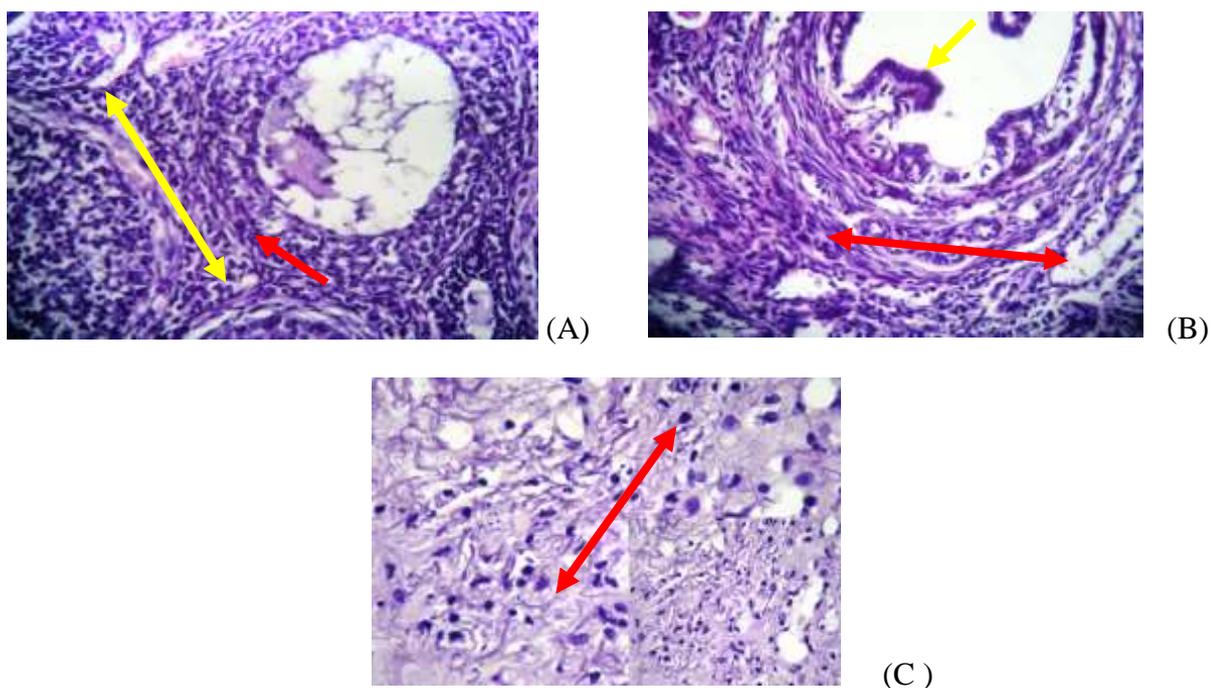


Fig.7: Histopathological section of (A) ovary (B) oviduct (c) uterus of low chronic toxic dose at 60 day shown (A) Slight degeneration in some ovarian follicle \rightarrow with slight stromal b.v congestion with evidence of stromal b.v congestion \leftrightarrow (H&E stain X40) , (B) MNCs infiltration , the oviduct wall consist of lymphocyte with b.v congestion \leftrightarrow & mucosal hyperplasia \rightarrow (H&E stain X40) , (C) Massive destruction in endometrial layer with necrosis of endometrial gland & tissue debris mixed with mucopurulent exudates \leftrightarrow (H&E stain X40).

The present work study showed severe pathological lesions in female reproductive organs of both acute & chronic toxic dose, these results may indicate that AlCl₃ caused alteration in metabolism of protein, CHO & nucleic acid as well as oxidative energy, these changes caused generation of oxygen metabolite –free radicals with increase lipid peroxidation & decrease SOD activity similar observation were reported (14; 9; 31). AL caused alteration in metabolism of (protein, carbohydrate, oxidative energy, nucleic acid) these changes were mainly caused increase lipid peroxidation, formation of oxygen free radicals and decreased in superoxide dismutase activity in ovary leading to degeneration changes and cell damage. AlCl₃ caused severe degenerative & necrotic changes in the ovarian tissue. Also (8, 32) demonstrate administration of AlCl₃ alone induced toxicity in female mice in effecting steroidogenesis in ovary, carbohydrate metabolism in uterus and causing hypercholesterolemic effect in mice. According to above pathological results we reported increase vascular changes with edema together with PMNCs infiltration mainly at (7) days post treatment with dose (221.83)mg/kg. bw this evidence may be supported by the idea that AlCl₃ may enhance the production of endogenous estrogen by stimulation the LH receptors & so increase of the theca cells to LH hormone (45; 48). Estrogen had a dramatic effect on the uterus it leads to increase blood flow & accumulation of extracellular fluid FSH and LH each bind to specific receptors on the surface of granulosa or theca cells and activate adenylate cyclase, increase

concentrations of cAMP in the cytoplasm activate protein kinase, which catalyzes phosphorylation of critical proteins leading to steroidogenesis, estrogen secretion primed the target tissues to respond to progesterone, estrogens induce the synthesis of progesterone receptors and without them progesterone has little biological effect (16). It has been known that estrogen exerts an important protein anabolic effect in chickens and cattle, possibly by stimulating the secretion of androgens from the adrenal, and estrogen treatment has been used commercially to increase the weight of domestic animals (6; 13). Also results showed hyperplastic reaction mainly seen in the wall of uterus & oviduct at (14) days with dose (221.83)mg/kg.bw. As well as increase diameter in some ovarian follicle mainly at (14) days with dose (221.83)mg/kg.bw. These changes may be attributed to different duration of treatment by AlCl₃ together with hormonal changes & the anabolic effect of accumulated AlCl₃ through the period of toxic effect (19) although evidence that mentioned by The presence of severe vacuolar changes in the epithelial lining of oviduct, uterus mainly at (14) days post treatment with dose (221.83)mg/kg.bw which could be correlated with decline in the activity of phosphorylase in the uterus and also reported earlier for other tissue in male & female mice (38;8). Although, there are some evidence mentioned by some authors suggest no information about presence of AlCl₃ toxicity in female organ such as oviduct (11). Changes in ovarian section are due to the effects of increasing levels of E₂, FSH and LH. The growth and development of follicular depend on releasing of E₂ (43). E₂ work within the follicle in an autocrine or paracrine pathway, that it stimulation proliferation of granulosa cells and increases their responsiveness to FSH, also activate proliferation of theca interna cells, these manners give the follicle progressively greater capacity to produce E₂ and made it increasingly sensitive to FSH as it matures, by these events, E₂ stimulate its own production, simultaneously, E₂ and FSH activate granulosa cells to synthesizing receptors for LH, in preantral follicles granulosa cells which have few receptors for LH and are didn't responsive for LH, in contrast, granulosa cells of preovulatory follicles have abundant LH receptors and consequently have acquired sensitivity to LH (15). We observed mucopurulent inflammation in the uterine tissue mostly at (15),(30),(60) days with (55.45) mg/kg.bw. & this finding in consistence with (41) who demonstrate that treated with AlCl₃ stimulate production of endogenous estrogen which important effects on mucus secretion of the cervix, which increasing the secretion of mucus, that become abundant, clear, and non viscous, all these characteristics are most clearly at ovulation and allow sperm depositing in the vagina to move easily through the mucus on their way to the uterus and uterine tubes, All these changes were clear in tissue sections of treated groups.

Oviduct alteration at (15), (30), (60) with (55.45) mg/kg. bw. can be attributed to the increase in the E₂ level. The significant effects of steroid hormone are similar on the fallopian tubes and uterine endometrium, they cause proliferation of the glandular tissues. also increase in the number of ciliated epithelial cells that line the fallopian tubes, also, activity of the cilia is considerably enhanced, these cilia always beat

toward the uterus, which helps propel the fertilized ovum in that direction (15; 32), and this is exactly detected in the treated groups compared with that of control one. The result of uterus at toxic (55.45)mg/kg.bw at (15),(30) (60) consist of hyperplasia of uterine epithelial lining with endometrial gland hyperplasia these result were agreement with (25) who reported the uterine epithelial hyperplasia & increase in diameter of endometrial glands effect of I/P treatment with AlCl₃ increase in the thickness of endometrium which increase pregnancy rate in females with a thin endometrium although there is a risk in treatment more than 10 wks because proliferation may lead to neoplasia (20)..In addition the present results reported prominence C.L together with proliferation of C.L particularly at (60) days with (55.45) mg/kg.bw. LH receptors, and so, it increases the affinity of theca interna cells to LH hormone (45).As AlCl₃ causes highly significant increase in the E₂, this may cause positive feedback action on gonadotropin secretion probably from both a direct effect of estrogen on the pituitary gonadotropins to secrete more FSH and LH in response to GnRH and indirectly by stimulating the hypothalamic neurons that secrete GnRH with modulation of the frequency and magnitude of the pulses of GnRH (41; 18).All results of this study revealed the effect of AlCl₃ on the different parts of the genital organs which might reflect on reproductions and the number of new generations .The administration of I/P of AlCl₃(55.45)mg/kg.Bwt/daily female mice (60 days) old, , resulting important microscopic alterations in the ovaries, oviducts and uterus.

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Table: 2. Clinical sings of AlCL3 (Acute toxic effect in mice)

1. G1(800 mg/kg.bw)														G2(900 mg/kg.bw)						G3(1000 mg/kg.bw)			
Toxic symptoms	Appearance time	Disappearance time	Dead and time /hrs		Toxic symptoms	Appearance time	Disappearance time	Dead and time /hrs		Toxic symptoms	Appearance time	Disappearance time	Dead and time/hrs										
Slow movement	30min	12 hrs	Animal	time	slow movement	40mint	12hrs	1	6hrs	slow movement	15mint	8hrs	3hrs										
Convulsion	50 hrs	9 hrs	0	0	paralysis in one Leg	4hrs	6hrs			Convulsion	60 mint	6hrs											
					Cramps	8hrs	2hrs			Paralysis	1hrs	18hrs											
paralysis in one Leg Cramps Diarrhea	12 hrs	24 hrs			Diarrhea with clonic Convulsion	6hrs	2hrs			Diarrhea	16hrs	1hrs											
	4 hrs	8 hrs			Dyspena	9hrs	death			Dyspena	8hrs	death											
	6 hrs	2 hrs			Salivation with greenish blue vomitus	death	death			Salivation with greenish blue vomitus	death	death											
G4(1100 mg/kg.bw)				G5(1200 mg/kg.bw)						G6(1300 mg/kg.bw)													
Toxic symptoms	Appearance time	Disappearance time	Dead and time /hrs		Toxic symptoms	Appearance time	Disappearance time	Dead and time /hrs		Toxic symptoms	Appearance time	Disappearance time	Dead and time /hrs										
Unbalance	15mint	12hrs	4	2-6hrs	spasms & leg paralyss	2hrs	24hrs	5	(1-11) hrs	severe spasms	2hrs	24hrs	(1/8)										
Paralysis	2hrs	24hrs			Convulsion and tremor	2hrs	11hrs			paralysis in all body	1hrs	24hrs											
Contraction of abdominal muscle	2hrs	10hrs			Bleeding from ear and eye	6hrs	20hrs			very rapid respiration	1hrs	death											
petechial hemorrhage in ear and eye	16hrs	18hrs			Crampy	3hrs	death			tachycardia	1hrs	death											

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convulsion and cramp	3hrs	16hrs		muscle weaknees and tremors	6hrs	death		Convulsion and tremor	1hrs	death	
polyuria then polydipsia	2hrs	22hrs		watery and Bloody diarrhea	14hrs	death		Severe Crampy, Abnormal posturing	2hrs ,death	death	
Hematuria	16hrs	24hrs		coma		death		Hemorrhage from ear and eye	death	death	
G7(1400 mg/kg.bw)				G8(1500 mg/kg.bw)							
Toxic symptoms	Appearance time	Disappearance time	Dead and time /hrs	Toxic symptoms	Appearance time	Disappearance time	Dead and time /hrs				
Depression	directly	death	7 2/4	Depression	directly	death	8	1/2-6			
paralysis in all body	20mint	death		paralysis in all body	20mint	death					
Asphyxia	30mint	death		Asphyxia	30mint	death					
Tachycardia	30mint	death		severeCrampy	20mint	death					
severeCrampy	1hrs	death		Sever convulsion and tremor	40mint	death					
Abnormal posturing	3hrs	death		Coma	1hrs	death					
Coma	death	death									