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EFFECT OF NIGELLA SATIVA OIL ON ALUMINUM CHLORIDE INDUCED NEPHROTOXICITY IN RABBITS

By

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ABSTRACT

This study aimed to evaluate the effect of Nigella Sativa oil (NSO) on aluminum chloride ($AlCl_3$) induced nephrotoxicity in rabbits. Twenty-eight adult White New Zealand rabbits were used and divided into 4 equal groups each of 7 rabbits. First group was given aluminum chloride 50 mg/kg BW daily for 21 successive days. Second group was given Nigella Sativa oil (NSO) 2ml/kg b.wt + aluminum chloride ($AlCl_3$) 50 mg/kg.bw. Daily for21 successive day's Third group was given. Nigella Sativa oil only and forth group was left as control group. Aluminum chloride ($AlCl_3$) and Nigella Sativa oil (NSO) were administered by oral gavages for 21 successive days. At the end of experimental period, blood samples were collected from 5 rabbits in each group to determine the kidney function tests and oxidative stress values, rabbits were slaughtered and kidneys were taken for pathological examination. Exposure to aluminum chloride ($AlCl_3$) for 21 successive days in a dose of 50 mg/kg bw induced nephrotoxicity as there was a significant increase in white blood cell counts and a marked decrease in erythrocyte counts, hemoglobin concentration in addition to a significant increase in serum creatinine, urea and uric acid concentration . Aluminum chloride ($AlCl_3$) induced oxidative stress as it significantly increased lipid peroxide MDA while caused a significant decrease in reduced glutathione (GSH) and superoxide dimutase SOD. Histopathological examination of. Aluminum chloride intoxicated group reveled Cloudy swelling, vacuolar and hydropic degeneration especially in renal tubules. Nigella Sativa oil (NSO) co- treatment with aluminum chloride ($AlCl_3$) exhibited significant improvement in all examined parameters compared with control and improves the histopathological picture of kidneys. In conclusion, Nigella Sativa oil (NSO) has beneficial effects and could ameliorate aluminum chloride ($AlCl_3$) nephrotoxicity in rabbits.

Key words:

Nigella Sativa oil, Aluminum chloride ($AlCl_3$), nephrotoxicity.

INTRODUCTION

Aluminum (Al) is one of the most abundant metal present on the earth's crust. It is extensively used in daily life and was found in drinking water probably due to water purification procedures, **(Walton 2012 and Kaddour *et al*., 2016)**. Several products containing Al salts like antiperspirants are major source of exposure; vaccines adjuvants and phosphate binders **(Duce and Bush 2010, Walton 2012, Shaw and Tomljenovie 2013 and Kaddour *et al*., 2016)**.The toxicity of Al is directly linked to its bioavailability. In biological systems, this element has been shown to accumulate in many mammalian tissues such as brain, bone, liver and kidney **(Shaw and Tomljenovie 2013)**. In the medical field, Al is used as a major constituent of drugs such as antacids, phosphate binders, buffered aspirins, vaccines and injectable allergens **(Turkez *et al.,* 2010 and Hussain 2016).** However, elimination of Al from the human body is very limited and urine is the primary route of its elimination. Higher doses of Al could increase the risk of renal Al retention and hence, induced nephrotoxicity **(Hussain 2016).** It has been reported that Al can cause degeneration of the renal tubular cells through generation of reactive oxygen species (ROS) which cause oxidative damage to cellular lipids, proteins, and DNAs. In addition, aluminum intoxication lowers the intracellular levels of reduced glutathione **(Geyikoglu *et al*., 2013)**. Al induced changes in biochemical parameters, increased lipid peroxidation and decreased the activities of the antioxidant enzymes in plasma and different tissues of rat **(Bouasla *et al*., 2014 and Newairy *et al* 2009)**. It was found that one of the main organs targeted by Al exposure is the kidney. Rats that were exposed to aluminum chloride induced nephrotoxicity, as evidenced by a decrease in the 24 -hour urine volume and uric acid levels in plasma and an increase of plasma creatinine and blood urea nitrogen levels. Nephrotoxicity was achieved by a significant increase in malondialdehyde level **(Ghorbel *et al*., 2016).** On the same contexest **(Liu *et al*., 2016)** indicates that $Alcl_3$ exposure induces oxidative stress and suppresses kidney function. Nigella Sativa (NS), known as black cumin or black seed, is an annual flowering plant that belongs to the Family ranunculaceae. In the Middle East, North Africa and Southwest Asia, seeds of NS and other derivatives have been used in food for a long time. The chemical composition of NS is very rich, containing fixed oils (84% fatty acids) and

volatile oils, amino acids, proteins, carbohydrates, alkaloids (nigellidine, nigellimine, and nigellicine), saponins, crude fiber, as well as minerals, such as calcium, iron, sodium and potassium. In addition, vitamins such as riboflavin, thiamine, pyridoxine, folic acid and niacin are present in the chemical composition. NS oil (NSO) is rich in polyunsaturated fatty acids (PUFA), such as omega-3 and omega-6 fatty acids, phytosterols and several other substances including thy moquinone (TQ) (up to 25% in volatile oil), carvacrol, t-anethole, sesquiterpenelongi folene and four terpinol. The principle active ingredients of NSO are TQ, dithymoquinone, thymohy droquinone **(Akram and Afzal, 2016).** Because NS and TQ have been found to be useful in combating a number of models of nephrotoxicity, such as those induced by gentamicin **(Yaman and Balikci 2010)**, there is a big interest in the role of these agents as preventive strategies for aluminum chloride induced nephrotoxicity in rabbits. Therefore, the present study was designed to clarify the protective effect of Nigella Sativa oil (NSO) on aluminum chloride ($Alcl_3$) induced nephrotoxicity in rabbits through studying complete blood picture, estimation of ovidative stress ,antioxidant enzymes and kidney function test in addition to the histopathological alteration in rabbit kidneys.

MATERIAL AND METHODS

Chemicals:

Aluminum chloride ($Alcl_3$):

Aluminum chloride ($Alcl_3$) used in the present study were purchased from El-Gomhoria Chemical Company. .

Nigella Sativa oil:

Nigella sativa oil used in the present study was purchased from Pharco Pharmaceuticals Alexandria- Egypt.

Experimental rabbits:

A total number twenty-eight White New Zealand rabbits, two months old weighing from 1.5 kg to 1.8 kg obtained from private farm. Rabbits were apparently healthy with no clinical signs to any other diseases. The rabbits were kept in stainless steel wire mesh cages under sanitary hygienic condition and fed on balanced commercial pellets diet (minimum of 15 g of crude protein per kg of dry matter (DM), 15 g of crude fibre per kg of DM, and 10.2 MJ of digestible energy per kg of DM). **(Xiccato and Trocino, 2010)** and provided water

ad-libitum during the experimental period. After 15 days of adaptation period, the rabbits were randomly divided into four equal groups each of 7 rabbits.

Experimental design:

The first group received aluminum chloride at a dose of 50mg/kg body weight r daily for21 days by oral gavages, according to, **(Arumugam Kalaiselvi *et al.,* 2014) and (Ghorbel *et al*., 2016)**. The second group received aluminum chloride by oral gavages as the same dose in-group 1(50mg/kg b.wt)**,** daily for 21 days with a concomitant dose of 2ml/kg b.wt Nigella Sativa oil (NSO) given directly orally. **(Bouasla *et al*., 2014)**.The third group received Nigella Sativa oil directly orally at the same dose as in-group 2. The forth group was the control group and kept without treatments for 21 days. Clinical signs were reported.

Sampling:

Blood and kidney samples were collected at end of experimental period (21 days).Blood samples were collected from five rabbits in each group from the marginal ear vien. Two types of blood samples were collected from each rabbit .The first blood sample was collected in test tube cotaining EDTA and used for hematological examination (RBCs count), hemoglobin concentration (Hb), packed cell volume (PCV) and total leukocytic count (WBCs). The second blood sample was collected in plain centrifuge tube for separation of clear serum. The obtained serum was used for determination of kidney function tests (creatinine, urea and uric acid) and estimation of antioxidant status (MDA, GSH and SOD). After blood sampling, rabbits were slaughtered and samples from kidneys were obtained and fixed in 10 % neutral buffered formalin for histopathological examination.

Methods:

Erythrocytic count (RBCs) hemoglobin (Hb) and packed cell volume (PCV) by **(Moxine and Benjamine, 1970)** and total leukocytic count (WBCs) according to **(Feldman *et al.*, 2000).** Serum urea **(Tabacco, 1979)**, creatinine **(Husdan and Rapoport, 1968)** and uric acid **(Fossati *et al*., 1980**. lipid peroxidation was estimated in terms of thiobar-bituric acid reactive substances (TBARS), using malondialdehyde (MDA) as standard by the method of **(Beuge and Aust 1978)**. Glutathione (GSH) concentration was determined in samples according to the method of **(Mates *et al.*, 2000)** and superoxide dismutase (SOD) was detected according to **(Misra and Fridovich 1972).**

Pathological examination:

At the end of experimental period (21 days) rabbits were slaughtered, gross examination of kidneys were performed and samples of kidneys of all groups were taken and preserved in formalin 10%. Formalin fixed paraffin embedded sections were processed routinely for H&E staining according to, **(Suvarna *et al.,* 2013).**

Statistical analysis:

The obtained data were statically analyzed using Student's t-test according to **(Petrie and Watson 1999).**

RESULTS AND DISCUSSION

Aluminum accumulates in all tissues of mammals such as the kidneys, liver, heart, blood, bones and brain **(Alkahtani 2010)**, and it was found that one of the main organ target by Al. exposure is the kidney, which plays a major role in preventing accumulation of Al by excreting it out through urine **(Stoehr *et al.*,2006).** Different mechanisms of renal excretion of Al. have been suggested. These include glomerular filtration **(Yokel and Namara 1985),** tubular reabsorption of filtered Al. and secretion in distal nephron and excretion in the distal tubules **(Shirley and Late 2005 and Al-Hashem 2009)**. Hence, Al. accumulates in the kidneys and induces renal toxicity **(Exley 2004).**

Signs of Toxicity:

No mortality occurred during the study period. In addition, no signs of toxicity were observed in $AlCl_3$ treated rabbits. Our results regarding absence of toxic signs in rabbits treated with $AlCl_3$ resembling the findings reported by **Ahn *et al.,* (1995).** They stated that Aluminum alone, (100 or 500 ppm Al as AlCl3), ad libitum for 10 wk. None of these treatments altered food intake or weight gain in these rabbits. Aluminum has also been implicated in neurotoxicity associated with amyotrophic lateral sclerosis and Alzheimer's disease **(Shilpi *et al.,* 2009)** and its elimination half-life from human brain is calculated to be seven years. Al caused significant short-term and long-term memory disturbances, a decrease in locomotor activity, a significant inhibition of acetylcholine esterase activity in brain and a significant depletion of antioxidant enzymes (catalase, glutathione reductase and glutathione peroxidase) and glutathione. It significantly increased lipid peroxidation levels in cerebrum and cerebellum. **(Kaddour, *et al.*, 2016).**The current results presented in **(**Table 1), showed that administration of $AlCl_3$ induced significant decrease in erythrocytes count, Hb and PCV.

There was also a significant increase in total WBC counts .Administration of Nigella Sativa oil (NSO) improved toxic effect of Al. Hematological effect of $AlCl_3$ may be a result of inhibition of haematopoiesis or defective haematopoiesis which is resulted from renal failure as kidney secrete the erythropoietin hormone that inhibit the bone marrow to further RBCs production. These findings were firmly proved by the histopathological findings in kidneys of $AlCl_3$ intoxicated rabbits. In addition, $AlCl_3$ treatment induced a significant increase in total WBC counts. This is indicative of immune system activation and that may reflect the incidence of tissue oedema and inflammation **(Mahieu *et al*., 2005 and Newairy *et al*., 2009).** The present results were in line with previous reports that demonstrated that heavy metal exposure altered erythropoiesis in rats **(Farina *et al*., 2005 and Zhu *et al*., 2012).** Thus, the NSO supplementation ameliorates this alteration, stimulation erythropoietine production and restores the erythropoiesis **(Bouasla *et al*., 2014)**. In fact, the beneficial effects of NSO are probably due to its antioxidant activity causing a decrease of $AlCl_3$ concentration in blood cells; inhibit its entry into erythrocytes and facilitating iron incorporation to the heme group. It is could be also related to the anti-inflammatory effects of NSO reported in several previous studies **(Salem 2005 and Krishnan and Muthu Krishnan 2012)**. The current results in (Table 2) showed significant increase in serum creatinine, urea and uric acid in $AlCl_3$ intoxicated group. Administration of Nigella Sativa oil (NSO) improve the levels of creatinine, urea and uric acid as compared to the control level. **Kowalczyk *et al.,* (2004),** previously recorded these findings**.** They reported that Al. has a significant role in the pathogenesis of renal dysfunction and in many clinical disorders. Chronic exposure to $AlCl_3$ in rats has produced nephrotoxicity. Glomerular tuft and renal tubules were reported to be the primary sites of renal damage **(Stacchiotti *et al.,* 2006 and Hussain 2016)**. Mainly the kidneys excreted aluminum chloride and it caused marked degeneration of tubules. Increased serum urea and creatinine concentration can be a consequence of critical accumulation of this metal in the kidneys, eventually resulting in renal failure **(Belaid *et al.,* 2013)**. The kidneys are involved in the excretion of various xenobiotics, pollutants, and toxins hence, they are prone to liberate high quantities of free radicals, which contribute to high oxidative stress that is involved in the pathogenesis of kidney damage **(Ghosh *et al.,* 2010 and Hussain 2016).** Administration of Nigella Sativa oil (NSO) improved the levels of creatinine, urea and uric acid .The protective effects of Nigella Sativa oil (NSO have been reported previously by other investigators as **Bouasla *et al*., (2014)**. Such improvement may be due to protection of the

kidneys from oxidative damage due to its antioxidant effect. Most of these protective actions have been associated with the anti-oxidant and anti-inflammatory proprieties of TQ **(Ragheb *et al*., 2009).** The free radical scavenging effect could be enhanced by many factors, including the redox properties of its Quinone structure, its ability to cross biological barriers and subsequent easy access to subcellular compartments **(Badary *et al*., 2003)** the present study revealed that oxidative stress may play a crucial role in $AlCl_3$ induced nephrotoxicity. (Table 3) Exposure of rabbits to $AlCl_3$, induced significant elevation (P<0.05) in MDA while Treatment of rabbits with nigella sativa oil lower the raised MDA level. GSH and SOD showed a significant decrease (P<0.05)in rabbits intoxicated with $AlCl_3$ .Rabbits receiving nigella sativa oil (2ml /kg. bw. daily for 21consecutive days) showed improvement in GSH and SOD toward normal control values. The toxic effects associated with $AlCl_3$ are due the generation of ROS, which in turns results in the oxidative deterioration of cellular lipids, proteins and DNA **(Sargazi *et al.,* 2006).** It was demonstrated that Al. may alter the activity and levels of a number of components of tissue antioxidants defense system, such as GSH, SOD leading to increase production of free radicals especially ROS and developing of lipid peroxidation **(Moumen *et al.*,2001 and Hussain 2016).** Lipid peroxidation of biological membranes leads to a loss of membrane fluidity, change in membrane potential, an increase in membrane and alterations in receptor functions **(Nehru and Anand 2005)**. Although. Al is a transition metal, and therefore, cannot initiate peroxidation; many studies have searched for mechanisms between Al. and oxidative damage in tissues **(Nehru and Anand 2005).** A previous study by **Ward *et al.*,(2001) and Hussain(2016),** suggested that exposure to Al could promote disruptions in the mineral balance, resulting in Al. ions replacing iron and magnesium, which would then lead to a reduction in Fe_2^+ binding to ferritin. Free iron ions released from biological complexes by Al. can catalyze hydroperoxides decomposition to hydroxyl radicals via Fenton's reaction. This high hydroxyl radical reactivity could initiate the peroxidation of membrane lipids, causing membrane damage. Administration of Nigella sativa oil at the same time with $AlCl_3$ significantly reduced the oxidative effect that dropped to the control level **(Bouasla *et al*., 2014)**. Our study revealed that oxidative stress might play a crucial role in $AlCl_3$ induced nephrotoxicity. **Bourgou *et al*.,(2008**) indicate that phenolic compounds are the most important active constituents of *N. sativa* seeds and its oil, that have been shown to be capable of scavenging free radicals and protecting lipids from being oxidized or destroyed during oxidative damage. Regarding to the pathological examination,

the kidneys of all groups were apparently normal except in $AlCl_3$ treated group. The kidney in $AlCl_3$ treated group was enlarged and dark red in color. The histopathological examination in the present study in $AlCl_3$ treated rabbits (group1), the kidneys of this group showed dilatation of some proximal, distal and collecting tubules together with perivascular edema Fig. (1A). Cloudy swelling, vacuolar and hydropic degeneration especially in renal tubules Fig. (1B). In $AlCl_3$ and NSO treated rabbits (group 2), the sections from kidneys of this group showed moderate degenerative changes in the proximal and distal convoluted tubular epithelium Fig. (2 A, B). Few numbers of the glomeruli showed hypertrophied mesangial cells with mildly dilated renal tubules and moderate hyaline cast in some renal tubules Fig. (2C). NSO treated rabbits (group3), examined sections from kidney of this group revealednormal nephron,medullary,papillaryandpelvicstructuresFig.(3A).The histopathological finding in kidney sections of $AlCl_3$ treated group was compatible with those reported by **(Somova *et al.,* 1997, Shilpi *et al.,* 2009 and Hussain 2016).** Indeed, Al accumulation in the kidney has been related to worsening renal function **(Exley 2004 and Al-Hashem 2009)**. It has been reported that, the kidney may be exposed to high concentration of Al during the normal process of renal excretion making the kidney vulnerable to Al-mediated toxicity, which is dependent on the route of exposure **(Krewski *et al.,* 2007).** In this regard, most of the published studies have investigated the toxic effect of $AlCl_3$ in animal after intraperitoneal or parenteral administration **(Krewski *et al.,* 2007 and Aguilar *et al.,* 2008)**. It is well reported that Al enters the body via 2 major routes, pulmonary and oral. Although only a small portion of Al is absorbed through the gastrointestinal tract, oral intake is associated with the greatest toxicological implication **(Krewski *et al.,* 2007and Aguilar *et al.,* 2008).** For this reason, **Chagnac *et al.,* (1987)** did not observe any change on the renal structure when injected $AlCl_3$ intraperitoneally to rats in a doses, 0.2mg/day and 2mg/day for 13 weeks, this difference may be attributed to, the dose administer and through the route of administration. In the present study administration of NSO to $AlCl_3$ treated rabbit's effectively improved renal function, as concluded from, improved hematological picture of rabbits and decreased serum urea and creatinine concentration. Ameliorated oxidative stress and decreased histological changes characteristic of $AlCl_3$ nephrotoxicity.The main mechanism by which NSO act in ameliorating $AlCl_3$ induced renal damage is due to its potent anti-inflammatory and antioxidant proprieties of thy moquinone (TQ). **(Bouasla *et al*., 2014)**. It has been reported that, the chemical composition of NS is very rich, containing fixed oils (84% fatty acids) and volatile oils,

amino acids, proteins, carbohydrates, alkaloids (nigellidine,nigellimine and nigellicine), saponine, crude fiber as well as minerals such as calcium, iron, sodium and potassium. In addition, vitamins such as riboflavin, thiamine, pyridoxine, folic acid and niacin. In addition, NSO is rich in polyunsaturated fatty acids (PUFA), such as omega-3 and omega-6 and several other substances including thymoquinone (TQ).These principle active ingredients may acts the protective effects of NSO **(Akram and Afzal, 2016).**

CONCLUSION

This study proved a protective effect of NSO against nephrotoxic effects of $AlCl_3$ through improvement of some hematological, biochemical, antioxidant parameters as well as histopathological picture.

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Table (1): The effect of Nigella Sativa oil (NSO), aluminum chloride ($AlCl_3$) and their combination on some hematological parameters (Mean ± SE) in rabbits of different treated groups: n=5.

Parameters / Groups	RBCs ($x10^6$)	Hb (g/dl)	WBCs ($x10^3$)	PCV%
Gp.1 ($AlCL_3$) Control +ve	**3.5± 0.26****	**8.1 ± 0.46****	**6.900 ± .18***	**34.4 ± 1.91***
Gp.2 (AlCl3+NSO) Treated group	**4.3 ±0.17***	**10.4 ± 0.90 ***	**6.350 ±0.43***	**37.7 ± 2.3**
Gp.3(NSO)	**5.4 ± 0.42**	**12.2 ± 0.72**	**5.82 ± 0.33**	**38.9± 1.97**
Gp.4 (-ve Control)	**5.24 ±0.51**	**12 ±1.33**	**5.5 ± 0.43**	**38.3 ± 1.95**

***Significant at (P ≤ 0.05) ** Significant at (P ≤ 0.01)**

RBCs= Red Blood Cell Count. Hb=Hemoglobin Concentration

PCV = Packed Cell Volume. Total leucocyte count (WBCs).

Table (2): The effect of Nigella Sativa oil (NSO), aluminum chloride ($AlCl_3$) and their combination on serum biochemical parameters (Mean ± SE) in rabbits of different treated groups: n=5.

Parameter / Groups	Uric acid (mg/dl)	Urea (mg/dl)	Creatinine (mg/dl)
Gp.1($AlCL_3$) Control +ve	8.9 ±0.32**	46.5 ± 1.12*	1.88 ±0.24**
Gp.2 ($AlCL_3$+NSO) Treated group	6.4±0.30*	33.2 ±1.38	1.34±0.15*
Gp.3 (NSO)	3.85±0.39	22.7 ±0.96	0.95±0.09
Gp.4 (-ve Control)	4.3±0.43	24.5 ±1.35	0.98±0.05

***Significant difference at (P ≤ 0.05) **Significant at (P ≤ 0.01).**

Table (3): The effect of Nigella Sativa oil (NSO), aluminum chloride ($AlCl_3$) and their combination on some antioxidant parameters (Mean ± SE) in rabbits of different treated groups: n=5.

Parameters / Groups	MDA(nmol/ml)	GSH(u/ml)	SOD(u/mg)
Gp.1($AlCl_3$) control +ve	24.6 ±1.45**	14.47±1.23**	1.00±0.12**
Gp.2($AlCl_3$+NSO) Treated group	16.85±1.0 3*	20.15±1.36*	1.7±0.11*
Gp.3(NSO)	10.3±0.7 2	31.15±3.52	2.5±0.24
Gp.4(-ve Control)	12.05±0.47	25.4±2.36	2.4±0.238

***Significant difference at (P ≤ 0.05) **Significant at (P ≤ 0.01).**

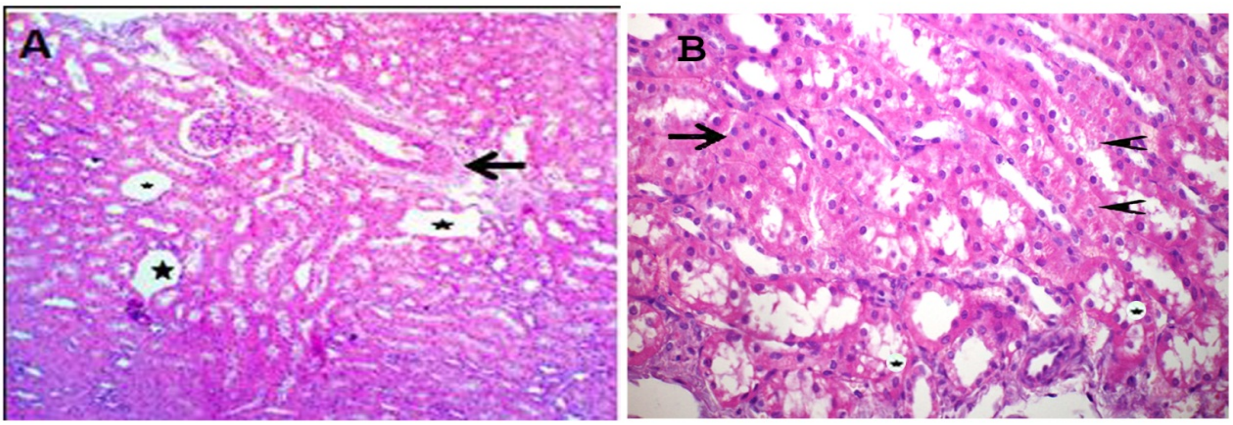

Fig. (1): photomicrograph of kidney received $AlCl_3$ (group1) showing: (A) mild dilatation of some cortical and medullary tubules (stars) with perivascular edema (open arrow) **(H&E) X100.** (B) Cloudy swelling (open arrow) together with vacuolar (star) and hydropic degeneration (head arrow) in renal tubules. **(H&E) X400.**

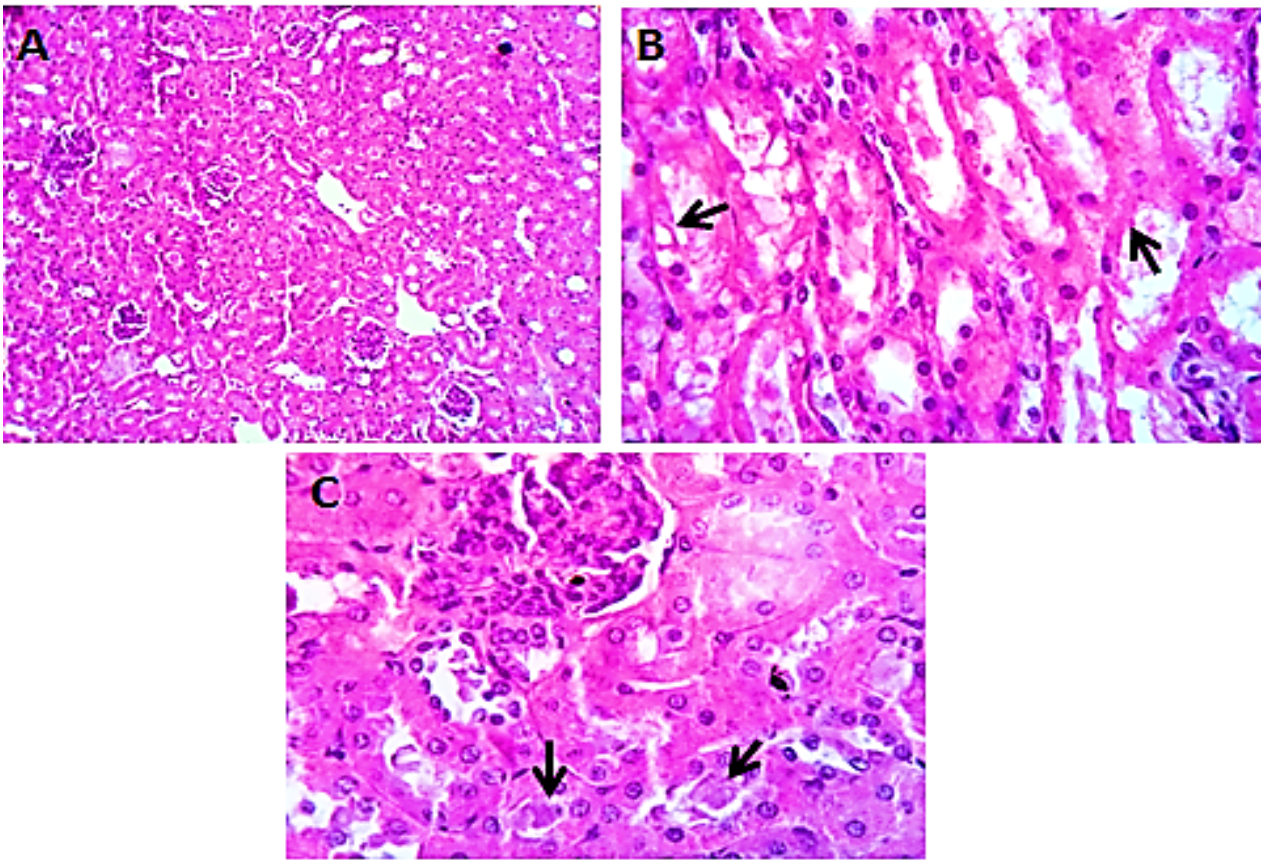

Fig. (2): photomicrograph of kidney received $AlCl_3$ and NSO (group2) showing: (A, B) degenerative and necrotic changes in cortical and medullary collecting tubules (arrows) **(H&E) X 100,400.** (C) Glomerular mesangial cells hypertrophy and dilated renal tubules with hyaline cast (arrows) **(H&E) X400.**

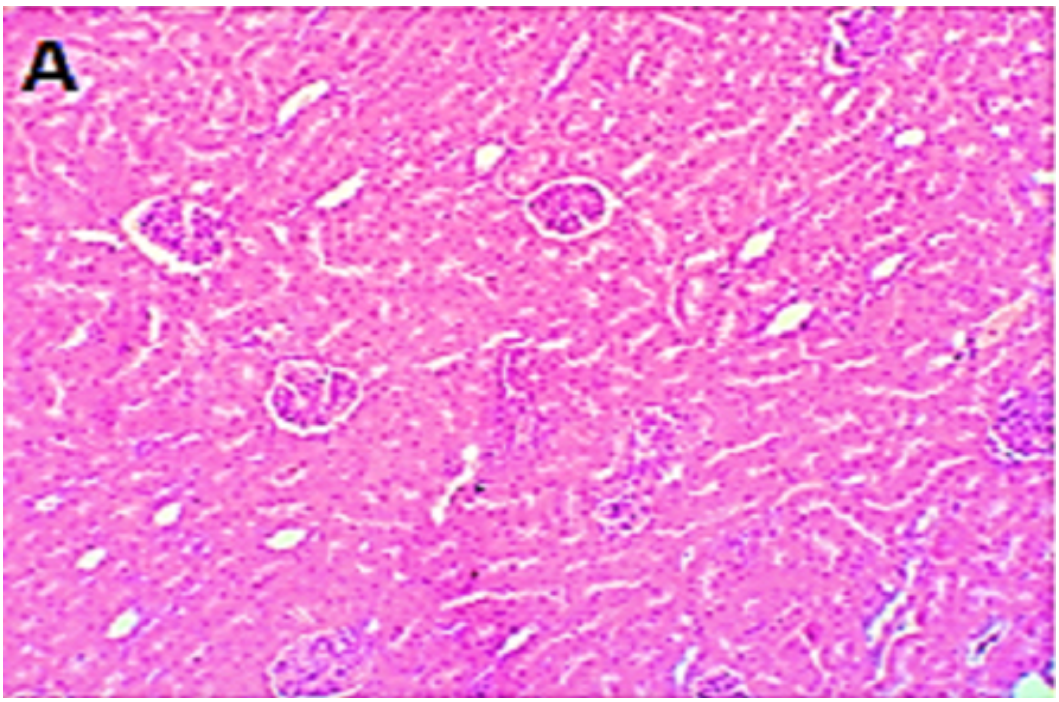

Fig. (3): photomicrograph of kidney received NSO (group3) showing: (A) Normal renal structures **(H&E) X100.**

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تأثير زيت حبة البركة على التسمم الكلوى بكلوريد الالومونيوم فى الارانب

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الملخص العربى

صممت هده الدراسة بهدف التحقق من التأثير الوقائى المحتمل لزيت حبة البركة على التسمم الكلوى المحدث فى الارانب نتيجة للتعرض لمادة كلوريد الالومنيوم.

أجريت هده الدراسة على عدد 28 أرنب أبيض نيوزيلاندى بالغ. قسمت بالتساوى الى اربع مجموعات.

المجموعة الاولي: أعطيت مادة كلوريد الالومنيوم بجرعة 50مجم/كجم من وزن الجسم فى ماء الشرب يوميا لمدة 21يوم.

المجموعة الثانية: أعطيت مادة كلوريد الالومنيوم فى ماء الشرب بنفس الجرعة كما فى المجموعة الاولى يوميا لمدة21يوم مصاحبا لدلك 2مل/كجم من وزن الجسم زيت حبة البركة عن طريق الفم.

المجموعة الثالثة: أعطيت فقط زيت حبة البركة بنفس الجرعة كما فى المجموعة الثانية عن طريق الفم لمدة 21 يوم.

المجموعة الرابعة: تركت ضابط للتجربة بدون اى علاجات لمدة 21 يوم. فى نهاية التجربة (21 يوم) تم سحبعينات الدم من جميع المجموعات. تم تجميع نوعين من عينات الدم. عينات الدم الاولى تأخد على ماده مانعه للتجلط ودلك لاجراء بعض الفجوصات الهيماتولوجية والجزء الاخر من الدم يتم سحبها فى انابيب اختبار لفصل السيرم واجراء بعض الاختبارات البيوكيميائية. تم دبح الارانب وعمل الصفة التشريحية فى نهاية التجربه وأخد عينات من الكلى من جميع المجموعات وحفظها في فورمالين 10% للقيام بالفحص الباثولوجى. اظهرت النتائج زيادة ملحوظة في العدد الكلىلكرات الدم البيضاء ونقص ملحوظ فى عدد خلايا كرات الدم الحمراء والهيموجلوبين كما لوحظ زيادة معنوية فى كل من اليوريا وحمض اليوريك والكرياتنين مع وجود زيادة واضحة فى تركيز المالونديالدهيد وانخفاض معنوى فى مستوى الجلوتاثيون المختزل وسوبر اوكسيد ديسميوتاز. وقد اظهر الفحص المجهرى للكلى وجود تنكسات فى الانبيبات الكلوية مع ارتشاح في جدر الاوعية الدموية ووجود زيادة فى الخلايا المكونة للكبيبات الكلوية.

وقد اظهرت الدراسة تحسن ملحوظ في جميع القياسات اللتى تم اجراؤها للمجموعة التى تم علاجها بزيت حبة البركة مقارنة بالمجموعة الضابطة وايضا تحسن في التغيرات الباثولوجية للكلية.

مما سبق نستنتج ان زيت حبة البركة ادى الى تقليل الاثار الجانبيه من التسمم الكلوى الدى احدثه مادةكلوريد الالومنيوم فى كلى الارانب