

PROPOLIS CARDIO PROTECTIVE ROLE FROM THE IMPACT OF ALUMINUM CHLORIDE IN FEMALE RABBITS

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ABSTRACT

The protective role of propolis against the side effects of Aluminum Chloride exposure on myocardium in adult female rabbits was studied. A total of 24 local breed rabbits at age 8 – 9 weeks , were divided in to three equal groups.1st group considered as control group , 2nd group received AlCl₃ solution (50mg/kg B.W) , 3rd group received AlCl₃ (50 mg /kg body weight) + propolis (50 mg /kg body weight).The experiment lasted for 60 days and at the end of experiment blood samples were collected for biochemical analysis and cardiac samples were collected histological examination. The results revealed that AlCl₃ caused a significant($p<0.05$) increase in total-cholesterol; LDL and VLDL –cholesterol; total triacylglycerol ; CK-MB; and CRI , and decrease in HDL-cholesterol and AAI. Otherwise propolis was efficient in restoration these variables to semi normal values. Histopathological analysis of heart sections showed deleterious changes in the myocardium which were improved in hearts of propolis received group

INTRODUCTION

Aluminum (Al), the third most common element as described by (1) found in the nature in different forms mostly bound to silicon, fluorine, oxygen, and other elements in the soil, rocks, clays and gems. Al compounds widely distributed in the environment and highly used in daily life, which causes its easy exposure to human beings and animals. The main ways of the human and animals body exposure via the gastrointestinal and the respiratory tracts(2). Al can accumulates in all tissues of the mammals, and has a significant toxic potential for humans and animal's tissues mainly due to it's deleterious effects on lipid peroxidation and induction of oxidative stress (3). Aluminum provoked neurotoxicity (4 ,5),hematotoxicity (6, 7) , nephrotoxicity(8, 9) , male reproductive toxicity(10)and cardiotoxicity (11,12). Al caused bone marrow chromosom abnormalities in rat bone marrow cells(13).

Although the mechanism of action is not known, Al has been shown to alter calcium (Ca^{2+}) flux and homeostasis, and facilitate the peroxidation of membrane lipids (3). Due to its toxicity, Aluminum, is involved in the pathogenesis of certain diseases, including Alzheimer's disease, encephalopathy, aplastic bone disease osteomalacia, microcytic anemia, myopathy, pulmonary fibrosis (5).

Propolis, a resinous wax-like, beehive product is collected by honey bees from plant exudates and also known as bee glue. Chemically propolis may contain varieties of compounds like resin, essential oils and waxes, and also contains amino acids, minerals, ethanol, vitamin A, B complex, E, and flavonoids (14). These chemical properties of propolis make it beneficial to bees and have general pharmacological value as a natural mixture (15). Several experimental and clinical findings point to the fact that propolis may be more effective against pathogenic microorganisms and counteract the damaging effects of toxic material including aluminum (16 and 7). The propolis as found by (17, 18, and 19) contains a variety of chemical compounds as poly phenols (flavonoids, phenolic acids, phenolic aldehyde and alcohols), coumarins and steroids, the many constituents of propolis are differ according to the geographical and botanical origins. For these constituents Propolis has the potential to be used in both treatment and prevention of leukaemia (14). In addition it used safely for its anti-inflammatory (20) cardioprotective (21) hepatoprotective (22) and neuroprotective (23). Inadequate information regarding mechanism of propolis against Aluminium chloride –induced cardiotoxicity. The aim of the present study was to evaluate the possible ameliorative role of propolis against negative effects of Aluminium chloride exposure could have effects on the structure and functions of myocardium in adult female rabbits.

MATERIALS AND METHODS

Experimental Animals :

The present study was carried out in the department of physiology and pharmacology in college of veterinary medicine /Baghdad university during the period from 1st Jan. to 10th of Feb. at 2013. A total of 24 local breed, adult female rabbits at age 8 – 9 weeks with body weight ranged between 1500 – 2000 grams, were divided in to three equal groups, 1st group considered as control group, 2nd (A) group received AlCl_3 solution (50mg/kg B.W). according to (24), 3rd group (AP) received AlCl_3 (50 mg /kg body weight) + propolis (50 mg /kg body weight). All experimental animals were given different treatment daily orally administered. At the end of experiment, after 60 days, blood samples were collected for biochemical analysis and immediately after animals were sacrificed, hearts were isolated and preserved in formalin solution of 10% for histopathological examination.

Propolis preparation: Crude propolis was obtained from local markets as Iraqi propolis and the soaking method (maceration) was used to prepare propolis extract, the dosage was designed according to previous studies by, (25).

Serum Lipid profile :

Serum total cholesterol and triacylglycerides levels were measured by a colorimetric methods involved enzymatic hydrolysis methods using commercial kits (Randox Laboratories Ltd., U.K.)

HDL-cholesterol measured by enzymatic colorimetric method with Randox diagnostic kit

The LDL and VLDL- cholesterol was determined by using Freidewald's formula(26):

$$\text{LDL cholesterol} = \{\text{total cholesterol} - (\text{triglycerides}/5) - \text{HDL-cholesterol}$$

$$\text{VLDL- cholesterol} = \text{Total triglycerides}/ 5.$$

Cardiac damage markers:

Creatin Kinase : Determined by a colorimetric method using commercial Kit (Randox Laboratories Ltd., U.K.)

Determination of antiatherogenic index (AAI) and coronary risk index (CRI) were calculated using the following formulae cited by (27 and 28):

$$\text{AAI} = \frac{[\text{HDL-C}]}{\text{Total cholesterol} - \text{HDL-C}} \times 100$$

$$\text{CRI} = \frac{\text{Total cholesterol}}{\text{HDL-cholesterol}}$$

Histopathological examination of the heart .

Sections of the heart were obtained from the experimental groups and were fixed for histopathological evaluation with 10% formaldehyde Solution. Serial sections were prepared as described in (29).

Statistical Analysis

The statistical Analysis System- SAS (30) was used to effect of different factors in study parameters. Least significant difference –LSD test was used to significant compare between means in this study.

RESULTS**Lipid profile**

Results of the present study in table -1, represented the variable parameters regarding to lipid profile. Results showed the significant ($p < 0.01$) increase in the total cholesterol, total triacylglyceride, LDL- cholesterol. VLDL- cholesterol. and significant ($p < 0.01$) decrease in HDL- cholesterol, in group received AlCl_3 when compare with the other experimental groups that either received propolis with AlCl_3 or control groups. Also results revealed a significant role for propolis to returned these variables to semi normal values in AP group.

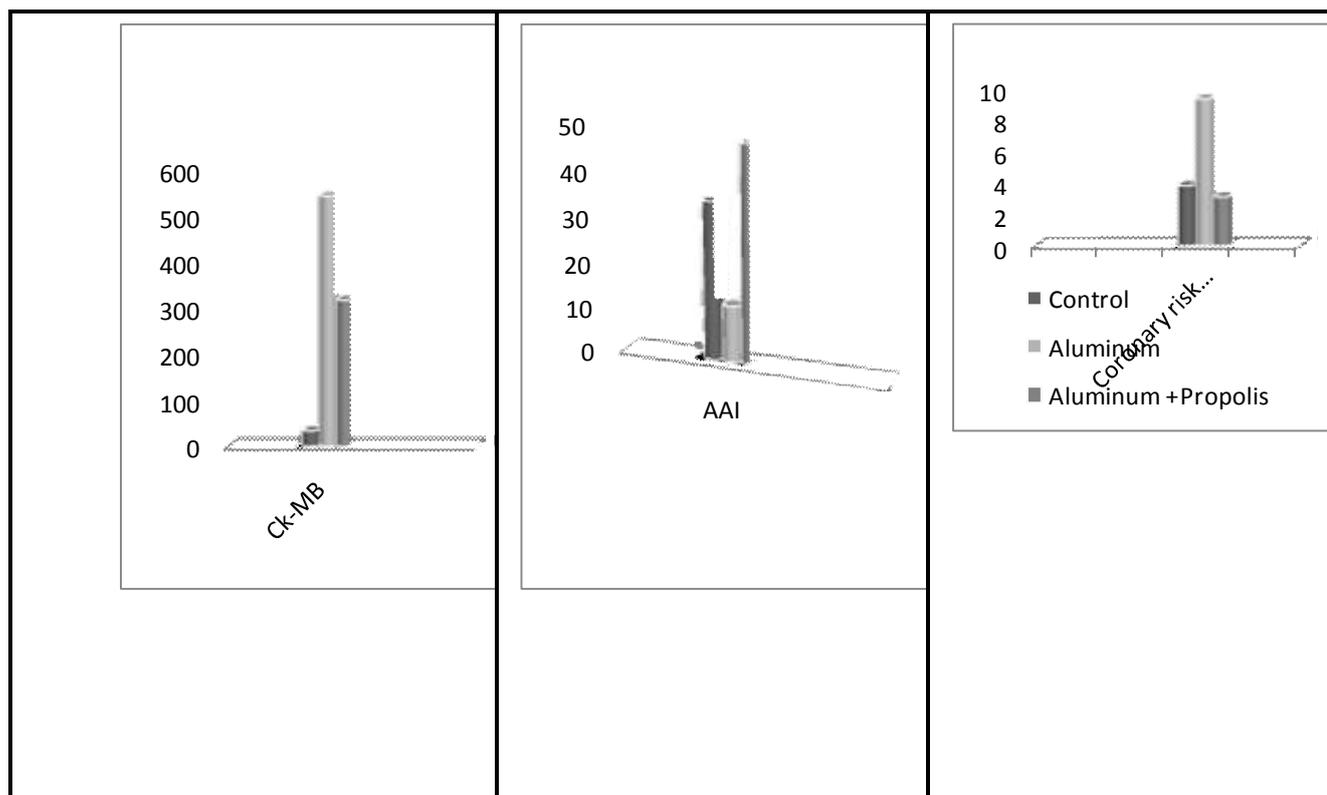
Table -1. Effect of Propolis on lipid profile against aluminum Chloride in femal rabbits for 60 days. Mean \pm SE . n=8

Parameters	Control	Aluminum chloride (50mg/kg)	Aluminum Chloride +Propolis (50mg+50mg/ Kg)	LSD value
Total Ch	116.67 \pm 10.21	202.50 \pm 10.93**	129.30 \pm 8.89	30.295
TAG	156.50 \pm 8.73	192.67 \pm 24.25**	112.50 \pm 5.35	45.812
HDL-Ch	29.00 \pm 0.58	22.33 \pm 1.31**	40.67 \pm 3.03	5.830
LDL- Ch	56.33 \pm 10.28	141.50 \pm 14.18**	66.50 \pm 8.51	33.899
VLDL-Ch	31.33 \pm 1.74	38.67 \pm 4.81*	24.17 \pm 2.62	10.005
* (P<0.05), ** (P<0.01).				

Cardiac damage Markers

There was a significant ($p < 0.01$) increase in mean CK-MB levels in the AlCl₃ administered group as compared to the control group . The comparison between propolis + AlCl₃ group with AlCl₃ group revealed a significant ($p < 0.01$) role of propolis in reduction of CK-MB levels was seen (figure-1).

Anti-atherogenic index significantly ($p < 0.01$) reduced in groupA and elevated in AP group. At the same time coronary risk index elevated significantly in group A when compared with AP and C groups.



Figur-1 : Effects of Propolis on cardiac damage markers against Aluminum Chloride in female rabbits

Histopathological examination of the heart

Analysis of the microscopic photograph in figure-2-A and B showed the normal heart of control group in terms of normal thickening of ventricular wall (fogur-2-A) and myocardium with a spindle shape with no significant pathological changes (figure-2-B). The deleterious effects of Aluminum in heart sections are shown in figure- 3- A and B. $AlCl_3$ caused as disorganization of cardiac muscle fibers due to sever vacuolar degenerative changes which appeared enlarged with different sizes and multiple vacuoles in their cytoplasm, congestion of interstitial blood vessels also seen, were dilated and filled with blood and contain inflammatory cells (mononuclear cells). The large and medium sized of cardiac blood vessels were increased in their sizes due to hyperatrophy of medium muscular coat and vacuolar degeneration of tunica antima with recognized flattening of the intimae elastic (figure-3-A). Other sections showed necrosis of cardiac muscle fibers and hyalinization, few inflammatory cells infiltrated between muscle fibers. These pathological changes caused an increase in the ventricles wall thickness and reduced the ventricle lumen (Figur-3-B). The histopathological examination of heart sections of group AP (Figure- 4-A) revealed that these deleterious changes were reduced in the myocardium, also showed the mild to moderate vacuolar degenerative changes with a fat deposit instead of the hyaline deposition found in myocardium of $AlCl_3$ group, which clearly affect on the wall thickness and lumen dialation(figure-4-B)

DISCUSSION

Lipids profile:

In generally known that increased serum lipids including total cholesterol and triacylglycerids ;and LDL and VLDL cholesterol could be resulted from two mechanisms , the 1st related to increase in the lipid food intake and/ or the biosynthesis of lipids by the liver cells , the 2nd mechanism is persist of the lipoproteins molecules that carried cholesterol and triacylglecrols circulating in the blood . Naturally up take of these molecules by different cells via a receptor mediated endocytosis. Decrease of uptake of cholesterol and the lipoproteins may be resulted from abnormal changes of these molecules or the receptors them self . In the present study the denoted elevation of serum cholesterol free or carried by LDL&VLDL and triacyleglycerol could be resulted from either increase the biosynthesis or decreased in the uptake of these lipid molecules . There are some evidences about the cholesterogenesis effects of some metals like Pb (31). As mentioned by many researchers) AlCl₃ induce pro-oxidants and antioxidant imbalance and act as predisposing factor for oxidative stress(32and 33) . This imbalance reflected by decreased of the activities of antioxidants enzymes as well as the non- enzymatic antioxidants in addition increased production of lipid peroxidation (34and 35), lead to formation of free radicals which further activates the lipid oxidative damage. Consequently this oxidative damage of cellular membranes and lipoproteins will persist the serum levels of LDLandVLDL cholesterol and triacylglycerids in high levels . HDL molecules which indicate the cholesterol transport from the peripheral tissues to liver for more metabolism and excretion as bile acids(36), the reduction of the biosynthesis of this molecule by hepatocytes is triggered by low intracellular cholesterol level (24). In the present study the reduced HDL-ch in AlCl₃ group could be attributed to defect in the intrahepatic cholesterol metabolism as a result to persisting of serum LDL and VLDL caused by AlCl₃ oxidative damage. Other wise, administration of propolis was efficient in keeping these lipids profile in semi normal levels. This restoration of LDLandVALDL cholesterol triacylglycerol to normal values indicated the protective role of propolis on these molecules and on the hepatocytes membrane receptors integrity . Although this protective role is not well clear, but it might be attributed to propolis antioxidant effects.The antioxidants activity of propolis represented by increase of antioxidant enzymes activities and/ or decrease reactive oxygen species as documented by many researchers (37, 38and 39) . On the other hand Cholesterol biosynthesis and esterification were concomitantly reduced by flavonoid, as indicated by the decreased HMG-CoA reductase and ACAT activities (40), and as mensioned previously Propolis is a rich flavonoiads material. Thus, the propolis rest orated the lipids profile to normal levels.

Cardiac damage Markers

In the present studt the significant elevation of serum creatine kinase-MB (CK-MB) confirming myocardial infarction in rabbits exposed to Aluminium Chlortide. When

cardiac muscle cells are damaged the membranes permeability will increased with increased muscle contractility, which results in increasing allowing cardiac enzyme to leak out into the bloodstream (41, 42)because CK-MB is the enzyme capable to transfer a phosphate group from the energy storage form of creatine phosphate, to a molecule of ADP, for production of ATP (43). CK-MB considered as a valuable diagnostic tool for myocardium, since damage specific to the myocardium would result in elevation of CK-MB levels because it is localized predominantly in the heart (44, 45).

The results also showed that the administration of AlCl₃ induced cardiotoxicity is manifested by a significant decrease of antiatherogenic index and increase of coronary risk index . This deviation in the cardiac damage markers from control denoted in the present study could be attributed to both oxidative stress of myocardium and deleterious lipid metabolism . Abnormal lipid metabolism usually correlated with cardiovascular diseases (46), moreover, lipid peroxidation is one of the consequences of free radical reactions which causes the dysfunction and damage of cell membranes (4).

Because of the high polyphenolic / flavonoid components of propolis that gave it the antioxidants activities are related to their ability to chelate metal ions and scavenge singlet oxygen, superoxide anions, proxy radicals, hydroxyl radicals and peroxy nitrite (37).Also , treatment of rabbits with AlCl₃ plus propolis decrease CK-MB and elevate the AAI correlated with a reduction of CRI. This suggest that administration of propolis to rabbits exposed to AlCl₃ can modulate the cardio toxicity induced by aluminum reflecting the antiatherogenic effect of propolis against AlCl₃. Similar results found by (47) who concluded that dietary propolis may be an antiatherogenic agent on experimental hypercholesterolemia in rabbits.

Histopathological Examination of heart sections

Histopathological findings of AlCl₃ administered rats showed a marked incidence of tissue injury with myocardial atrophy, nuclear pyknosis, cytoplasmic vacuoles and cytoplasmic eosinophilia sever vacular degenerative with edema and hyaline accumulation. A similar results cited by (48) they found that administration a high dose of AlCl₃ by injection for 7-8 days influence on the rhythm of the heart activity to the point of death . As discussed by (49) AlCl₃ is greater accumulated in the heart than other Aluminium salts and consequently deviate the heart physiology from normal functioning

The analysis of the histopathological findings confirmed the biochemical data of the present study and showed that AlCl₃ administration caused cardiac damage ,The possible mechanisms for this damage changes is due to the oxidative stress induced by Aluminium . The cardiac myocyte is highly susceptible to free radical oxidative damage because of their high oxidative metabolism and relatively poor antioxidant defenses (50). The myocardial amelioration was seen in rats received propolis , suggesting a protective effects of propolis in myocardial damage .These effects

resulted due to the increase of antioxidants enzymes, non enzymatic antioxidants , and decrease of reactive oxygen species as suggested by (51), further more the restoration

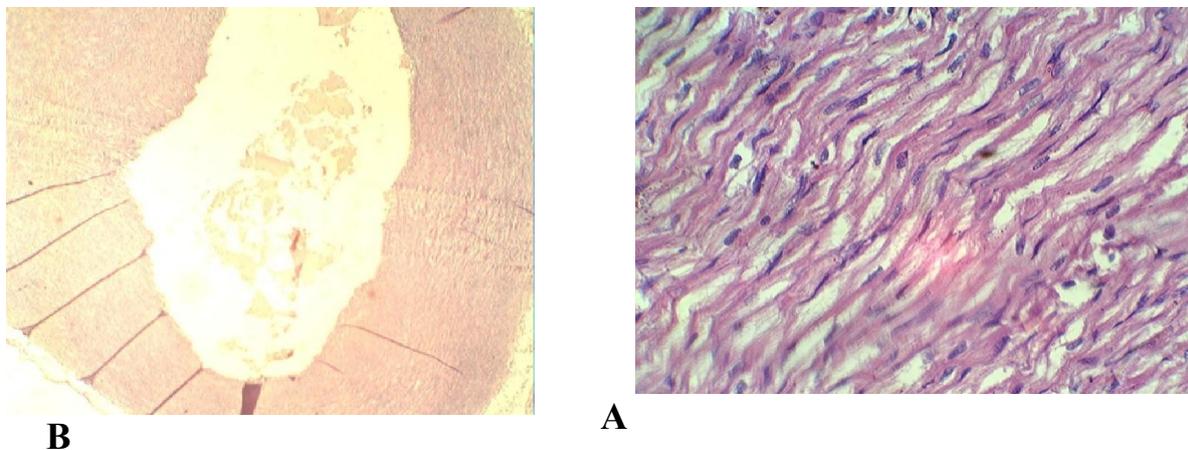


figure-2-A and B showed the normal heart of control group in terms of normal thickening of ventricular wall (A) and myocardium with a spindle shape (B) with no significant pathological changes

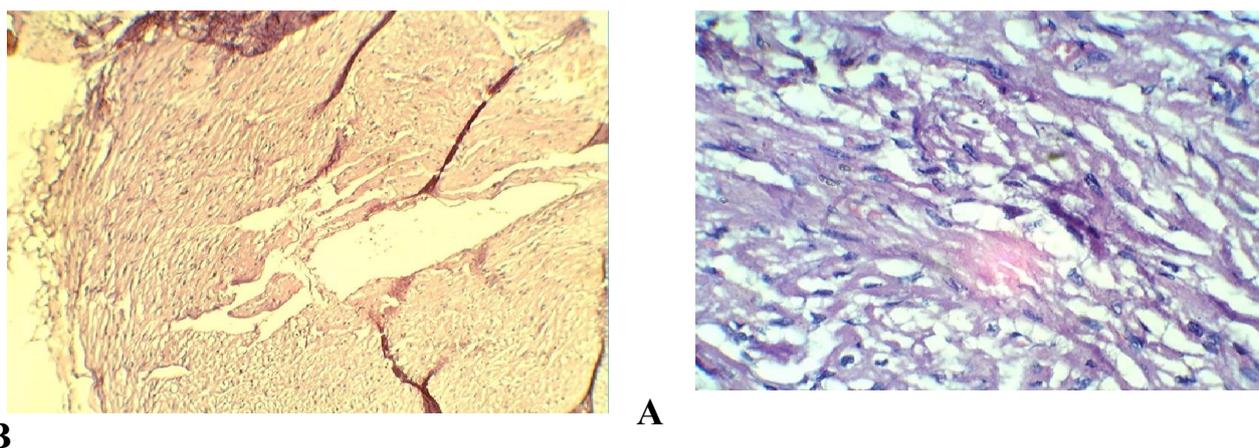
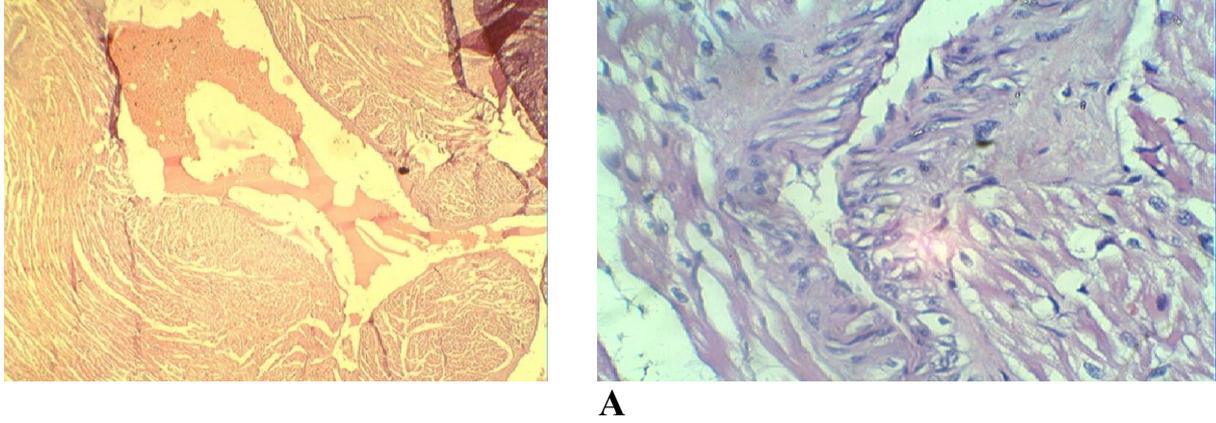


figure- 3. Light microscopic photograph of heart sections of $AlCl_3$ group. A- showing disorganization of cardiac muscle fibers, severe vacuolar degenerative , necrosis and hyalinization of cardiac muscle fibers. B- show increase in the ventricles wall thickness and reduced the ventricle lumen .



B

A

Figure-4:Light microscopic photograph of heart sections of group AP. A- show the mild to moderate vacuolar degenerative changes . B- semi normal ventricular wall thickness and lumen dialation

of lipids to normal values in the present study .

In conclusion , the present study suggests that *propolis* has potent blood lipid-lowering capability, in addition, it has significant antiatherogenic effect against hyperlipidemia caused by AIC13. Further more propolis improved the histopathological deleterious changes of myocardium.

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دور العكبر في وقاية القلب من تأثير كلوريد الألمنيوم في إناث الأرانب

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الخلاصة

تم دراسة دور العكبر في وقاية القلب من تأثير الألمنيوم كلورايد في إناث الأرانب البالغة . اجريت الدراسة على ٢٤ أرنبية من العرق المحلي بعمر ٨-٩ اسابيع و وزعت بالتساوي الى ثلاثة مجاميع التوالى اعتبرت سيطرة والثانية جرعت ب ٥٠ ملغم / كغم وزن جسم من كلوريد الألمنيوم والمجموعة الثالثة جرعت بالعكبر ٥٠ ملغم/كغم وزن جسم + ٥٠ ملغم / كغم وزن جسم من كلوريد الألمنيوم تم تجريع الحيوانات فمويا ويوميا لمدة ٦٠ يوما و في نهاية التجربة جمعت عينات الدم لغرض اجراء التحليلات الكيموحيوية ونماذج من القلب لغرض الفحص النسيجي. أظهرت نتائج الدراسة بأن الألمنيوم كلورايد سبب في زيادة معنوية في كولستيرول الدم الكلي والمرتبط مع LDL & VLDL و CK-MB و CRI وانخفاض في الكولستيرول مع HDL و AAI في حين كان العكبر فعال في المحافظة على هذه المتغيرات ضمن الحدود الطبيعية كما أظهر الفحص النسيجي مرضي لمقاطع القلب لتأثيرات الألمنيوم المحرفة في عضلة القلب والتي تم تحسينها في قلوب الحيوانات التي تناولت العكبر

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