The Protective and Therapeutic Effect of Resveratrol in Improving Renal and Hepatic Failure Induced by Aluminum Chloride in Experimental Animals

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Abstract: Aluminum is a toxic agent to humans and animals. It is found in the processed foods and medicines. It is added to the drinking water for purification purposes, thus increasing human exposure to this toxic metal. Resveratrol belongs to the polyphenol group, found in red grapes, peanuts and dark chocolate, has anti- oxidant and anti-inflammatory effect. This study was conducted to determine the protective and therapeutic role of resveratrol in improving renal and hepatic failure induced by aluminum chloride. The experimental rats were divided into four groups, as follows: Group 1: 1 ml Saline solution was given orally until the end of the experiment set as healthy control group. Group 2: Mice were given aluminum chloride (17 mg / kg B.W) orally daily for 45 days set as toxicity group. Group 3: Rats of this group were given resveratrol for 45 days then aluminum chloride was given for 45 days. This group set as protective effect of resveratrol. Group 4: This group was given aluminum chloride for 45 days then resveratrol was given for another 45 days as a therapeutic effect. At the end of the experiment, serum was used to perform biochemical analyses to measure liver and kidney functions. The results of this study showed a significant increase in the activity of liver enzymes, (AST, ALT, ALP) in AlCl3 induced group compared to the control group. The results illustrated a high level of urea, creatinine and uric acid was also shown in group treated with aluminum chloride compared to the healthy group. While, the results showed a significant improve in both liver and kidney function in the resveratrol treated group either as protecting or therapeutic effect. The current study provides an important insight into the use of natural products to treat and eliminate the toxicity of aluminum chloride. Where Albumin showed significant decrease in AlCl3 induced group.
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1. Introduction

Aluminum is one of the most common minerals. About 8% of the earth's crust is in combination with oxygen, silicon, fluorine and other elements found in soil, rock, clay and precious stones [1]. This element has no known biological significance [2]. Aluminum cookware is widely used in developing countries [3], and this can lead to a higher proportion of aluminum in food, especially if cooked foods are salty, acidic or alkaline [4].

Aluminum has been widely used in everyday life and is widely believed to have little toxicity and is quickly eliminated by urine, but it turns out that aluminum has a detrimental effect on human health [5].

This component is mainly found in food products and drinking water (6). It is also sourced from corn, yellow cheese, table salt, herbs, spices, tea, cosmetics and cooking utensils made of aluminum. It is also used in the manufacture of food additives and toothpaste [7]. Aluminum is used medically in the synthesis of certain antacids, some analgesics and in the synthesis of certain vaccines [8-10].

Environmental pollution with different aluminum compounds plays a significant role in increasing the concentration of this element from the limits allowed by people, especially those living close to cement factories [11-12]. The average allowable aluminum for food and water for an adult is 1-10 mg / day [13].

Toxic studies have indicated that aluminum is distributed and deposited in the bone, liver, testicular, kidney, and brain [14]. This element can be deposited in the aorta and cerebral arteries [15]. It was also found that increasing the level of aluminum in the serum and body tissues is directly associated with the occurrence of neurological diseases such as Alzheimer's disease [16]. One study indicated that the administration of aluminum chloride to rabbits led to increased activity of liver enzymes in the serum [17]. This may be due to the obstruction of the bile duct due to the accumulation of aluminum in the liver (5) or the result of necrosis in the hepatic tissue, which causes an increase in the activity of liver enzymes (18- 20). Other studies have shown that accumulating aluminum in the kidneys destroys renal tubular cells, causing renal toxicity [11 and 21]. The toxic effects of aluminum may be due to the generation of free radicals that have a detrimental effect on health.

Polyphenols are active biotechnologies found in nature that capture free radicals [22]. Resveratrol belongs to the polyphenols and is found in red grapes, peanuts and dark chocolate. The first separation of resveratrol from the white beret roots was in 1940, and later, in 1963, this substance gained considerable attention when it was found to have an effect on heart disease [23]. Resveratrol is an antidote to certain cancers, such as breast cancer, and prevents cis-platinum poisoning. It is also anti-aging, moreover resveratrol was found as an antioxidant and anti-inflammatory [24]. More recently, dietary supplements containing resveratrol have been manufactured [25]. Oral resveratrol can be taken, and the metabolism is performed directly in the intestines and liver within one hour [26], and metabolic products are excreted by the kidneys. In addition, it is important to note that resveratrol is transferred to tissues by plasma proteins, with albumin being the main carrier [27].

The aim of this study is to explore the protective and therapeutic effect of resveratrol against the toxicity of aluminum chloride in experimental animals. This target was achieved by measuring biochemical markers of some vital parameters including renal function (urea, creatinine, uric acid, and albumin) and liver functions: aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) in the serum of mice.

2. Materials and methods:

2-1 Chemicals:

Aluminum chloride was purchased from Loba Chemi Mumbai, India, while Resveratrol was purchased from Sigma Aldrich Chemical Company, St, Louis. USA

2-2 Preparation of chemicals:

1.7 g of aluminum chloride was dissolved in 100 ml distilled water to obtain a final concentration of 17 mL of aluminum chloride [28]. Toprepare resveratrol, 5 mg of resveratrol was dissolved in 35 ml DMSO and then completed to 100 ml with distilled water [29].

2-3 Experimental animals:

The study used 24 male white albino rats, weighing between 120-130 grams, obtained from King Fahad Medical Research Center in Jeddah. The mice were placed in special cages at appropriate temperature and humidity, noting that the space in these cages was sufficient for freedom of movement, and the room for breeding was well ventilated, noting the lighting sequence between darkness and light. These mice were left for two weeks before they began experiment, until they adapted to the standard conditions, and in the meantime they were eating a natural diet.

2-4 Experimental Design:

The experimental mice were divided into four groups, each containing 6 mice, as follows:

Group 1: Healthy control group, feeds daily on natural food and saline with a dose of 1 ml orally until the end of the experiment.

Group 2: Mice were given aluminum chloride (17 mg / kg B.W) orally daily for 45 days.

Group 3: Rats were given resveratrol for 45 days then aluminum chloride was given for 45 days. This group set as protective effect of resveratrol.

Group 4: This group was given aluminum chloride for 45 days then resveratrol was given for 45 days. This group describes the therapeutic effect of resveratrol.

Biochemical measurements:

Kidney functions (urea, uric acid, creatinine, albumin) and liver functions (ALT, AST, ALP) were measured using ultraviolet / visible ultraviolet spectrometer (UV-1240 / UVmini- 1240 V from Shimadzu Corporation, Kyoto- Japan. Using kits purchased from Crescent Diagnostics Jeddah KSA.

At the end of the experiment of period (90 days) later, the blood samples were withdrawn from the retro orbital plexis in the early morning and preceded by the mouse for at least eight hours, using non-coagulant capillary tubes after simple anesthesia of the mice with diethyl ether. Blood samples were collected from all groups in tubes contain only gel. The serum was separated by a centrifuge at 4000 l / min for a quarter of an hour, and the serum was kept in a refrigerator at -20°C. The serum was used to perform various biochemical analyzes.

Statistical analysis:

Statistical analysis of the samples was done using SPSS, Issue (19), and the method of multiple comparisons was used in the analysis of the statistical data for the different groups. The difference was significant when $P \leq 0.05$.

3. Results and Discussion

Table (1) shows the effect of aluminum chloride and resveratrol on kidney function in different groups. The results of this study showed that giving the aluminum chloride to the mice with a daily oral dose for 45 days resulted in a significant increase in serum urea, creatinine and uric acid compared to the control group ($p \leq 0.05$).

The high level of urea, creatinine and uric acid in the serum of mice treated with aluminum chloride indicates a defect in renal function. This is consistent with the findings of several researchers who indicated that the accumulation of aluminum in the kidneys would break down renal tubular cells, Renal nephrotoxicity [11and 21]. The change in serum urea level is due to metabolic disorders in renal function.

The researchers explained the increased level of urea in serum treated with aluminum chloride to its detrimental effect on liver function, where the urine is the final product of protein destruction and may result in renal function dysfunction [30]. This is consistent with previous studies [31].

Table (1): Concentrating of kidney function biomarkers (urea, creatinine, uric acid, albumin) in serum of all different groups. (Results expressed as mean ± standard error)

Group	Albumin mg\dl	mg\dluric acid	Creatinine mg\dl	mg\dlurea
Control	3.303 ±0.1393	1.258±0.1174	0.6340±0.0051	19.91±1.42
AlCl3	2.966±0.1605	2.146±0.2301 *	1.127±0.1465 *	30.38±1.56 *
Res. (Pro) +AlCl3	3.025±0.0069	1.171±0.2105**	0.5343±0.0025 **	19.65±1.16 **
AlCl3 + Res. (Treat)	3.0133±0.0066	1.158±0.207 **	0.5700±0.0052 **	18.53±1.11 **

* Significant difference compared to control group.
** Significant difference compared with treated group of aluminum chloride.

Albumin is the most common protein in human blood plasma, accounting for 55-65% of total protein. The albumin is made in the liver, while a small amount of it is filtered through the glomeruli and the majority of it is re-absorbed by proximal tubule cells where the albumin are broken by lysosome enzymes into simpler molecules and then return to the circulatory system.

The results of this study showed a significant decrease in albumin in mice treated with aluminum chloride compared with the control group (Table 1). This indicates a defect in liver function or a deficiency in the production of albumin in the liver. This causes lack of absorption of amino acids leading to malnutrition.

In addition, the low level of albumin in the aluminum chloride-treated rats group may be due to abnormalities in the production or metabolism of proteins in the liver [19]. In addition, aluminum may lead to nephrotic syndrome or acute renal glomerulonephritis and this contributes to the loss of proteins by urine.

Furthermore, results in table (1) showed a significant decrease ($p \leq 0.05$) in the level of urea, creatinine and uric acid in groups treated with resveratrol.

The results of this study showed that resveratrol treatment resulted in a marked improvement in renal function. The level of urea, uric acid and creatinine decreased in this group compared to the group treated with aluminum chloride. This is due to the effective role resveratrol as an anti-inflammatory agent. These results are consistent with many previous studies which have shown that this material has the ability to capture free radicals, such as superoxide and toxic hydroxyl radicals [32]. In addition to the ability of resveratrol to improve the activity of antioxidant enzymes such as superoxide oxidase, glutathione, peroxidase and catalase, which improves renal function [33].

Table (2) shows the effect of aluminum chloride and resveratrol on liver function in all studied groups. The extent of liver damage by aluminum chloride has been measured by measuring some biomarkers, including some liver enzymes: AST, ALT which are known as amino group carriers. The level of alkaline phosphatase enzyme (ALP) was also measured in one of the cholestatic liver enzymes.

Enzymes carrying amino groups, which include AST and ALT, are important enzymes in vital processes [35]. The results of the present study showed a significant increase in serum AST and ALT in serum treated with aluminum chloride compared with the healthy group, which is consistent with previous studies [18-19]. The researcher explained that exposure to aluminum chloride causes liver necrosis [36]. The reason for the rise of the AST enzyme is also attributed to damage to hepatic cells. The increase in the level of this enzyme is a sign of liver damage and changes in its function.

Table (2): Concentrating of Liver function (AST, ALT, ALP) in serum for different groups. (Results expressed as mean ± standard error)

Group	ALP U\L	AST U\L	ALT U\L
Control	227.55±10.288	81.91±1.566	23.95±1.219
Alcl3	326.65±26.11 *	134.76±3.572 *	36.77±0.860 *
Res. (Pro) +Alcl3	236.52±11.48 **	99.106±2.82 **	24.65±1.2030 **
Alcl3 + Res. (Treat)	238.05±6.706 **	105.36±5.115 **	23.48±1.874 **

* Significant difference compared to control group.
** Significant difference compared with treated group of aluminum chloride.

ALP is a vital indicator of mineral salts, an enzyme linked to the cell membrane that transfers many metabolic substances [37]. The activity of the ALP is related to the metabolic activity and biological processes within the body, so the lack of activity of this enzyme refers to the energy processes within the cell [38]. The significant increase in the activity of this enzyme in the group treated with aluminum chloride compared to the control group is consistent with the previous findings [31,36 and 39]). While, researchers believe that the reason is that aluminum may be associated with nucleic acids, deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) damage, which inhibits the activity of acid and basal phosphatase.

On the other hand, the study showed that resveratrol significantly reduced liver function (ALT, AST, ALP) ($p \leq 0.05$) in the groups treated either as preventive or therapeutic treatment compared to the group treated with aluminum chloride only (Table 2).

There is a close relationship between the high level of ALT enzyme, insulin resistance and metabolic syndrome [40]. The current study indicated a low level of AST and ALP in the resveratrol-treated groups and improved condition.

It may be due to the protective effect of this substance, which has a number of properties: Resveratrol has an antioxidant activity by stimulating antioxidants within the body, as well as anti-freeradicals effect through lipid oxidation and prevent the formation of dildehyde monouns, Resveratrol has an anti- inflammatory effect, leading to an improvement of liver function [41-42].

4- Conclusion and Recommendations:

The results conclude that the protective and therapeutic effect of resveratrol in reducing the toxicity of aluminum chloride by improving renal function and liver function enzymes in groups treated with aluminum chloride and resveratrol compared to the group treated with aluminum chloride only. For that, the study recommends, avoiding products containing aluminum and its compounds, especially cooking utensils. As well as, the use of natural compounds (resveratrol) in red grapes, peanuts and dark chocolate to avoid the damage caused by poisoning of many compounds and keep the liver and kidneys in good health.

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