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GREEN TEA EXTRACT AND OMEGA-3 FATTY ACIDS AS PROTECTIVE AGENTS AGAINST ALUMINUM CHLORIDE–INDUCED NEPHROTOXICITY IN RABBITS

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Abstract

Background: *Aluminum chloride ($AlCl_3$) is one of the most common environmental pollutants that has been reported to cause renal injury through the formation of oxidative stress, inflammatory response, and cellular destruction. In the current paper, the nephroprotective properties of green tea extract (GTE) and omega-3 fatty acids were compared to prevent renal toxicity induced by aluminum chloride in rabbits.*

Methods: *There were 32 healthy rabbits who were randomly assigned to four equal groups: control group, $AlCl_3$ treated group (300 mg/kg/day), green tea extracts (50 mg/kg/day) with the addition of $AlCl_3$ group, and omega-3 (20 mg/kg/day) with $AlCl_3$ group. Four weeks of treatment were followed by evaluation of the body weight, kidney morphometry, serum renal bio markers such as BUN, creatinine, uric acid and a histopathological change. Data were analyzed using one-way ANOVA.*

Results: *$AlCl_3$-exposed rabbits showed decreased weight gain, pronounced kidney weight and size gains, highly enhanced renal biomarkers, and the worst histopathology including tubular necrosis, glomerular atrophy, inflammation and fibrosis of the interstitium, and vascular damage. Conversely, rabbits with either green tea extract or omega-3 had significant improvement in all the parameters assessed. Green tea extract demonstrated stronger effects of protection against kidney hypertrophy and oxidative damage, whereas omega-3 demonstrated better anti-inflammatory and anti-fibrotic effects and better lowered the level of serum creatinine. Both of the agents to some degree repaired the traditionally normal renal architecture. These results suggest that green tea extract and omega-3 fatty acids are nephroprotective with significant potential and can be used as safe, natural treatment options to prevent the renal effects of aluminum.*

INTRODUCTION

The most common metal in the crust of the earth is aluminum (Al) which is found everywhere in water, soil, food, pharmaceuticals, cosmetics, utensils, and most industrial products (Duce and Bush 2010, Shaw and Tomljenovie, 2013). Once it enters into the body, either by ingestion, inhalation or dermal absorption, it is likely to be localized in multiple vital organs, such as liver, brain, bones and kidneys (Anane *et al.*, 1997). The kidneys are particularly susceptible since they are the key to the excretion of the aluminum. Although the major route of elimination is renal excretion, it is comparatively slow, and thus, it allows aluminum to build-up and cause chronic toxicity (Hussain, 2016).

Aluminum chloride ($AlCl_3$) is an easily absorbed form of aluminum that is known to induce oxidative stress by producing reactive oxygen species. Over-oxidative stress impairs cellular proteins, lipids, as well as DNA leading to impaired functioning of kidneys (Barabasz *et al.,* 2000). Also, aluminum is proved to cause mitochondrial dysfunction, inflammatory reaction, apoptosis, and changes in renal tubular transport functions. In extreme cases, the exposure to aluminum causes nephrotoxicity, which is characterized by heightened blood urea nitrogen levels, creatinine levels, uric acid levels and gross histological changes including tubular necrosis, glomerular damage, and interstitial infiltration (Ahmed *et al.,* 2021).

An increasing interest on the toxicity of aluminum has led to efforts to find natural substances that can reduce the negative impact of this toxicity. High levels of polyphenols, in particular, catechins including epigallocatechin-3-gallate (EGCG), are found in green tea (*Camellia sinensis*) and have potent anti-oxidant and anti-inflammatory effects (Moen *et al.,* 2017). Another group of natural compounds, which are known to have anti-inflammatory, antioxidant, and cell-protective effects, are omega-3 fatty acids, especially eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (Mozaffarian *et al.,* 2018). These fatty acids are incorporated into cell membranes, enhance cell membrane stability, reverse lipid peroxidation, and stimulate the production of anti-inflammatory mediators. The protective effects of omega-3 in drug induced and chemical induced model of nephrotoxicity have been proven renal protective.

In spite of the fact that both green tea extract and omega-3 have great protective effects, their relative effectiveness against the influence of aluminum chloride-induced kidney damage is not fully studied. The current research was aimed to assess and compare the protection of these two natural antioxidants on the biochemical, morphological and histopathological parameters of rabbits that were subjected to exposure to aluminum chloride.

Aims and Objectives

- To induce nephrotoxicity in rabbits using aluminum chloride.
- To administer green tea extract or omega-3 fatty acids as protective agents.
- To measure specific biochemical parameters including blood urea nitrogen (BUN), serum creatinine and uric acid level.
- To evaluate histopathological changes in the kidney tissues of experimental animals.
- To compare the degree of renal protection offered by green tea extract, omega-3, and their combination.

Materials and Methods

The healthy adult rabbits (32 of them) were kept in the standard laboratory conditions in a properly ventilated area with bedding and food. The four groups of rabbits were randomly matched with animals. There was no treatment of the control group. The $AlCl_3$ group was orally administered with 300 mg/kg/day of the aluminum chloride that triggered nephrotoxicity. The group of green tea extracts was administered green tea extract (50 mg/kg/day) along with aluminum chloride and the group of omega-3 were administered omega-3 fatty acids (20 mg/kg/day) with aluminum chloride. The therapies lasted four weeks and body weight recorded every week.

Freshly prepared aluminum chloride was used as its administration. The extracts of green tea were made daily out of the tea leaves, which were available in the market and the omega-3 fatty acids were prescribed in the form of powdered omega-3 capsules. Biochemical analysis of the blood samples taken at the end of the experimental period was done at the marginal ear vein. Standard diagnostic kits were used to determine serum levels of blood urea nitrogen, creatinine, and uric acid. Kidneys were taken out and washed as well as assessed in terms of weight, length, width, and thickness. They were then fixed, sectioned, and stained using the hematoxylin and eosin stains and observed under the microscope concerning the pathological changes, such as tubular degeneration, necrosis, glomerular lesion, inflammation, and fibrosis. Analysis of variance was used to make the statistical comparisons of groups and the differences were regarded to be significant at $p < 0.05$.

Results

Rabbits of the control group were normal and steady in their weight gain during the study. On the contrary,

there was much lower weight gain when the rabbits were subjected to aluminum chloride, which indicates a systemic effect of the aluminum toxicity. Rabbits fed on green tea extract or omega-3 exhibited a better progression of their weight as compared to $AlCl_3$ group and the rabbits fed on omega-3 exhibited a slightly better recovery in the body weight trends.

Compared to the control group, aluminum chloride induced considerable kidney weight gain, length, width, and cortical thickness, which is an indication of renal hypertrophy and edema. Green tea extract significantly inhibited these morphological changes, demonstrating more effect on the parameters of kidney size than omega-3 but both treatments were effective at alleviating the hypertrophic response.

Aluminum chloride caused significant increases in blood urea nitrogen, creatinine and uric acid biochemically. These rises proved high disruption of renal functioning. The administration of Omega-3 and the green tea extract had a significant effect of reducing these markers. The green tea extract had been having a slight more impact on the reduction of the BUN and uric acid and omega-3 had more significant impact on the reduction of serum creatinine, which indicated more significant improvement of glomerular filtration.

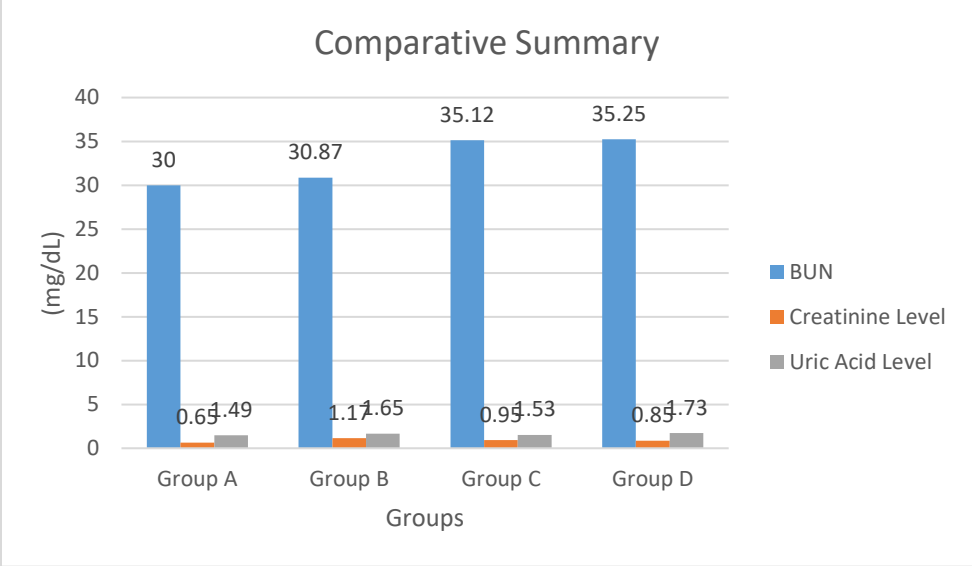

Figure 1: Graph showing Comparison of Biochemical Parameters of Experimental Groups

Histopathological analysis of kidney samples in the $AlCl_3$ group demonstrated severe maladaptations in the tubules, such as tubular necrosis, vacuolation, glomerular atrophy, inflammation, and infiltration by inflammatory cells and fibrosis of the interstitium. These pathological damages were a clear indication of damage of the kidneys caused by aluminum. Green tea extract group also demonstrated significant improvement whereby there was only mild tubular degeneration, glomerular structure was preserved and there was minimal inflammation. The omega-3-treated rabbits had significantly lower inflammation in the kidney, improved glomerular and tubular architecture, and fibrosis. The protective effectiveness of both treatments was shown, but their strength was different as regards
to the particular pathological features enhanced.

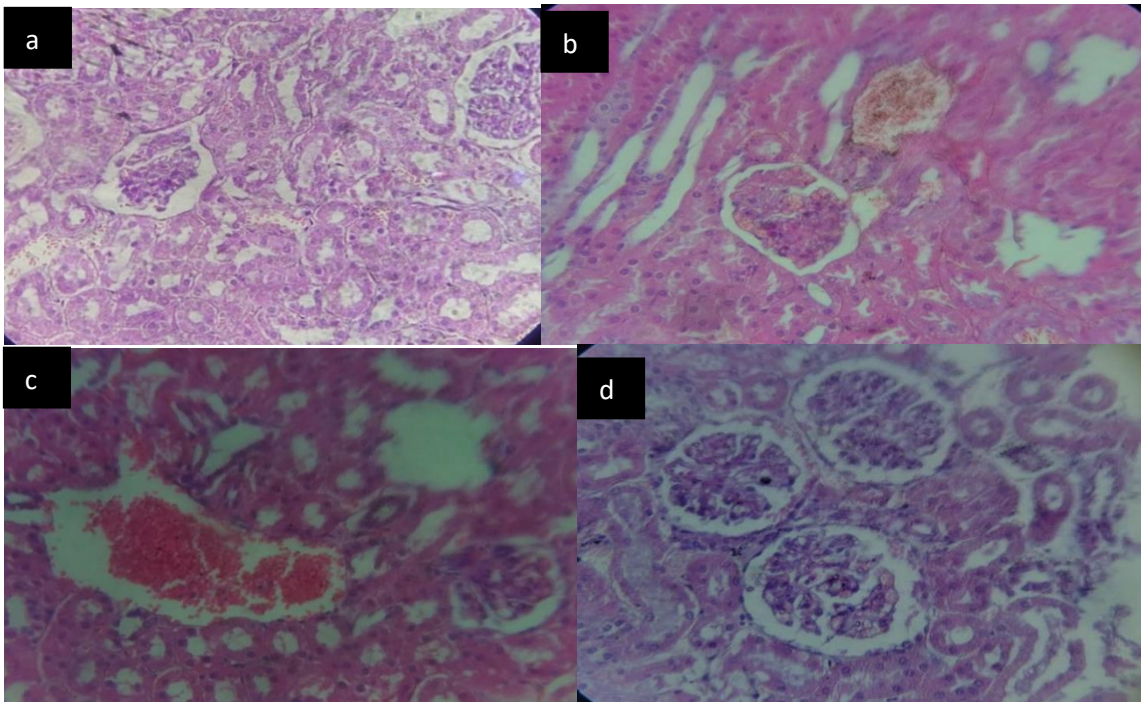

Figure 2(a) Control group showing normal histology. (b) $AlCl_3$-treated group showing tubular necrosis and glomerular atrophy. (c) Green Tea + $AlCl_3$ group showing mild tubular degeneration with partial protection of renal architecture. (d) Omega-3 + $AlCl_3$ group showing reduced tubular damage and preserved glomeruli compared to $AlCl_3$ group (H&E stain)

Discussion

The results of the current research exhibit the nephrotoxicity of aluminum chloride and the preventive measures of the green tea extract and omega-3 fatty acids. Exposure to aluminum chloride resulted in the development of dysfunctional renal status that was manifested by increased biochemical indicators and severe changes in renal tissue framework. These findings are in line with earlier research results of aluminum-induced oxidative stress, hypertrophy of the kidney, tubular necrosis, and glomerular injury.

Green tea extract improved renal damage caused by aluminum considerably. Its prodiguous amounts of catechins and especially EGCG might have been the factor behind its ability to counter oxidants, stop lipid peroxidation, and boost antioxidant defenses on cells. The decrease in swelling and increase in morphological and histological changes of the kidney indicate that green tea extract is a good antioxidant to oxidative damage and renal tissue protection.

There were extensive protective effects generated by omega-3 fatty acids, too. Probably their effectiveness was facilitated by their well-known anti-inflammatory effects, improvement of the membrane fluidity, and stabilization of the renal cellular structures. The omega-3 group has shown a greater decrease in the level of serum creatinine, which implies that omega-3 could better improve glomerular filtration and lessen the effects of inflammation on renal dysfunction.

Conclusion

This paper concludes that green tea extract and omega-3 fatty acids are significant in alleviating nephrotoxicity induced by a combination of aluminum chloride and rabbits. They are antioxidant and anti-inflammatory and thus can be used to restore renal function, lessen biochemical derangements and enhance renal tissue structure. The extract of green tea is superior in decreasing oxidative stress and kidney hypertrophy, whereas omega-3 is more potent in increasing the filtration process and minimizing fibrosis. These results

endorse the possible therapeutic importance of natural dietary supplements like green tea extract and omega-3 in the prevention or management of

nephrotoxicity occasioned by environmental toxins like aluminum.

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