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AMELIORATIVE POTENTIAL OF GREEN TEA AND OMEGA-3 AGAINST ALUMINIUM CHLORIDE-INDUCED HEPATOTOXICITY IN RABBITS

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ABSTRACT

Background: *Aluminium chloride has been reported to cause toxic effects on the liver by triggering oxidative stress, damaging hepatocytes, and impairing normal hepatic functions. Some natural antioxidants, like green tea and omega-3 fatty acids, can stop such toxic effect, because of their superior anti-inflammatory and free radical scavenging capabilities. The purpose of the current study was to determine whether green tea and omega-3 fatty acids could reduce the hepatotoxicity caused by $AlCl_3$ in rabbits.*

Methods: *32 mature rabbits were homogeneously divided into four groups (n = 8). Group I (control group) was given regular diet and distilled water. Group II received $AlCl_3$ (300 mg/kg) orally, one time in a day for 30 days. Group III was orally given $AlCl_3$ (300 mg/kg) with green tea extract (50 mg/kg/day), while Group IV was orally given $AlCl_3$ (300 mg/kg) with omega-3 fatty acids (20 mg/kg/day). Body weight measurement at regular intervals, liver function tests (ALT, ALP, and total bilirubin level), and histopathology were studied.*

Results: *Aluminium chloride led to decrease in weight, while green tea and omega-3 supplementation preserved body weight closer to control values. Aluminium chloride greatly increased RLW when compared with control values, while green tea and omega-3 co-supplementation greatly reduced RLW to near normal values. $AlCl_3$ greatly elevated ALT, ALP, and total bilirubin values, while both treatments greatly reduced these parameters to near normal values with respect to their efficacy with omega-3 reporting maximum activity. By Histopathological analysis, hepatocellular necrosis, inflammation, and fibrosis were observed in $AlCl_3$ group.*

Conclusion: *Green tea or omega-3 supplementation greatly rehabilitated biochemical parameters with improved histological architecture. These hepatoprotections can be attributed to green tea and omega-3's antioxidant and anti-inflammatory activities, thereby they potently act as natutal antioxidants (NATs) for chemically induced liver damages.*

Keywords: *Aluminium chloride, Antioxidants, Green tea extract, Hepatoprotection, Hepatotoxicity, Histopathology, Liver function tests, Omega-3 fatty acids, Oxidative stress, Rabbits*

INTRODUCTION

Aluminium chloride is a chemical compound that has wide applications in several industries. Its primary use is in the manufacture of aluminium metal by acting as a catalyst to transform mineral-form aluminium into the form of aluminium metal known as bauxite. It has also played a huge

role in petrochemical processes where the action of aluminium chloride catalyses reactions in critical chemicals and fuel production. Aluminium chloride is used in a number of methods on chemical syntheses such as Friedel-Crafts alkylation and acylation. Among major causes for concern with exposure to $AlCl_3$ pertains to long-term functionality of liver. Since the liver acts as an organ of detoxification and metabolic homeostasis, dysfunctionality of the organ may give rise to states of diseases with far-reaching ramifications, one of which comprises hepatic failure. Among major causes for concern with exposure to $AlCl_3$ pertains to long-term functionality of liver. Since the liver acts as an organ of detoxification and metabolic homeostasis, dysfunctionality of the organ may give rise to states of diseases with far-reaching ramifications, one of which comprises hepatic failure. The operating mechanisms of pathologies are believed to emanate from the aluminium-induced perturbations in cellular processes, including folding of protein, mitochondrial functions, and redox stress response. In hepatotoxicity, liver regenerative capacity is destroyed that gives rise to cumulative injuries with progressive time.

Green tea is obtained from the plant Camellia sinensis species, ranking among the globally highly consumed beverages for its numerous health benefits. The consumption of green tea has immense value in human well-being. It possesses a wide variety of bioactive compounds such as flavonoids, catechins, alkaloids like caffeine, amino acids such as L-theanine, and basic vitamins and minerals. The compounds have been found to possess various physiological actions that contribute to health and disease prevention (Zhao et al., 2022). Green tea is an important agent against chronic diseases because it neutralizes unstable molecules called free radicals, which are responsible for oxidative damage of the cellular, protein, and DNA levels. This oxidative stress has been implicated in aging and most diseases, such as cancer, diabetes, cardiovascular disease, and neurodegenerative diseases.

Omega-3 fatty acids are a family of essential polyunsaturated fatty acids critical to numerous physiological processes in the human body. They are considered "essential" in nutritional terms because the human body does not possess the ability to synthesize them de novo (meaning synthesis from scratch) and hence, they need to be provided through dietary sources. Structurally, all omega-3 fatty acids have a double bond three carbon atoms away from the terminal methyl group of the fatty acid chain. The three most important types of omega-3 fatty acids include ALA (alpha-linolenic acid), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA). While ALA is relatively available in plant oils like flaxseed, soybean, and canola, EPA and DHA sources are mostly marine-based, including fatty fish like salmon, sardines, and mackerel, fish oils, and specific types of algae (Qin et al., 2022).

Green tea and omega-3 fatty acids show a very promising potential in preventing $AlCl_3$-induced liver injury, although the exact mechanisms remain obscure. Studies on animals have shown that the agents possess the ability to be hepatoprotective, but further studies are as yet required to say whether they may act similarly in humans. A number of experimental models have been utilized in trying to study the pathogenesis of the injury caused to the liver by the administration of $AlCl_3$, one of the most frequently used laboratory animals being the rabbit.

Aims and objective:

- To study the influence of aluminium chloride on growth performance, body weight, and overall health status of rabbits.
- To determine the effect of aluminium chloride on liver function markers (ALT, AST, ALP, bilirubin) and how green tea and omega-3 supplementation can ameliorate.
- To examine the histopathological changes in liver tissue due to aluminium chloride and to identify the extent of protection provided by green tea and omega-3.
- To compare the relative hepatoprotective efficacy of omega-3 and green tea towards preventing aluminium chloride-induced biochemical and structural damage.
- To present experimental evidence favouring the application of dietary antioxidants

(green tea and omega-3) as therapeutic tools against hepatotoxicity.

METHODOLOGY:

This is an experimental study involving 32 healthy domestic rabbits because, in hepatotoxicity and pharmacological experiments, there is a well-documented use of rabbits. Rabbits were acquired from a licensed breeder for the assurance of uniformity of age (around 10–12 weeks old) and body weight (1–2.0 kg) to minimize biological variability that may affect the outcomes of the experiments. The experimental animals were kept under standard laboratory cages in controlled environments. Each cage has a tray that was filled with soil placed under it to absorb excreta for keeping conditions hygienic. The housing conditions had to be stringently controlled to reduce confounding variables, hence the maintenance of optimal health: temperature was kept between 61°F to 72°F (16°C to 22°C); relative humidity was between 45% and 65%.

The experimental animals received a standard diet including seasonal green fodder: spinach- Spinacia oleracea, carrot- Daucus carota, and fenugreek- Trigonella foenum-graecum. Fresh drinking water was provided throughout the period. Feeding was done twice a day in adequate amounts to fulfil the nutritional requirements of animals.

Green tea sachets, commercially packed, were procured from the local market. A required number of sachets were opened and the contents weighed. The weighed tea leaves were soaked in distilled water and kept for 30 min for proper extraction. The solution was filtered, and the prepared green tea extract was administered orally to the rabbits.

Omega-3 capsules were in powder form. Dosage was measured accurately based on body weight. Supplements were dissolved in distilled water, mixed well, and given orally to rabbits. Aluminium chloride ($AlCl_3$) was used to induce hepatotoxicity in rabbits. Stock solution was freshly prepared by dissolving aluminium chloride powder in distilled water. The prepared solution was administered to rabbits in their drinking water. Animals were divided into four groups, each of which received a different treatment for one month;

- **Group 1:** is the control group and received the normal diet and tap water without any treatment; it acted as the baseline control.
- **Group 2** (Aluminium chloride group): Rabbits were given aluminium chloride in the drinking water at the dose level of 300 mg/kg/day for three weeks. This group was applied to cause hepatotoxicity and for the monitoring of toxicological effects of aluminium chloride.
- **Group 3** (Green tea treated group): Rabbits received aluminium chloride in the drinking water with the same dose as Group 2, besides green tea extract at a dose of 50 mg/kg/day orally for three weeks. This group served for assessing the hepatoprotective effect of green tea against aluminium chloride toxicity.
- **Group 4:** Omega-3 treated group. Rabbits were given aluminium chloride in the drinking water at the same dose as Group 2, with supplemental omega-3 (20 mg/kg/day oral for three weeks). This group was applied for measuring the protectant effect of omega-3 in aluminium chloride poisoning.

At the end of the treatment period, rabbits were euthanized. Livers and blood samples were collected for research purposes: anatomical identification and histopathological observation. LFTs were analyzed to study the effects of aluminium chloride, green tea extract, and omega-3 fatty acids on hepatic health. The information was also statistically analyzed using computer statistical software such as SPSS version 25 and Excel. Continuous data were presented as mean ± standard deviation (SD). Comparisons among some groups were managed with the assistance of one-way analysis of variance (ANOVA). With a resultant p-value less than 0.05, a statistically significant difference in the parameters analyzed was identified due to the treatments.

RESULTS:

The results derived from the experimental research carried out to assess the protective action of green tea and omega-3 fatty acids against aluminium chloride-induced hepatotoxicity in rabbits are shown in this chapter. Results are structured systematically, with the descriptive statistics on the parameters evaluated to begin

with, followed by comparison among the experimental groups.

Comparison of averages of weight among experimental groups:

Fig 1 illustrates the comparison of mean values of weight between different experimental groups for four weeks. In the control animals, the body weight remained steady during the whole period with minor undulations around the baseline value. Rabbits treated with $AlCl_3$ revealed a progressive decrease in mean body weight from the second week and the minimum values were observed during the fourth week reflecting toxic impact of $AlCl_3$ on normal physiological growth. Surprisingly, both treatment groups receiving AlCl3 plus either omega-3 or green tea had body weights that were much closer to the control group. AlCl3 + omega-3 mean weight showed very little variation all weeks of study while AlCl3 + green tea also showed a protective effect wherein the mean body weight values were consistently higher than AlCl3 group and nearly comparable to the control group. Thereafter, it was concluded that supplementation of either omega-3 or green tea effectively counteracted the weight loss produced by AlCl3 toxicity and favored the maintenance of normal growth in rabbits. One-way ANOVA was utilized to compare weekly changes within each group. The analysis revealed no significant differences across the four weeks in the Control ($p = 1.000$), but it revealed significant differences across the four weeks in the ALCL3 ($P<0.001$).

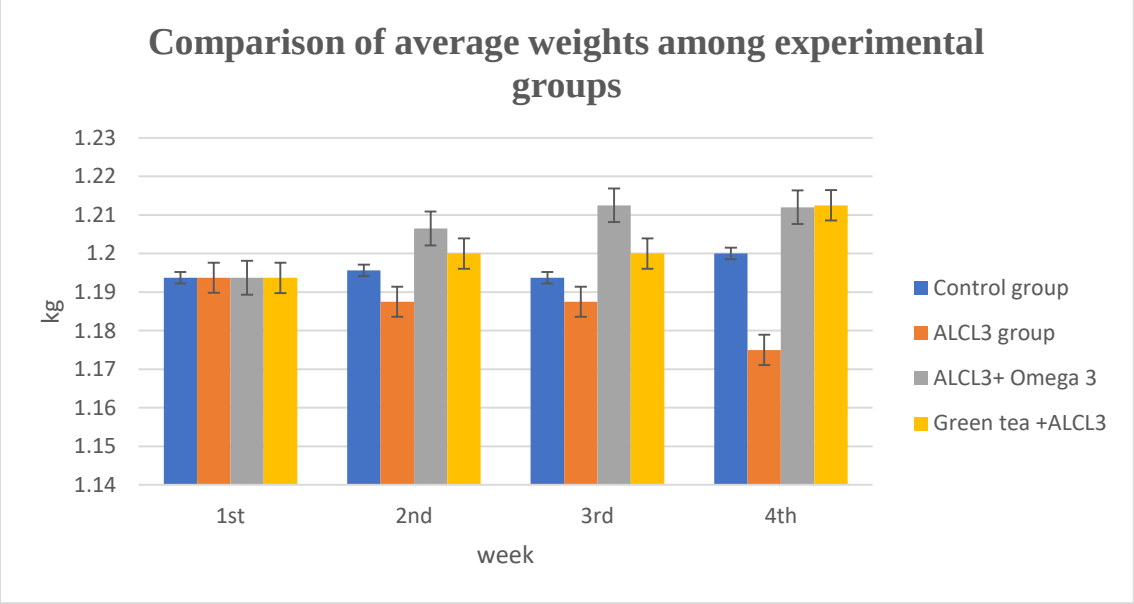

Figure 1: Comparison of average weights among experimental groups

Relative weight of liver:

The normal relative liver weight as measured in the control group was 3.57 ± 0.38%, reflecting good liver function and a normal hepatosomatic index. Contrarily, in the $AlCl_3$-treated group, the relative liver weight was significantly increased in comparison to the control group, reaching 5.09 ± 0.92%, implying hepatomegaly, which confirms the toxic impact of aluminium chloride on the liver tissue. Co-administration of omega-3 with $AlCl_3$ decreased its relative liver weight to 3.56 ± 0.43%, close to the control values, indicating the strong protective effect of omega-3 against $ALCl_3$-induced hepatotoxicity. Co-treatment with green tea lowered the relative liver weight to 4.03 ± 0.82%, which, though lower than that of the ALCL3 group, was still slightly higher than control values. Thus, it could be said that green tea also provided some measure of hepatoprotection, albeit not as effectively as omega-3 in terms of bringing down the relative liver weight to nearly normal values. A comparison of mean values among the different groups (Control, ALCL3,

Omega-3+ALCL3, and Green tea+ALCL3) was done through one-way ANOVA, and this demonstrated a very significant difference among the groups at $p<0.001$.

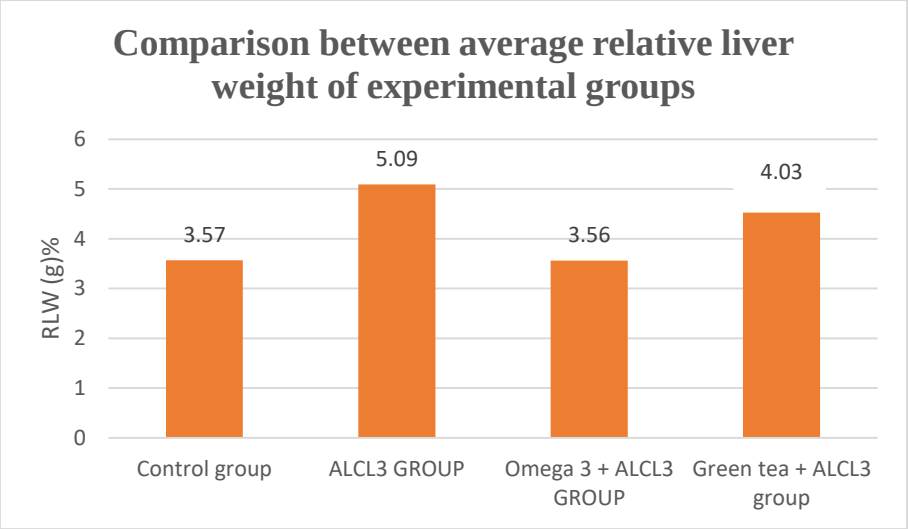

Figure 2: Comparison of average relative liver weights among experimental groups

Liver function test analysis:

Combined Comparison of averages of Liver Function Parameters (Bilirubin,ALT,ALP): Figure 3 presents the comparative study of the combined parameters in liver function, including total bilirubin, ALT, and ALP, among different experimental groups. In the control group, the values for bilirubin (0.58 mg/dL), ALT (77.3 U/L), and ALP (27.75 U/L) were all within the reported normal physiological ranges, hence assuring normal hepatic function. On the other hand, the group treated with $AlCl_3$ recorded a sharp increase in ALT (116.5 U/L) and ALP (69.87 U/L), although there was a slight increase in bilirubin (0.79 mg/dL), evidencing pronounced hepatocellular damage due to aluminium toxicity, which in turn is supported by altered liver metabolism. Supplementation with omega-3 fatty acids partially restored liver function, as reflected by a moderate reduction in ALT (102.5 U/L), a considerable decrease in ALP (41.5 U/L), and maintenance of bilirubin at near-normal levels (0.66 mg/dL). Similarly, the green tea + $AlCl_3$ group showed ALT (115.5 U/L) and ALP (47.5 U/L) levels lower than the $AlCl_3$-only group, along with bilirubin (0.68 mg/dL) remaining close to control values, suggesting some degree of hepatoprotection.

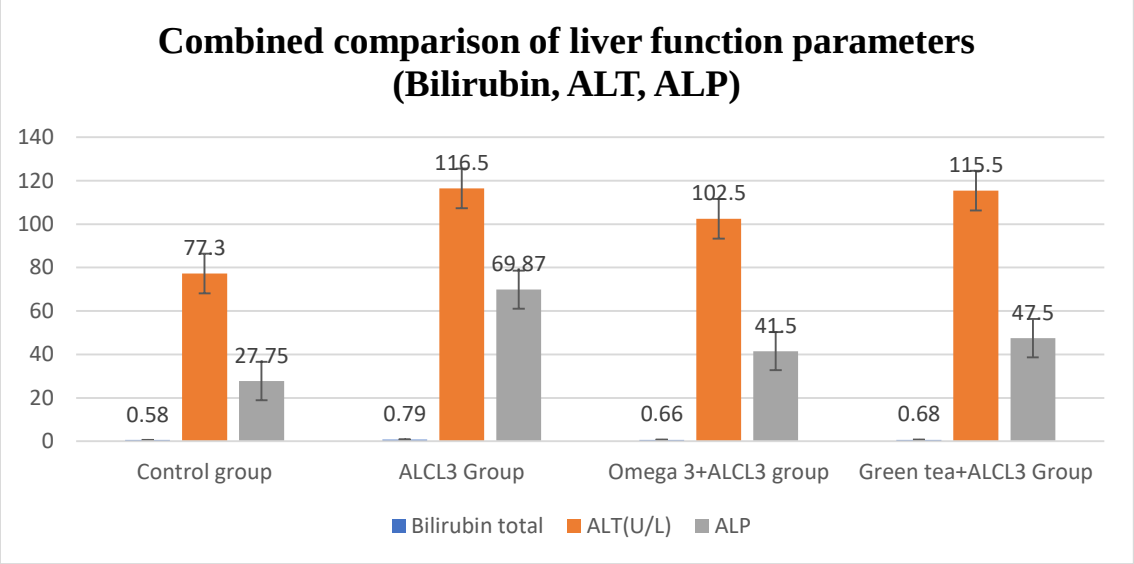

Figure 3: Combined comparison of liver function parameters (Bilirubin, ALT, ALP)

Histopathological Finding:

Histopathological evaluation of liver tissue was carried out to assess the structural integrity and microscopic changes induced by aluminium chloride administration in comparison with the control group. Hematoxylin and eosin (H&E) stained sections were examined under light microscopy to evaluate the arrangement of hepatocytes, the condition of sinusoids and central veins, and the presence or absence of degenerative changes such as necrosis, inflammation, cytoplasmic vacuolation, and fibrosis. The control group provided the baseline for normal liver architecture, against which pathological alterations in the treated groups were identified. The findings are presented in a group-wise manner, beginning with the control animals to establish normal hepatic histology, followed by a detailed description of the experimental groups to highlight deviations from normal morphology.

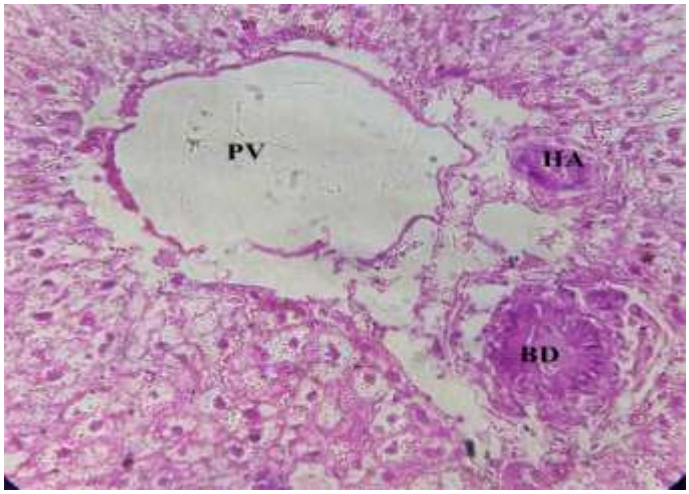

Figure 4: Photomicrograph of liver section from the control group showing the portal triad consisting of the portal vein (PV), hepatic artery (HA), and bile duct (BD). The portal vein appears as a large thin-walled structure, the hepatic artery as a smaller thick-walled vessel, and the bile duct lined with epithelium. Surrounding hepatocytes appear normal with intact polygonal morphology. (H&E stain, X40)

Aluminium chloride group:

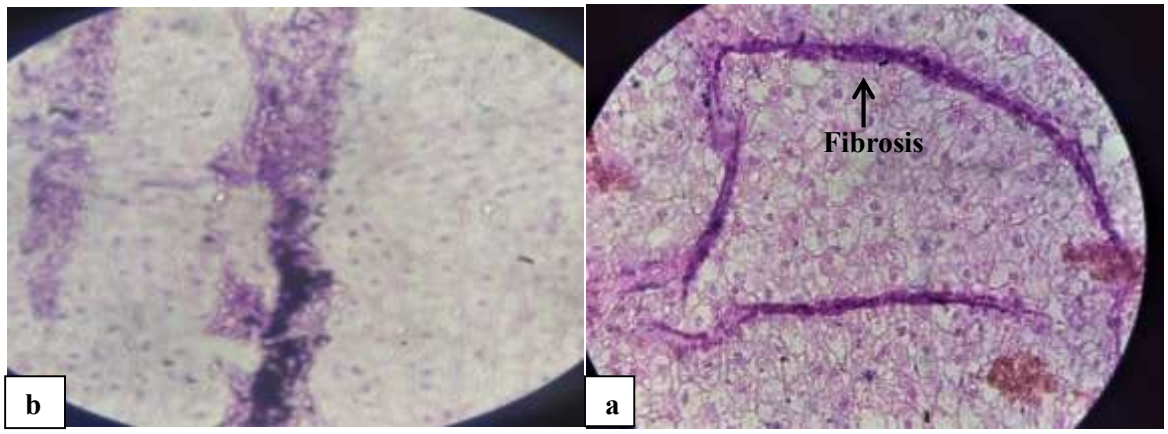

Figure 5: The photomicrograph of liver tissue (40X, H&E) shows fibrosis characterized by dense fibrous septa disrupting normal lobular architecture and forming irregular nodules. Hepatocytes appear swollen with vacuolated cytoplasm and degenerative changes. These alterations, due to excessive collagen deposition by activated stellate cells, indicate progressive liver injury that can advance to cirrhosis

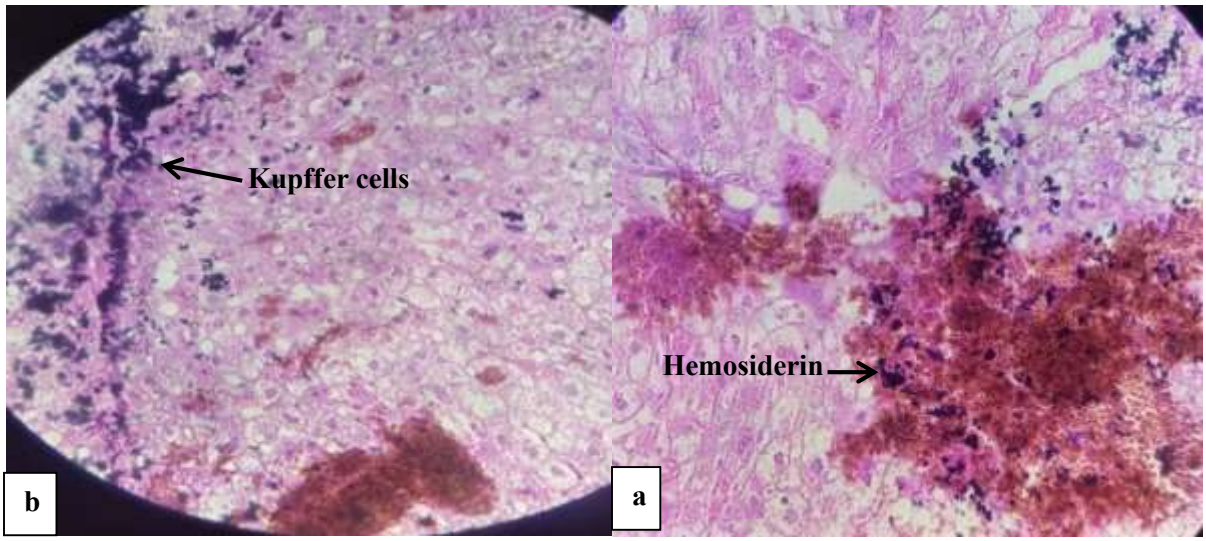

Figure 6: Histological liver section shows Kupffer cells with brownish-golden hemosiderin granules, indicating phagocytosis of erythrocyte breakdown products. The presence of enlarged, pigment-laden Kupffer cells reflects altered iron metabolism and highlights th their role in iron homeostasis during chronic liver injury

Green tea + ALCL3 group:

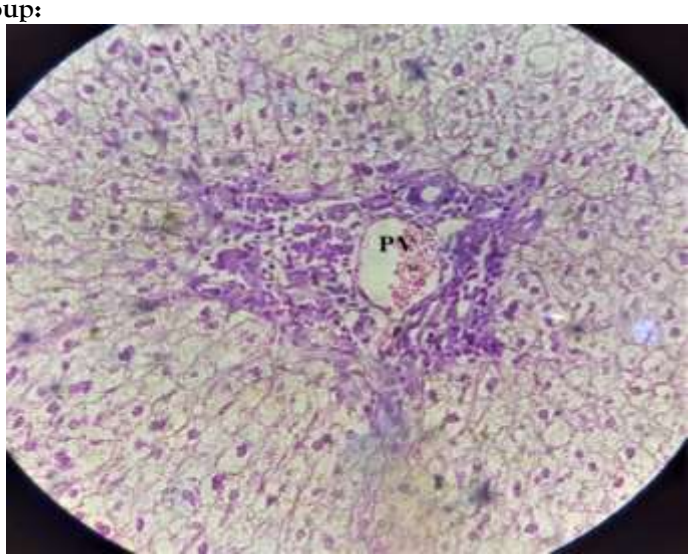

Figure 7: Photomicrograph of liver section from green tea treated group showing portal triad with bile duct (BD), portal vein (PV), and hepatic artery (HA). The structures appear preserved with reduced histopathological alterations, indicating recovery towards no normal architecture

Omega 3+ALCL3 group:

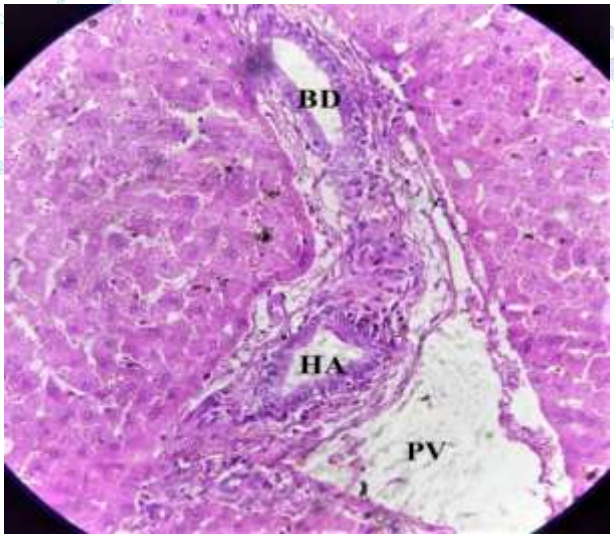

Figure 8: Liver section from Omega-3 + $AlCl_3$ group: The portal vein is almost normal in appearance, vascular walls intact, and shows minimal fibrosis with limited inflammatory infiltration. This suggests omega-3 fatty acids could effectively attenuate the $AlCl_3$-induced hepatic injury.

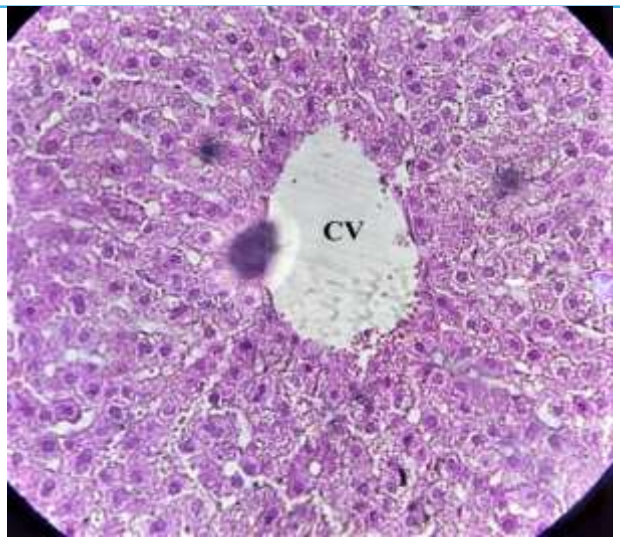

Figure 9: Histological section of liver tissue from the Omega-3 treated group, showing an overall well-preserved architecture. The central vein is present and sharply defined, with well-defined radiating hepatic cords of hepatocytes and intervening sinusoids. Hepatocytes also appear relatively normal, with intact cytoplasm and nuclei, while the degenerative changes are minimal. The overall organization suggests protective and restorative effects of Omega-3 against hepatotoxicity.

DISCUSSION:

The present study was conducted to find out the green tea and omega-3 protective role against AlCl3–induced hepatotoxicity in rabbits. Exposure to AlCl3 caused significant hepatotoxic injury as it was revealed through body weight loss and increase in all liver biomarkers such as ALT, ALP, and total bilirubin ($p < 0.001$). The results entirely confirm that AlCl3 reliably induces hepatotoxicity, omega 3 demonstrated more consistent and robust protection, particularly in correcting biochemical abnormalities, whereas the green tea also improved liver function but generally provided partial recovery. These findings have been corroborated with histopathological assessments showing that both green tea and omega-3 reduce necrosis, inflammation, and fibrosis compared to the $AlCl_3$ group. It is most likely that protection comes via the antioxidant and anti-inflammatory action of both agents in mitigating aluminium induced liver damage.

The hepatoprotective potential of both green tea and omega-3 has been established in separate studies. Polyphenols within green tea have shown the ability to protect mice against a wide array of toxin-induced hepatotoxic effects through mechanisms that include lowering oxidative stress, suppression of inflammatory mediators, and the maintenance of hepatocellular integrity for overall liver function (Wang et al., 2022). Omega-3 fatty acids, because of their high antioxidant and anti-inflammatory potencies, protect the liver through mechanisms which include reduction of lipid peroxidation, cellular membrane stabilization, and attenuation of inflammatory responses, hence giving protection in numerous experimental models of hepatotoxicity (Oda 2016). The present study further extends the findings above by showing that co-treatment with green tea and omega-3 confers enhanced hepatoprotection when the liver is exposed to $AlCl_3$ in rabbits.

Despite the positive results, there are weaknesses found in the research. Sampling for population was limited, and this could affect generalizing findings. Besides that, the duration was restricted to a period of time, and long-term consequences of green tea and omega-3 supplementations on the liver health need to be identified. Moreover, exact molecular mechanisms associated with the hepatoprotective action observed were not studied, and more research is required to find out the pathways involved. Finally, the study was conducted on only one animal model, and the

applicability of results to other species and humans would require validation by clinical studies.

CONCLUSION:

This study demonstrates that green tea and omega-3 supplements afford sound protection to the liver against AlCl3-induced injury in rabbits. These substances reduced markers of liver damage, oxidative stress, and impaired structure when used alone or in combination. Of significance is the fact that the combination of green tea with omega-3 exhibited a synergistic protective effect by affording greater protection than either agent used alone. The findings are in agreement with previous reports indicating that green tea and omega-3 fatty acids possess antioxidant, anti-inflammatory, and immunomodulatory activities that may support liver health. The results suggest potential for using natural compounds in preventing and managing hepatotoxicity from environmental toxins. However, several limitations exist in the present study, such as a small sample size and short duration of treatment. Moreover, no molecular level analyses were available. Long-term effects, comprehensive mechanisms, and clinical studies need to be determined in further studies, which can accurately demonstrate the translation of such findings to humans. In summary, the investigation hereby offers relevant information on how green tea and omega-3 fatty acids might be used as complementary interventions in liver protection and limiting the activity of hepatotoxic agents through natural pathways.

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