A STUDY ON HEPATOTOXICITY AND NEPHROTOXICITY INDUCED BY CHRONIC ALCOHOL TOXICITY IN WISTAR RATS

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Abstract:
Chronic alcohol toxicity has many harmful effects, and chronic consumption of alcohol is deleterious for alcohol consumers in the long term. The purpose of our research is to examine the hepatotoxic & nephrotoxic effects of chronic alcohol toxicity in Wistar rats. The animals consisted of an aggregate of twelve male Wistar rats divided into two distinct sets (n = 6 in each group); control and chronic alcohol toxicity. In this chronic alcohol toxicity model, 20% ethanol in water was provided as the only water source in the proportion of 2 gm/kg body weight for a duration of 90 days. During this period, normal food was provided throughout. These animals had their blood collected towards the conclusion of the research session and were sent to the lab for biochemical assays. The levels of urea and creatinine were analyzed and tabulated using Microsoft Excel. Calculations were made to determine the mean & standard deviation regarding the information at hand. Statistical analysis showed a substantial decline ($p< 0.05$) within the urea levels inside the chronic alcohol toxicity group as contrasted to the control group. When comparing the between the groups, the concentration of creatinine witnessed a noteworthy rise ($p< 0.05$). These findings emphasize that there is a need for timely diagnosis and treatment of chronic alcohol toxicity to prevent organ damage and to improve patient outcomes. A deeper understanding of mechanisms underlying the toxicity of alcohol consumption for extended periods of time is essential towards the creation of effective treatment plans. Further investigations revealing the enduring consequences of chronic alcohol toxicity could provide valuable insights into the molecular mechanisms altered, and the pathways involved in chronic alcohol toxicity.

Keywords: *Alcohol toxicity, Binge drinking, Wistar rats, Nephrotoxicity, and Hepatotoxicity.*

INTRODUCTION:

Alcoholism is an addictive condition prevailing throughout our societies as well as has become a serious global health issue related to drinking. This condition affects morbidity and mortality in multiple races, is socially damaging, and is related to various health conditions. The major organs affected include the liver and the kidneys leading to fatty liver, liver cirrhosis, hepatitis, and extreme conditions may lead to failure of kidneys in alcohol intake in very high levels. As a result, the covalent modification of cellular macromolecules leads to damaging morphological modifications causing tissue and kidney damage on a whole. The metabolic reactions of alcohol with substances produce multiple products along with urea, creatinine, uric acid etc., which are filtered by kidneys as a natural way of eliminating these toxins at high concentrations. Among these vital functions of kidneys, other important functions include regulation of water balance within the body for the maintenance of acid base balance. On an average, about 200 litres of blood is cleared of toxic materials daily, and the excess liquid of up to 1.5 litres is removed as urine. Any alteration in this functioning of kidneys can deteriorate the balance in these fluids and can lead to kidney disorders. Any such effect on the normal functioning of kidneys leads to accumulation of toxins within the blood and this in turn aggravates the condition worse. Kidney function tests

are very important in such conditions and these help in assessing the degree of damage or normal functions, and the levels of functioning of kidneys. These tests assess the residual products within blood and urine, which helps in evaluating the condition of the kidneys in removing the excess liquids and toxins from the body.

Aim/Objectives: This research's objective has been to assess the effect of chronic alcohol toxicity induced nephrotoxicity and hepatotoxicity within adult masculine Wistar rats.

MATERIALS & METHODS:

Adult male, Wistar rats had been obtained from the Saveetha University, Biomedical Research Unit and Laboratory Animal Centre. The rats had been raised within typical lab settings, which included constant temperature setup (25º ± 2ºC), humidity in air (70% ± 4%), 12 hrs of light and dark cycles, with uncontrolled access to feed and water. A total of 12 Wistar rats were randomly chosen and grouped within control & alcohol toxicity groups (n = 6 in each). Alcohol for the entire study period was purchased from Southern India Scientific Corporation.

Ethical approval:

The approval (Ref-SU / CLAR / RD / 08 / 2024) for animal experimental procedures was obtained from the Institutional Ethics Committee (IAEC), Department of Research & Development, Saveetha Institute of Medical & Technical Sciences, Chennai. Animal handling and procedural protocols were followed during the entire study, according to the ethical and regulatory guidelines for animal research.

Experimental Design

The investigation used adult male Wistar rats that were obtained through the central animal house unit. The Rats had been selected at random, categorised under control & chronic groups, and acclimatized to the standard laboratory conditions before the beginning the experiment. Two distinct sets of six rats apiece had been selected at random from among the rats. The rats in group 1 were treated as normal controlsand provided with normal water and food. The animals in group two were treated for the creation of the chronic alcohol toxicity model. An ethanol solution (e.g., 20% v/v in saline) was administered daily for 90 days, to deliver through drinking water. The solution dosage was calculated based on the weight of the rats (1-2 g/kg body weight) and then administered. The animals were maintained in cages with three rats in each cage. A total of four cages were maintained with two for normal control and two for alcohol toxicity model. When the experimental period was over, blood had been collected through retro-orbital sampling & sent to the biochemistry laboratory for estimations of urea and creatinine in the samples.

Preparation of 20% alcohol for inducing chronic alcoholism:

The chronic alcohol toxicity model was created by providing drinking water mixed with ethanol. The alcohol containing water had been created by combining 20 ml of ethanol in 80 ml of water that had been maintained as the only source of water during the entire study period.

Collection of Blood Samples:

After twelve weeks of oral alcohol administration, the rats were mildly

anesthetized to minimize stress and injury. This was done by keeping the rat in a chamber with a small amount of chloroform on a cotton ball, allowing the rat to inhale the chloroform until it reached a state of mild anesthesia (loss of movement and relaxed posture). The rats were closely monitored during the entire procedure to avoid excessive exposure.

Serum biochemistry:

The retro-orbital collection of blood was performed in rats; where in blood sample (2 ml) from each rat was collected & centrifuged for ten minutes @ 3000 revolutions per minute. The serum obtained after centrifugation was used for biochemical analysis with an autoanalyzer. The serum urea and creatinine tests were done to assess liver and kidney function. The remaining serum was stored at -80 degrees Celsius for further evaluation. The estimation of urea was done according to Berthel's method and creatinine estimation was done according to Jaffe's method.

Results:

All the animals were continuously monitored for any behavioural changes and alterations in health conditions. The external environmental conditions were maintained at recommended standards. During the study period, a total of two deaths of animals from the chronic alcohol toxicity groups were recorded, which could have been due to multiple internal factors that are modified in such experimental groups. The chronic groups' urea levels were considerably ($p<0.05$) lower than those of the control group (figure. 01). The creatinine concentration had been significantly ($p<0.05$) increased within the chronic group as contrasted against the control group (figure. 02).

Statistical analysis: Statistical analysis was done using Excel. Showing the mean & standard deviation obtained p-value.

Table.01: Displaying the mean & standard deviation of urea and creatinine levels of the two groups. Urea displayed a substantial decrease among chronic & control groups. Creatinine displayed a considerable increase amongst the chronic and control groups. [* = $p<0.05$]

Parameter	Control (mean ± SD)	Chronic (mean ±SD)
Urea	63	40.47*
creatinine	0.42	0.58*

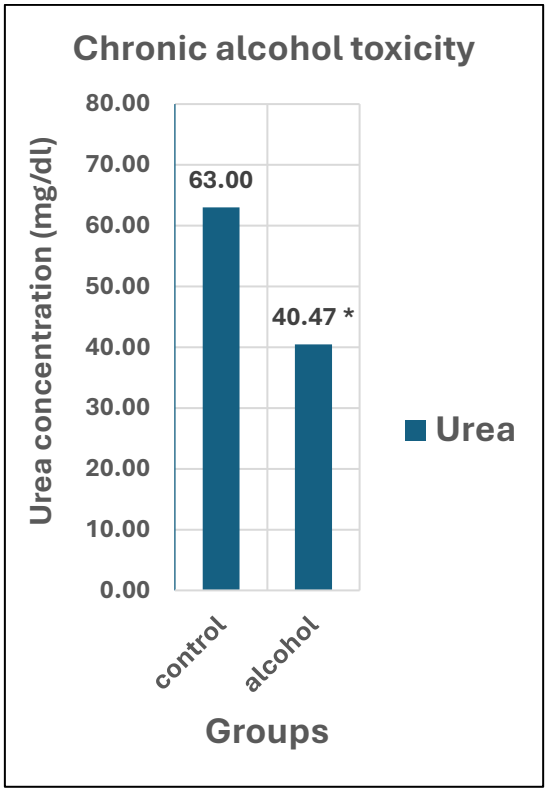

Figure 1: Bar graph showing the serum concentrations of urea in the control & chronic groups.

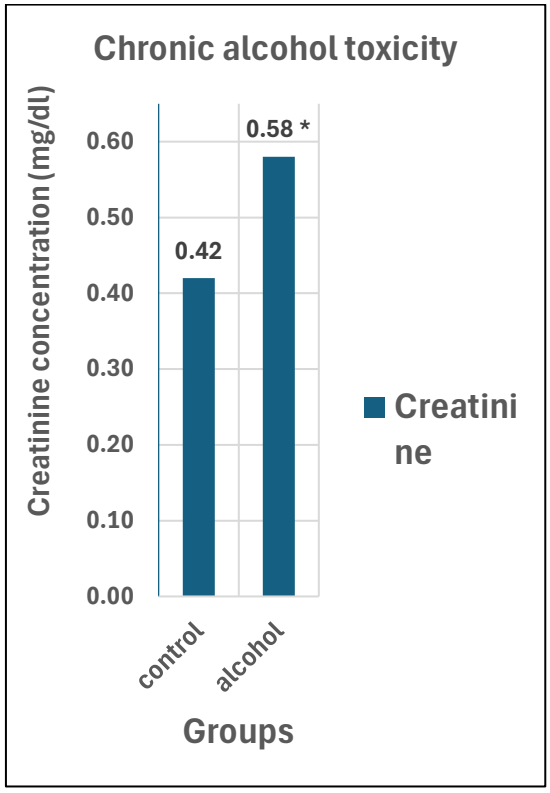

Figure 2: Bar graph showing the serum concentrations of creatinine in the control & chronic groups.

DISCUSSION:

Alcohol toxicity is known to affect the normal functioning of liver and kidneys, which in chronic alcohol toxicity conditions could lead to even kidney failure. The effect of functioning of liver and kidneys can be assessed through their functioning tests. In this study, the chronic alcohol toxicity models were subjected to exposure to longer periods of alcohol toxicity, which were evident in the results. The effect of alcohol toxicity on kidneys was significantly evident from the decrease in the urea levels and increase in the creatinine levels.

The organic molecule urea (carbamide) has the molecular formula CO (NH2)2. The carbonyl (C=0) functional group connects both of the NH2 groups within the molecule. During the urea cycle, the liver combines two ammonia (NH3) molecules alongside a carbon dioxide (CO2) molecule to create urea.

The reduced urea levels in the alcohol toxicity group may be attributed to impaired protein metabolism which could be linked to hepatic dysfunction. This effect on the liver and the alteration in the metabolism of proteins can be linked to chronic exposure to alcohol. Ethanol is said to have a direct influence on the functioning of the liver and in turn, leads to the disruption of protein metabolic pathways. This could be the probable cause for the decreased levels of urea seen in the alcohol toxicity group. Even though the model created was chronic with twelve weeks of alcohol exposure, the findings suggest that even this long-term exposure to alcohol toxicity can compromise the protein metabolism, which is related to nitrogen clearance, and reflects the underlying cause for hepatic dysfunction or stress.

On comparing the levels of creatinine, it was seen to have a significant increase in the alcohol toxicity group which indicates renal impairment. Creatinine is a reliable marker for glomerular filtration rate (GFR) and an elevation in the serum creatinine indicates a potential reduction in GFR. A significant increase in creatinine affecting GFR indicates the toxic effect of alcohol on kidneys indicating nephrotoxicity. The probable cause of this nephrotoxicity could be the vasoconstriction and oxidative stress caused by alcohol on kidneys, and this in turn leads to glomerular damage affecting

the glomerulus. The toxic effects could also affect the GFR by causing tubular dysfunction. In both these conditions, the impairment in normal functioning of kidneys can be seen and this could be linked to filtration inefficiency. The increase in the Reactive Oxygen Species (ROS) could be a reason for the increase in oxidative stress, which could be the potential reason for injury or damage to the tubular and glomerular components of kidneys. From the immune system point of view, the triggering of neutrophils & macrophages could be the cause of producing inflammatory cytokines & chemokines, which in turn can harm the kidneys.

The study findings indicate a significantly decreased urea levels and a significantly increased creatinine levels. The mechanisms responsible for these changes could be mitochondrial dysfunction, or a result of oxidative stress, inflammatory signalling, or could be a direct result of acetaldehyde toxicity. The disruption in these mechanisms which help in the maintenance of the internal homeostasis could be the primary cause of the effects on the liver and kidneys. These changes underscore the vulnerability of the liver and kidneys to the chronic damage caused by the changes seen within multiple systems.

The kidneys are highly susceptible to the effects of toxins mainly due to two reasons. The high volume of blood which passes through them is subjected to filtration of these toxins, and in due course these toxins can accumulate in the kidney tubules leading reduced functioning of the kidneys. In such conditions, the nephrotoxicity caused depends on the extent of damage caused to the renal tubules and the inefficiency of these tubules in filtering the toxins into the urine. The residual toxins accumulate within these tubules and remain within the blood system. This inefficiency in removal of the toxins indirectly leads to damaging multiple organs in such conditions. Other effects of nephrotoxicity include reduced excretion of body waste, and failure to keep the fluid-electrolyte equilibrium.

Measurement of creatinine & Blood Urea Nitrogen (BUN) serve as prominent markers in the evaluation of the normal functioning of kidneys. With reduced filtration capacity of the kidneys, the residual levels of the toxins build up within the body are reflected in the blood. These alterations in the levels of these substances in the blood are used in the assessment of the normal functioning of the kidneys.

Creatinine:

Creatinine is formed because of breakdown of creatinine phosphate within muscle, where its production is steady & contingent upon muscle mass. The levels of creatinine in the serum can be measured and serves as a crucial determinant of kidney health, as it is a readily measurable by-product produced because of metabolic process in the muscle and excreted through the kidneys. Any increase in the levels of creatinine indicated affected kidney functions and this impairment in renal function could result in chronic nephritis, and excess accumulation can also lead to shocks, and muscle atrophy.

CONCLUSION:

Regarding the current investigation, the chronic implications of ethanol upon the normal kidney functions are evaluated

using urea and creatinine as parameters. The results from this study, the chronic alcohol toxicity group against the normal control indicates that ethanol has very high toxic effects on the normal functioning of kidneys when exposed to longer durations. The understanding of the potential toxic mechanisms of ethanol on kidneys in the long term could help in the development of potential therapies in the management of nephrotoxicity, and treatment for renal toxicity.

The important function of kidneys in the removal of toxins from the body is affected in chronic alcohol toxicity, and this impaired function results in various disorders. This effect of accumulation of toxins is not only seen in the kidneys but is also reflected in other systems of the body. The initial indications of accumulation of toxins act as a sign of nephrotoxicity, which lead to chronic conditions affecting the total kidneys, and even causing death.

Long term use of alcohol results in damage to liver and kidneys. The results (Table. 01) show the alterations in the urea and creatinine levels, which indicate the deteriorating effects of alcohol upon the liver and kidneys. The chronic duration of alcohol exposure for a period of 12-weeks has shown negative affects upon the kidneys' & liver's regular operation. Initial effects of alcohol on the kidneys and liver as seen in our study are consistent with the literature, and extended study periods as in this study show increased damage to the normal functioning of liver and kidneys.

Overall, the effects of long-term exposure to alcohol are clear on the damage caused to the liver and kidneys. The build-up of urea and creatinine is evident from the alterations in the levels in the serum at the end of 12 weeks study period. Alterations in the ALT and AST, which indicate the normal functioning of liver are also seen to be affected, indicating liver damage. This impairment of normal liver function is seen as elevated urea levels, and the impairment of normal kidney function in seen in the impairment of increased creatinine levels.

Further studies evaluating the molecular mechanisms of toxicity of alcohol could help in associating multiple conditions which are a result of chronic alcohol toxicity. These molecular mechanisms could provide us with the insights for the development of promising therapies in managing the damage caused in chronic alcohol toxicity conditions.

Conflict of interest

Regarding this work, the authors disclose no conflicts of interest.

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