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Evaluating the protective effects of choline chloride on biochemical alterations induced by paracetamol toxicity in mice: A pilot study

Elif Baris[1], Yasemen Adali Rusen[2]*

[1]İzmir University of Economics, Faculty of Medicine, Department of Medical Pharmacology, İzmir, Türkiye
[2]İzmir Democracy University, Faculty of Medicine, Department of Pathology, İzmir, Türkiye
*Corresponding: yasemenadali@hotmail.com

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Abstract
Paracetamol (acetaminophen, APAP) toxicity is a significant clinical concern due to its hepatotoxic and nephrotoxic effects, which can lead to disruptions in serum protein synthesis, metabolic changes, and electrolyte imbalances. This study aimed to investigate the biochemical impacts of paracetamol toxicity and evaluate the potential protective effects of choline chloride. Using an in vivo model with balb/c mice, three groups were examined: A control group, an APAP-toxicity group (300 mg/kg paracetamol), and a choline-treated group (300 mg/kg paracetamol with 30 mg/kg choline chloride). Biochemical analyses revealed that paracetamol administration caused a slight but non-significant decrease in serum total protein and albumin levels, reflecting impaired hepatic function. While the toxicity model also showed significant reductions in glucose and triglyceride levels, cholesterol and electrolyte changes were non-significant. Notably, treatment with choline chloride led to a significant increase in serum potassium levels but did not significantly alter other biochemical markers within 24 h. These findings suggest that while choline chloride may support electrolyte balance and modulate certain metabolic disruptions, the short-term model may not capture long-term or more profound biochemical alterations. The study underscores the importance of further research to explore the protective role of choline and extended observation periods to better understand paracetamol-induced toxicity and recovery mechanisms..
Keywords: *Paracetamol, toxicity, choline, lipids, protein, electrolyte*

1. Introduction

Acetaminophen (N-asetil-p-aminofenol, APAP, parasetamol) is one the leading causes of drug-induced liver damage and acute liver failure as a widely used analgesic and antipyretic drug. While doses up to 4 g/day are considered safe, exceeding this threshold can result in severe liver and kidney damage, particularly due to accidental ingestion in children or intentional overdose in adults. This toxicity leads to centrilobular necrosis, primarily driven by glutathione depletion during the liver metabolism of acetaminophen through CYP2E1, producing the toxic metabolite N-acetyl-p-benzoquinone imine (NAPQI). N-acetylcysteine is the primary treatment, replenishing depleted glutathione.[1,2]

Liver injury from APAP overdose disrupts hepatic synthetic function, causing measurable decreases in different biochemical parameters. Its toxicity poses a significant clinical concern due to its hepatotoxic effects, impacting serum protein synthesis, particularly albumin and total protein. Although initial reductions in serum albumin may not appear significant, they reflect long-term hepatic dysfunction.[3,4] APAP is known to bind to serum albumin, the most abundant protein in the blood. Serum albumin serves mainly as a carrier protein, binding various substrates and playing a crucial role in drug pharmacokinetics.[5]

APAP-induced liver damage also involves disruptions in lipid profiles such as cholesterol and triglycerides. Research has shown that APAP overdose elevates total cholesterol and triglyceride levels in blood while reducing high-density lipoprotein (HDL) cholesterol and albumin.[6] Impaired hepatic function results in the accumulation of triglycerides due to secretion issues.[7] Additionally, it exacerbates glucose metabolism disturbances, as liver dysfunction hinders glucose homeostasis.[8,9] Moreover, the fluctuation of serum electrolytes like sodium, potassium, and chloride, can also occur in case of tubular damage in kidneys which are responsible for electrolyte balance. This is critical as electrolyte imbalances can further complicate the clinical picture, potentially leading to arrhythmias or other cardiovascular issues.[10]

Choline, as a precursor for acetylcholine (ACh), increases cholinergic neurotransmission and bound to ACh receptors at higher doses. Clinically, choline and choline donors (citicoline, phosphatidylcholine, etc.) supplementation are recommended for conditions such as cerebral ischemia, head trauma, Alzheimer's disease, cognitive disorders, and Parkinson's disease[11] Phosphatidylcholine, crucial for cell membranes, has been shown to improve APAP toxicity related complications. In animal studies, soybean phosphatidylcholine reduced serum transaminase levels post-APAP exposure, and polyene phosphatidylcholine has shown efficacy in treating drug-induced liver injury.[12–14]

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The aim of this pilot study was to evaluate the protective and therapeutic effects of choline chloride on biochemical alterations induced by acetaminophen (APAP) toxicity.

2. Materials and methods

2.1. In-vivo experimental protocol

Balb/c mice (8–12-week-old, N=15) were maintained under standard laboratory conditions with a 12-h light/dark cycle and an ambient temperature of 22±2ºC. Groups of five mice were housed in Plexiglas cages, and all in vivo experiments were conducted at İzmir Biomedicine and Genome Center (IBG) laboratories. The animals had free access to food and water during the study. Paracetamol (Atabay®) and Choline (Sigma Aldrich, C7017) were prepared and diluted with physiological saline (0.5 mL total volume) and administered intraperitoneally (i.p.) according to the weight of the animals at the beginning of the experiments.

The experimental design consisted of 3 groups: Group 1 (control, n=5) received 0.9% NaCl at 0, 1, 5, and 9 h. Group 2 (APAP toxicity, n=5) received 300 mg/kg paracetamol at 0 h and 0.9% NaCl at 1, 5, and 9 h. Group 3 (choline chloride, n=5) received 300 mg/kg paracetamol at 0 h and 30 mg/kg choline chloride at 1, 5, and 9 h.[2,15,16] All animals were sacrificed 24 h after the protocol initiated with ketamine/xylazine anesthesia (80/12.5 mg/kg, i.p.).

2.2. Biochemical analyses

Blood samples were collected via cardiac puncture into serum separator tubes, and then centrifuged at 3,000 rpm (or approximately 1,500 × g) for 10 minutes to obtain serum. The serum samples were obtained was stored at -20ºC until analysis. The serum samples were used to evaluate routine biochemistry panels of total protein (Randox/TP8336), albumin (Randox/AB8301), sodium (Randox/NA8327), potassium (Randox/PT8329), calcium (Randox/CA8309), glucose (Randox/GL8318), cholesterol (Randox/CH8310), triglyceride (Randox/TR8332) levels were analyzed by using Randox Rx daytona+ Clinical Chemistry Analyzer device.

2.3. Statistical analysis

Statistical analysis was conducted using GraphPad program. Biochemical parameters were evaluated using the Kruskal-Wallis and Student's T-test. Data presented as mean ± standard error of mean (SEM) and p-value of less than 0.05 considered significant.

3. Results

3.1. Serum proteins

The APAP-toxicity group exhibits a slight but non-significant decrease in both total protein and albumin levels compared to the control. Treatment with 30 mg/kg choline chloride also results in non-significant changes compared to the APAP and control groups for 24 h. protocol (Figure 1, Table 1).

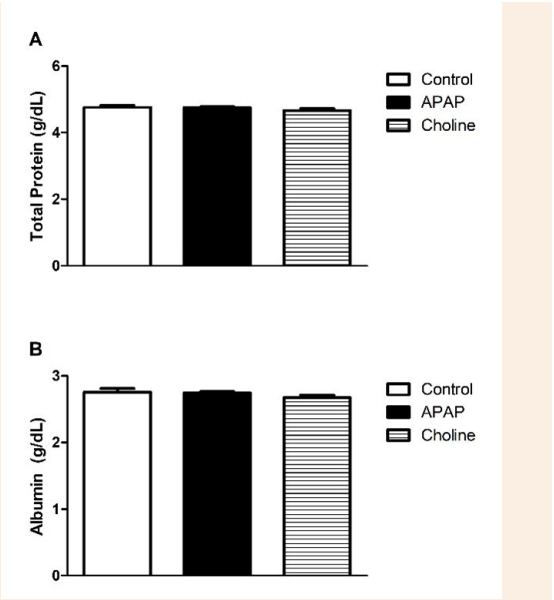

Figure 1: Effects of chloride on protein levels in rats with paracetamol toxicity. Shown effects of 30 mg/kg chloride treatment on albumin (A) and total protein (B) levels in rats with paracetamol toxicity and controls.

3.2. Metabolism

The APAP-toxicity group shows a slight, non-significant increase in cholesterol levels, while glucose and triglyceride levels are significantly decreased compared to the control groups. Treatment with 30 mg/kg choline chloride leads to non-significant changes in cholesterol levels compared to both the APAP and control groups over a 24-h protocol, while glucose and triglyceride levels are significantly decreased in the choline-treated group compared to the controls (Figure 2, Table 1).

Table 1: Biochemical analysis summary of experimental groups

Parameters	Control	APAP	Choline 30 mg/kg
Albumin (g/dL)	2.7 ± 0.12	2.7 ± 0.04	2.7 ± 0.08
Calcium (mg/dL)	10.3 ± 0.28	10.1 ± 0.21	10.1 ± 0.34
Cholesterol (mg/dL)	109.3 ± 7.01	122.6 ± 19.05	108.1 ± 10.67
Glucose (mg/dL)	331.4 ± 19.29	301.3 ± 25.48	281.3 ± 11.53
Sodium (mmol/L)	160.0 ± 3.38	158.4 ± 2.013	155.8 ± 0.83
Potassium (mmol/L)	5.3 ± 0.17	5.2± 0.23	5.7 ± 0.40
Triglyceride (mg/dL)	191.0 ± 23.83	144.3 ± 43.04	134.3 ± 21.63
Total Protein (g/dL)	4.8 ± 0.12	4.7 ± 0.08	4.6 ± 0.13

The table presents the biochemical parameters measured across experimental groups: Control, APAP, Choline 30 mg/kg.

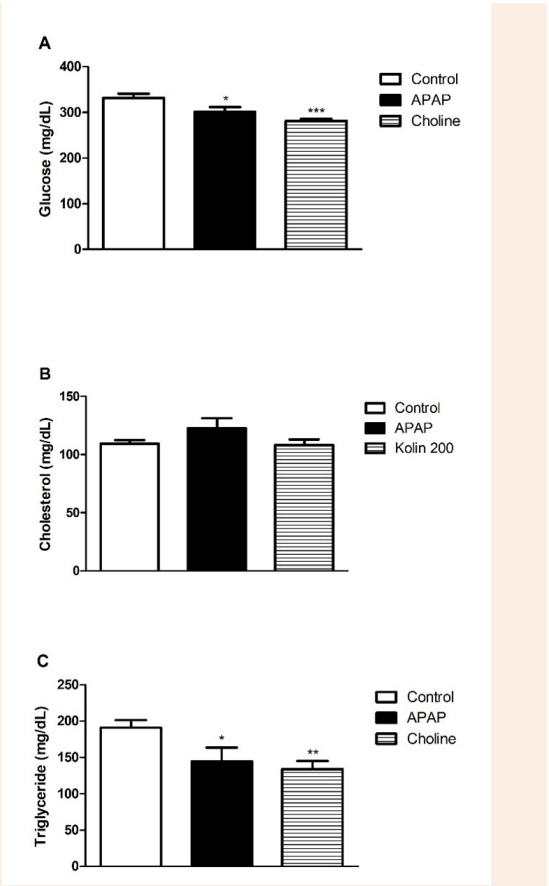

Figure 2: Effects of choline chloride on glucose and lipid metabolism in rats with paracetamol toxicity. Shown effects of 30mg/kg choline chloride treatment on glucose (A), cholesterol (B) and triglyceride (C) levels in rats with paracetamol toxicity and controls. *p < 0.05, **p < 0.01 and ***p < 0.001 vs control group

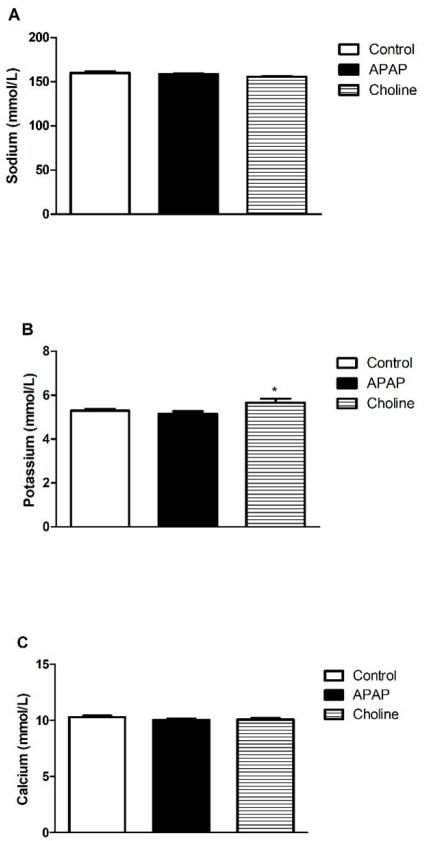

Figure 3: Effects of choline chloride on electrolytes in rats with paracetamol toxicity. Shown effects of 30 mg/kg choline chloride treatment on sodium (A), potassium (B) and calcium (C) levels in rats with paracetamol toxicity and controls. *p < 0.05, vs APAP group.

3.3. Electrolytes

The APAP-toxicity group shows non-significant changes in electrolyte levels, including sodium, potassium, and calcium, compared to the control group. Treatment with 30 mg/kg choline chloride only increase serum potassium level significantly compared to the APAP group for 24 h. protocol (Figure 3, Table 1).

4. Discussion

Paracetamol (acetaminophen) toxicity is a significant clinical concern, primarily due to its hepatotoxic effects, which can lead to alterations in serum protein levels, particularly albumin and total protein. The liver's ability to synthesize these proteins is compromised during paracetamol-induced hepatic injury, resulting in measurable changes in serum biochemistry. Studies have demonstrated that paracetamol overdose leads to a decrease in serum albumin and total protein levels. The impairment of albumin synthesis is a well-documented consequence of liver damage.[17] The study pointed out that while the decrease in serum albumin following paracetamol-induced hepatotoxicity may not be immediately significant, it reflects the liver's compromised synthetic function over time.[18] Similarly another study reported significant reductions in total protein, albumin, and globulin levels due to hepatic dysfunction following paracetamol administration.[19] This decrease is attributed to the liver's inability to maintain normal protein synthesis under toxic conditions.[4] Moreover, the nephrotoxic effects of paracetamol also contribute to serum protein changes. A study indicated that paracetamol toxicity can lead to metabolic disturbances, including alterations in serum total protein and albumin levels, alongside increases in urea and creatinine.[20] This nephrotoxicity is further supported by another study that observed elevated blood urea and serum creatinine levels, alongside increased urine protein and albumin excretion, indicating renal damage associated with paracetamol overdose.[17,21] The biochemical markers of liver injury, such as aspartate aminotransferase (AST) and alanine aminotransferase (ALT), are also elevated in cases of paracetamol toxicity, which correlates with the observed decreases in serum proteins.[22] Another study emphasized that the elevation of these enzymes serves as a quantitative marker for assessing the extent of hepatic damage. The release of these enzymes into circulation is indicative of cellular necrosis and loss of functional integrity in hepatocytes, further exacerbating the decline in serum protein levels.[23] In this study, the APAP-toxicity group exhibited a slight but non-significant decrease in both total protein and albumin levels compared to the control group. Similarly, treatment with 30 mg/kg choline chloride resulted in non-significant changes in these protein levels. These findings underscore the importance of monitoring serum proteins and enzyme levels, indicating that while

changes may be present, a 24-h toxicity model might not be sufficient to cause statistically significant alterations in serum protein levels. From a pharmacokinetic perspective, it is noteworthy that APAP has been shown to bind to serum albumin, which carries significant clinical implications. Albumin, being the most abundant plasma protein, functions as a transport vehicle for a range of endogenous and exogenous substances, including medications.[24] The binding of paracetamol to albumin can alter its pharmacokinetic profile by influencing the drug's distribution and bioavailability in systemic circulation. This interaction may impact serum albumin levels, especially under conditions of paracetamol-induced hepatic damage, which impairs the liver's ability to synthesize albumin and may result in reduced production over time. However, due to the reversible nature of paracetamol's binding to albumin, acute toxicity models may not fully capture the extent of this interaction. Further research is needed to understand the long-term effects of paracetamol-albumin binding and how it contributes to protein level alterations in the context of paracetamol toxicity.

The toxicity mainly involves significant elevations of serum creatinine and urea, indicative of renal impairment rather than electrolyte changes.[20] Studies reported that high doses of paracetamol led to increased blood urea and serum creatinine levels, confirming renal damage and elevated levels of serum enzymes such as aspartate aminotransferase and alanine aminotransferase, which are markers of liver function deterioration.[22,23] In this study, the APAP-toxicity group showed non-significant changes in electrolyte levels, including sodium, potassium, and calcium, compared to the control group. However, treatment with 30 mg/kg choline chloride led to a significant increase in serum potassium levels compared to the APAP group. This result suggests that chloride choline may influence potassium regulation under conditions of paracetamol-induced stress, potentially aiding in the maintenance of electrolyte balance. Currently, there is limited research directly linking choline administration to hyperkalemia. However, studies have shown that certain choline analogs, such as succinylcholine, can induce hyperkalemia, particularly in patients with neuromuscular diseases or severe trauma.[25–27] These findings highlight the need for careful potassium monitoring during choline administration, especially in cases of renal compromise or electrolyte disturbances. Further research is required to clarify whether choline's impact on potassium levels is a direct pharmacological effect or a result of interactions with APAP-induced pathological processes.

The observed increase in potassium levels could indicate an interaction between choline and cellular ion transport mechanisms, highlighting its potential role in preventing disruptions in electrolyte homeostasis associated with liver and kidney damage. Further research is needed to elucidate the pathways through which choline exerts these effects and to determine its broader implications in the management of APAP toxicity and related metabolic disturbances.

Paracetamol-induced liver damage significantly alters lipid profiles, increasing serum cholesterol and triglyceride levels, particularly in overdose cases. It has been reported that paracetamol toxicity led to increased levels of total cholesterol and triglycerides, alongside a decrease in high-density lipoprotein (HDL) cholesterol and albumin levels.[6] Another study confirmed that paracetamol-induced hepatotoxicity was associated with elevated biochemical parameters including total cholesterol and triglycerides, indicating a disruption in lipid metabolism due to liver damage.[28] The increase in triglycerides can be attributed to impaired hepatic function, which disrupts the secretion of triglycerides into the plasma, leading to their accumulation. Moreover, the hepatotoxic effects of paracetamol also extend to glucose metabolism. The liver plays a crucial role in glucose homeostasis, and its impairment can lead to dysregulation of glucose levels. Although specific studies directly linking paracetamol toxicity to glucose changes are limited, the overall hepatic dysfunction observed in paracetamol overdose suggests potential alterations in glucose metabolism as well.[29] The depletion of glutathione, a critical antioxidant in the liver, further exacerbates oxidative stress and cellular damage, contributing to the dysregulation of both lipid and glucose metabolism.[8] In addition to the biochemical changes, histopathological studies have shown that paracetamol toxicity can lead to structural alterations in liver tissue, which may further complicate metabolic processes.[9] The resultant fatty liver condition, characterized by the accumulation of triglycerides, is a direct consequence of the liver's inability to process lipids effectively due to cellular injury. In this study, the APAP-toxicity group showed a slight, non-significant increase in cholesterol levels, while glucose and triglyceride levels significantly decreased compared to the control group. Treatment with 30 mg/kg choline chloride led to non-significant changes in cholesterol levels compared to both the APAP and control groups, while glucose and triglyceride levels were significantly decreased in the choline-treated group compared to the controls. These findings suggest that while choline chloride may not markedly affect cholesterol levels, it could play a role in modulating glucose and triglyceride levels, potentially offering a protective effect against certain metabolic disruptions associated with paracetamol toxicity. This indicates the need for further studies to explore the mechanisms behind these changes and the potential clinical implications of choline as a therapeutic agent in managing APAP-induced metabolic disturbances.

Previous studies have shown that an intravenous glucose tolerance test was performed on fourteen patients with mild to moderate liver damage resulting from paracetamol overdose. Patients managed conservatively displayed glucose intolerance and a reduced early insulin response, suggesting inadequate nutritional intake between the time of overdose and testing. In contrast, those who received intravenous glucose supplementation for nutritional support also exhibited glucose intolerance but maintained normal insulin responses. The observed reduction in the fractional disappearance rate was attributed to an expanded glucose distribution volume and a decrease in the absolute rate of glucose disappearance.[29] Additionally, mild fasting hypoglycemia observed in four patients indicated impaired gluconeogenesis.[30] In animal models, following a toxic dose of acetaminophen (500 mg/kg) in mice, blood

glucose levels initially surged to 225% at three hours, returned to normal by six hours, and dropped to 45% of baseline levels by 24 hours.[31] In this study, findings align with these prior observations, as the APAP-toxicity group demonstrated significant disruptions in glucose metabolism. The significant decrease in glucose levels compared to controls highlights the potential impairment in hepatic gluconeogenesis and glucose regulation following paracetamol-induced liver damage. Moreover, the treatment with 30 mg/kg choline chloride resulted in a decrease in glucose levels, which, while notable, indicates that choline's role may not fully restore glucose metabolism within a short observation period. These results suggest that paracetamol's impact on liver function extends to metabolic processes, reinforcing the need to explore supportive treatments that could mitigate such metabolic disruptions over longer recovery periods or in more extensive models.

5. Limitations

One of the main limitations of this study is the short observation period of 24 hours, which may not fully capture the long-term effects of paracetamol toxicity on serum protein levels, electrolyte balance, and metabolic changes. This limited timeframe may have contributed to the non-significant findings in certain biochemical parameters, potentially underestimating the broader implications of hepatic and renal damage. The study also focused on acute paracetamol toxicity without exploring chronic exposure or repeated dosing, which may yield different outcomes in terms of liver function and protein synthesis. Furthermore, while the study assessed the impact of choline chloride treatment, the specific mechanisms underlying its influence on metabolic pathways were not explored in depth. Future studies should include longer observation periods, and a more detailed analysis of the biochemical pathways involved to provide a more comprehensive understanding of the effects of paracetamol toxicity and potential therapeutic interventions like choline.

6. Conclusion

In conclusion, paracetamol toxicity presents significant clinical challenges which disrupt serum protein synthesis and lead to metabolic disturbances. This study highlighted that while there were non-significant changes in total protein, albumin, cholesterol, and electrolyte levels following APAP exposure, significant decreases in glucose and triglyceride levels were observed, emphasizing paracetamol's broad impact on metabolic functions. The administration of choline chloride did not significantly alter most parameters within the 24-h model but showed potential benefits in modulating serum potassium and mitigating metabolic disruptions in glucose and triglyceride levels within 24-h. These findings underline the need for extended observation periods and further investigation into the role of choline as a therapeutic agent. Additionally, the pharmacokinetic interaction of paracetamol binding to serum albumin and its potential influence on drug distribution and protein levels requires more comprehensive study. Understanding these mechanisms may contribute to improved management strategies for paracetamol toxicity and better support for hepatic and renal function.

Ethical approval

Ethical approval for the study was obtained from Izmir Biotype And Genome Center Local Ethics Committee For Animal Experiments IBG-HADYEK/2023-016.

Authors contribution

EB and YAR: Research, planning, article scanning, writing original draft and review. All authors contributed to the article and gave final approval of the version to be submitted.

Conflict of interest

There are no conflicts of interest associated with this re-search publication, according to the authors.

Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Acknowledgments

This situation does not exist.

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