

Original Research Article

Comparative efficacy of N-Acetylcysteine (NAC) and rutin in ameliorating paclitaxel-induced hepatotoxicity in male rats

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Abstract

Purpose: To analyze the hepatoprotective properties of N-acetylcysteine (NAC) and rutin (RU) via a rat model of paclitaxel (PAC)-induced hepatotoxicity.

Methods: Forty male Swiss albino rats were divided into four groups containing ten rats each. Paclitaxel (2 mg/kg) was administered intraperitoneally (ip) to all groups on days 1 and 7, except the control group, which received only distilled water orally. Rutin (40 mg/kg/day) was administered orally to the paclitaxel + rutin group, while 50 mg/kg/day of N-acetylcysteine was administered orally to the paclitaxel + N-acetylcysteine group. The treatments lasted for 14 days, during which liver weight, body weight change, Serum levels of Tumor necrosis factor-alpha (TNF- α) and Interleukin-10 (IL-10) were measured using enzyme-linked immunosorbent assay (ELISA), and histopathology of liver tissues was examined to evaluate tissue damage.

Results: Groups that received PAC had significantly reduced liver weight and increased serum IL-10 as well as TNF- α levels in comparison to control group ($p < 0.05$). The use of NAC and rutin mitigated these effects. Paclitaxel + N-acetylcysteine group showed a more significant reduction in inflammatory markers, with levels approaching values in control group ($p < 0.05$). Histologically, PAC caused severe hepatic damage, whereas NAC and RU preserved liver architecture, with NAC offering better protection.

Conclusion: N-acetylcysteine and rutin were equally effective in attenuating PAC-induced hepatotoxicity, with N-acetylcysteine showing superior anti-inflammatory effect and rutin better structural protection. These findings suggest that both are promising adjuvants for PAC chemotherapy.

Keywords: Paclitaxel-induced liver injury, Rutin, N-Acetylcysteine, Cytokine modulation, Hepato-protectants

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INTRODUCTION

Chemotherapy-induced liver injury encompasses a spectrum of hepatic damage resulting from the hepatotoxic effects of chemotherapeutic drugs. These injuries range from mild, transient abnormalities to severe and irreversible liver dysfunction. The underlying mechanisms

include direct hepatocyte toxicity, oxidative stress, cholestasis (bile flow obstruction), vascular injury and immune-mediated damage, collectively impairing the metabolic, detoxifying and synthetic functions of the liver [1,2].

Paclitaxel is a broad-spectrum chemotherapeutic agent that prevents cancer cell division through the inhibition of mitosis. It is used for managing a

variety of cancers, including lung, ovarian, and breast cancers [2,3]. While it is effective in targeting cancer cells, paclitaxel is associated with several dose-limiting adverse effects, among which hepatotoxicity is a major concern [3,4]. Paclitaxel-induced liver damage is primarily mediated by oxidative stress, mitochondrial dysfunction, and activation of inflammatory pathways, leading to hepatocellular injury and altered liver function [3,4]. Paclitaxel is one of the most used chemotherapeutic medications because it stabilizes microtubules and prevents microtubule depolymerization during cell division [2]. However, the use of PAC leads to various side effects, such as neuropathy, cardiotoxicity, and hepatotoxicity, as well as the development of resistance in cancer cells to paclitaxel treatment [3,4].

Oxidative stress occurs in response to excess reactive oxygen species (ROS), which disrupt the cellular redox balance and encourages lipid peroxidation and hepatic tissue death [5]. Concurrently, hepatic impairment development is significantly influenced by the inflammatory response. One important pro-inflammatory cytokine that is frequently elevated under hepatic stress is tumor necrosis factor (TNF- α), which stimulates inflammation and necrosis. However, by preventing the synthesis of pro-inflammatory mediators and promoting tissue repair, interleukin-10 (IL-10), a cytokine with anti-inflammatory qualities, contributes to immune response modulation [6]. An imbalance between these cytokines often reflects the severity of liver inflammation and the extent of hepatocellular damage.

Given the roles of oxidative and inflammatory pathways in paclitaxel-induced hepatotoxicity, antioxidants and anti-inflammatory agents offer promising therapeutic approaches [5,6]. N-acetylcysteine (NAC), an established antioxidant that restores intracellular glutathione levels, is a precursor of glutathione that scavenges free radicals and attenuates cytokine-mediated inflammation.

Rutin is a dietary bioflavonoid present in different fruits and vegetables with potent antioxidant and anti-inflammatory properties, such as the capacity to reduce the production of ROS and modulation of cytokine expression [3,6]. The antioxidant property of rutin arises from its capacity to counteract ROS, thus reducing oxidative harm within cells. Moreover, rutin shows significant anti-diabetic properties by blocking α -glucosidase, resulting in lower postprandial glucose levels [8].

N-acetylcysteine (NAC) is an amino acid primarily used as a mucolytic agent and to treat overdoses of paracetamol (acetaminophen). N-acetylcysteine is relatively safe, with toxicity being rare and typically dose or administration-dependent [9]. N-acetylcysteine exhibits potent anti-inflammatory, antioxidant, antimicrobial and immunomodulatory properties. Thus, NAC is advantageous in inflammatory and oxidative stress-related diseases.

The direct antioxidant effects arise from the capacity of NAC to neutralize ROS like nitrogen dioxide, hydrogen peroxide, and hydroxyl radicals. Indirectly, NAC serves as a precursor for glutathione (GSH), which enhances cellular antioxidant defenses. In liver injury, particularly acetaminophen toxicity, NAC mitigates oxidative damage by scavenging free radicals and replenishing glutathione (GSH) levels in mitochondria and cytoplasm. Furthermore, it reduces inflammation by inhibiting the levels of cytokines, chemokines and NF- κ B, a critical transcription factor that stimulates inflammation. These mechanisms support its therapeutic role in both acute and chronic liver injuries [9,10].

Although NAC and rutin have shown hepatoprotective effects against various hepatotoxic agents, reports on their efficacy in ameliorating paclitaxel-induced hepatic damage are scanty. This study aims to assess the protective effects of rutin and NAC in a rat model of paclitaxel-induced hepatotoxicity. Histopathological evaluation of liver tissue will further support the mechanistic insights into their protective roles.

EXPERIMENTAL

Animals

Forty mature male Swiss Albino rats weighing between 160 and 170 g were acquired from the Animal Facility of the College of Pharmacy, University of Basrah, Iraq. The rats were randomly assigned to four groups, with each group containing 10 rats. The rats were acclimatized for one week in ventilated plastic cages under controlled conditions (22 ± 4 °C; 30 ± 15 % relative humidity, and 12 hours of light and dark cycles). Water and standard chow were constantly available.

Ethical approval

Approval for this study was granted by the Animal Ethics Committee of the College of Pharmacy, University of Basrah, Basrah, Iraq (approval no. 181 at 3-2-2024). All procedures

adhered to the institutional ethical standards and the guide for the care and use of laboratory animals [11].

Induction of hepatotoxicity and treatment

All rats were deprived of feed and water for 2 hours before drug administration. Paclitaxel (Aqvada, Hamburg, Germany) was administered at a dose of 2 mg/kg body weight (BW) intraperitoneally on days 1 and 7 to induce hepatotoxicity in all the rats except for those in control group (G1) which received only distilled water orally. Paclitaxel group (G2) received only 2 mg/kg paclitaxel, intraperitoneally on days 1 and 7. The third group (RU + PAC; G3) received PAC (2 mg/kg, intraperitoneally on days 1 and 7) along with rutin (40 mg/kg/day) orally, while the fourth group (NAC + PAC; G4) received NAC (50 mg/kg/day orally) along with intraperitoneal PAC administration.

The rats were anesthetized with chloroform at the end of treatment, specifically on Day 15. Blood (3 mL) was collected by cardiac puncture, collected in gel tubes and centrifuged for ten minutes at 4,000 rpm. Sera obtained was stored at -20 °C prior to biochemical examination.

Evaluation of TNF- α and IL-10 levels

To assess TNF- α and IL-10 levels, a sandwich enzyme-linked immunosorbent assay (ELISA) was employed. Rat-specific commercial kits (Elabscience Biotechnology Inc., China) were utilized. The measurements were completed in three hours, and each test was done following the guidelines provided by the manufacturer.

Following blood collection, the liver was excised and cleaned with 0.9 % saline, and then stored in 10 % neutral-buffered formalin. Paraffin embedding, and subsequent sectioning to 5 μ m thickness was carried out and the tissues were subsequently stained with hematoxylin and eosin (H&E).

Statistical analysis

Data were imported into an Excel spreadsheet running Microsoft Windows 2010 and then analysis was performed using the Statistical Analysis System (SAS) and the Statistical Package for the Social Sciences (SPSS). The results are displayed as mean $\pm$ standard deviation (SD). One-way analysis of variance (ANOVA) was used to evaluate group differences with a $p < 0.05$ considered significant.

RESULTS

The effect of treatment on body and liver weights in all groups is reported in Table 1. Liver weight of rats in PAC group (G2) was significantly lower than that of the control group (G1; $p < 0.05$). This outcome was confirmed by the Least Significant Difference (LSD) test at a threshold of 2.65. Liver weights of rats in the Rutin-paclitaxel (RUPAC) group (G3) and N-acetylcysteine-paclitaxel (NAPAC) group (G4) were not significantly different from the control group ($p > 0.05$).

Control group (G1) demonstrated the highest body weight (BW) increase (13.87 %), whereas all treatment groups (G2, G3, and G4) showed markedly lower increments (4.49, 4.64, and 5.79 %, respectively). However, there were no significant differences in BW changes among groups. ($p > 0.05$). These findings suggest that Paclitaxel alone significantly reduced liver weight, while the combination with NAC or rutin mitigated this effect. The BW gain was suppressed in all treatment groups, though not significantly ($p > 0.05$).

The least significant difference (LSD) for liver weight was 2.65, indicating that only the paclitaxel group showed a statistically meaningful deviation from control.

Table 1: Effect of treatment on body and liver weights in all groups

Group	Liver weight (g)	Body weight increment (%)
G1 (Control)	12.36 $\pm$ 0.638	13.87
G2 (PAC)	10.38 $\pm$ 2.606*	4.49
G3 (RUPAC)	12.43 $\pm$ 1.993	4.64
G4 (NAPAC)	11.66 $\pm$ 1.657	5.79
LSD	2.650198	NS

Results are mean $\pm$ standard deviation (SD), * $P < 0.05$ vs control. LSD: Least Significant Difference; NS: not significant

The PAC group (G2) exhibited a significant rise in IL-10 and TNF- α compared to control group (G1: 40.97 $\pm$ 9.66 and 42.03 $\pm$ 13.98 pg/mL, respectively), indicating a pronounced inflammatory response ($p < 0.05$).

The TNF- α and IL-10 levels decreased in RUPAC and NAPAC administration (G3 and G4), with TNF- α level, showing a significant reduction when compared to G2 ($p < 0.05$), suggesting modulatory effects of RU on proinflammatory cytokines. Similarly, the NAC co-treatment group (G4) exhibited further suppression of inflammatory markers, with IL-10 and TNF- α levels reduced to 42.65 $\pm$ 14.27 and 41.94 $\pm$ 14.

76 pg/mL, respectively. Both values were statistically equivalent to control group and much lower than those seen in the PAC group (G2). The least significant difference (LSD) values for IL-10 and TNF- α were 26.73 and 30.55, respectively.

These findings demonstrate that RU and NAC attenuated PAC-induced elevation in inflammatory markers, with NAC showing a more pronounced normalization effect.

Table 2: Effects of treatments on TNF- α and IL-10 cytokine levels in different groups

Group	IL-10 (pg/mL)	TNF- α (pg/mL)
G1 (control)	40.97 $\pm$ 9.66	42.03 $\pm$ 13.98
G2 (PAC)	75.54 $\pm$ 49.35 ^a	82.66 $\pm$ 51.55 ^a
G3 (RUPAC)	50.37 $\pm$ 23.18	41.57 $\pm$ 14.49 ^b
G4 (NAPAC)	42.65 $\pm$ 14.27 ^b	41.94 $\pm$ 14.76 ^b
LSD	26.72547	30.54581

^a*P* < 0.05 vs control group; ^b*P* < 0.05 vs G2 (b)

Histopathological findings

Hepatic tissue appears normal in architecture in the control group (Figure 1). Hepatocytes are arranged in well-organized cords or plates radiating from the central vein. Sinusoidal spaces between the cords are intact and unremarkable. No signs of inflammation, necrosis, fatty changes, or fibrosis are noted. The portal area and central vein show no pathological alterations (Figure 1).

Liver sections from rats administered paclitaxel (G2) revealed significant histopathological alterations. Notable changes observed include dilated central veins, marked sinusoidal congestion, and disruption of the normal hepatic lobular and trabecular architecture. The hepatocytes revealed a pyknotic nucleus (apoptosis or necrosis) and cytoplasmic vacuolization (fatty degeneration). The sinusoidal walls indicated an increase in the number of Kupffer cells. In addition, focal sites of microabscess formation were observed, characterized by necrotic debris and infiltrating inflammatory cells indicative of localized hepatic inflammation.

In contrast, liver tissues from rats treated with rutin in combination with paclitaxel (G3), as well as those that received paclitaxel plus NAC (G4), exhibited relatively intact hepatic histoarchitecture. Typical trabecular hepatic lobules consisting of orderly hepatocytes were seen. There was no remarkable dilation, and the vascular structures were normal. Mild changes were occasionally noted as slight widening of the sinusoids and mild proliferation of Kupffer

cells. Some hepatocytes manifested a slight degree of cytoplasmic vacuolation for mild fatty change. In general, hepatocellular morphology was preserved, with cells showing prominent nuclei, distinct nucleoli, and well-defined nuclear membranes.

Liver tissue in control group, showing normal hepatic architecture with intact hepatocytes arranged more or less radially from the central vein (Red arrow). The G2 (PAC) hepatic cells showed vacuolar and hydropic changes that were predominantly distributed in the periportal area. These alterations were associated with focal necrosis and infiltration of inflammatory cells (blue arrow), as well as nuclear pyknosis and cytoplasmic eosinophilia signs (white arrow). Generally, G3 presented as centro-lobular hazy swelling (arrowhead) with minimal degenerative changes and an observable reduction in hepatic vacuolation.

DISCUSSION

Paclitaxel (PAC) is known for the induction of oxidative stress, inflammation and mitochondrial dysfunction in hepatic tissue, leading to hepatotoxic effects [12]. The present study was designed to evaluate the efficacy of two antioxidants, namely rutin and N-acetylcysteine (NAC), in preventing paclitaxel-induced hepatic damage in rats. The results indicated that both NAC and Rutin were effective in preventing liver damage, but with varying degrees of effectiveness.

N-acetylcysteine enhances liver function by increasing the levels of glutathione which is responsible for removing toxic metabolites and free radicals from the body. Since NAC is a cysteine derivative, it contains or thiol groups that fill up the cellular glutathione tank. It also enhances detoxification within cells and has the capacity to scavenge free radicals directly. There are reports that the lipophilicity of NAC enables it to easily cross the cell membranes, chelate copper ions (a free radical generator), scavenge free radicals, and protect against oxidative damage [13].

Rutin pretreatment attenuated PAC increase in the production of proinflammatory cytokines, such as TNF- α , IL-1 β , and IL-6 in macrophages [12]. Moreover, Rutin has been reported to inhibit a concentration-dependent induction of iNOS and NO release in BPA-GMA [12]. Rutin is employed for the therapy of vascular inflammatory diseases due to its capacity to attenuate TNF- α production and NF- κ B activation. Rutin has been shown to effectively

inhibit the activity of phospholipase A2 (PLA2), a crucial inflammatory enzyme that aids in the formation of arachidonic acid (AA) in human synovial fluid [3].

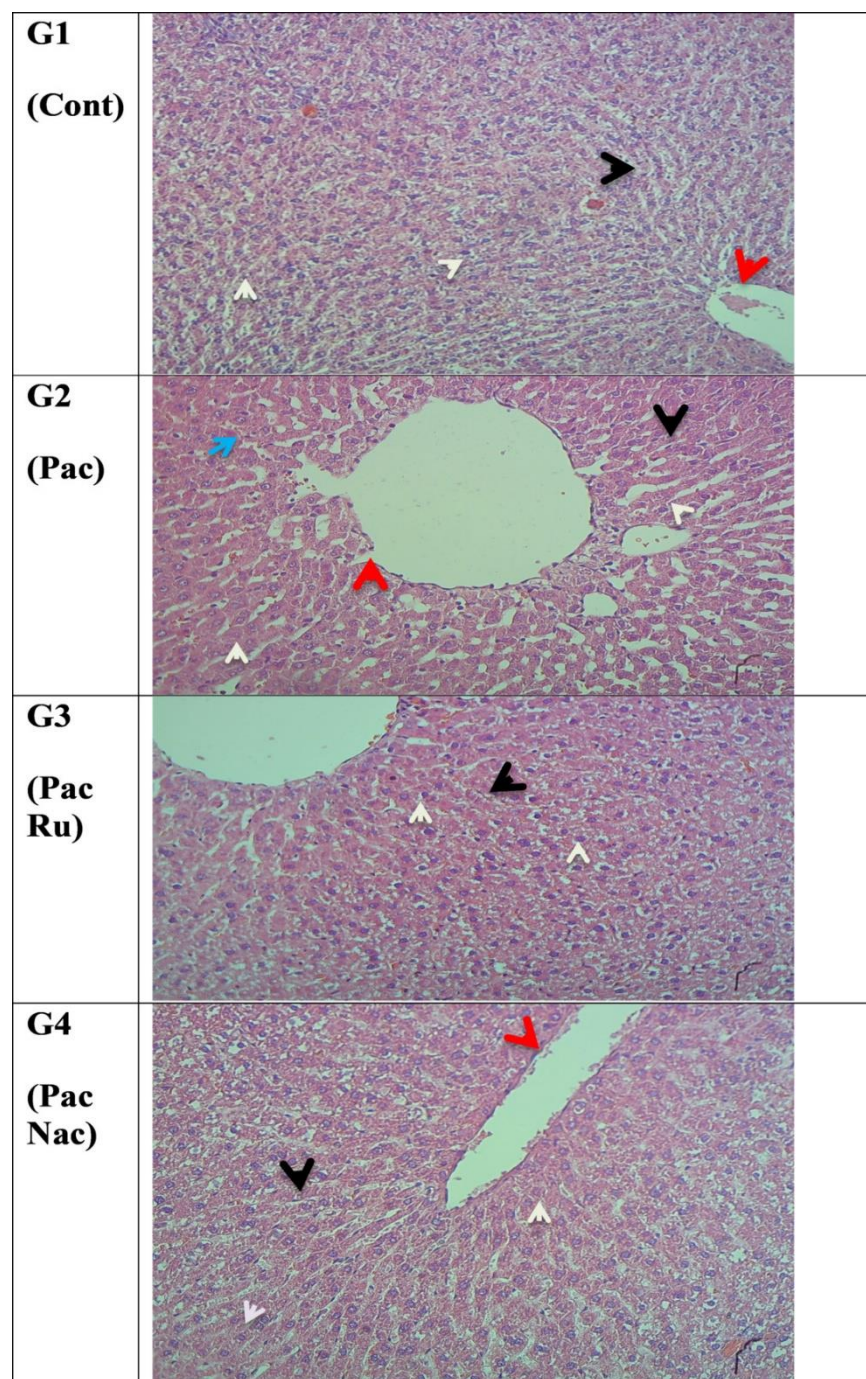


Figure 1: Liver histopathology of the different treatment groups

The anti-inflammatory properties of rutin are amplified by the inhibition of established pro-mediators and other related inflammatory molecules. Oxidative stress and inflammation are two events that increase the chance of tissue failure, eventually damaging the target tissue. Therefore, attenuation of inflammation and reduction of oxidative stress are the main mechanisms to mitigate such toxic effects [4,5]. Choi *et al* [12] showed that Rutin exhibited

slightly stronger anti-inflammatory effect than Rutin – glycoside, because of the different cell viabilities of the two compounds, the effects could not be determined. Nonetheless, the relatively low toxicity of Rutin glycoside justifies its use [13]. *In vitro* studies [12,13] previously reported that Rutin decreased intracellular ROS, nitric oxide levels, and pro-inflammatory cytokines (TNF- α and IL-6) at low dosages without compromising the survival of healthy

cells. This lends credence to the notion that Rutin is a strong anti-inflammatory and antioxidant substance that has a remarkable capacity to block mediators of inflammation and oxidative damage.

Histopathological analysis of liver tissues from the paclitaxel-treated group showed extensive damage characterized by sinusoidal congestion, pyknotic nuclei, cytoplasmic vacuolization, and focal inflammatory infiltrates. These results align with a previous report demonstrating that PAC-induced hepatotoxicity is mediated through increased reactive oxygen species (ROS) generation and the expression of proinflammatory cytokine, particularly TNF- α and IL-1 β , leading to cellular apoptosis and necrosis [14].

Fararh *et al* reported that the antioxidant properties of rutin may be responsible for its hepatoprotective properties which shield the liver from damage [5]. The NAC aids in liver repair through immunomodulatory and anti-inflammatory properties. By stabilizing membranes and preventing liver enzymes from escaping, NAC stops hepatic damage [5]. In the present study, treatment with NAC significantly attenuated histological and biochemical markers of liver injury. This is consistent with an earlier study that shows the hepatoprotective role of NAC via replenishment of intracellular glutathione levels, suppression of lipid peroxidation, and reduction of proinflammatory cytokines [5]. The observed normalization of TNF- α and restoration of anti-inflammatory IL-10 levels in the NAC-treated group further support its potent immunomodulatory action [6].

CONCLUSION

N-Acetylcysteine and Rutin are effective in mitigating paclitaxel-induced hepatotoxicity, with N-Acetylcysteine demonstrating a slightly superior anti-inflammatory and antioxidant profile, while Rutin showed notable structural preservation of hepatic tissue. These results encourage further clinical exploration of N-Acetylcysteine and Rutin as adjunctive therapies in Paclitaxel-based chemotherapy regimens.

DECLARATIONS

Acknowledgement/Funding

None.

Ethical approval

Ethical approval was obtained from the Animal Ethics Committee of the College of Pharmacy, University of Basrah (approval no. 181, dated 3-2-2024).

Use of Artificial intelligence/Large language models

We also declare that we did not use Generative artificial intelligence (AI) and AI-assisted technologies in writing the manuscript.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

We declare that this work was done by the author(s) named in this article, and all liabilities pertaining to claims relating to the content of this article will be borne by the authors.

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