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Phyllanthin identified from *Phyllanthus amarus* attenuates arsenite-induced liver and kidney damage: Role of NF-kB pathway inhibition.

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Keywords: Antioxidant; Hepatotoxicity; Nephrotoxicity; NF-κb; Oxidative stress; *Phyllanthus amarus;* Sodium arsenite.

Abstract. Sodium arsenite is a common and highly toxic inorganic arsenic compound that causes liver and kidney damage. *Phyllanthus amarus* is well known for its protective effects on these organs. This study aimed to identify the active phytoconstituents of the methanolic extract of *P. amarus* (PAME) and to explore their effects on arsenite-induced liver and kidney toxicity in experimental rats. The standardization of *P. amarus* extract was performed using high-performance liquid chromatography (HPLC). Male Wistar rats developed liver and kidney toxicity after daily oral administration of sodium arsenite (5 mg/kg) for 4 weeks. The rats were simultaneously given coenzyme Q10 (CoQ10; 10 mg/kg) or PAME (50, 100, and 200 mg/kg). Results showed that HPLC analysis detected phyllanthin at a retention time of 25.41 minutes with an area of 71.84%. Arsenite treatment caused a significant ($p<0.001$) increase in hepatic enzymes (ALT, AST, and ALP), renal markers (BUN, uric acid, and creatinine), and direct and total bilirubin in the serum. It also significantly increased hepatic and renal levels of malondialdehyde, nitric oxide, NF-κB p65, interleukins (ILs), and TNF-α ($p<0.001$), while decreasing hepatic antioxidant enzymes (GSH and SOD) and overall hepatic antioxidant capacity. Notably, P. amarus extract (200 mg/kg) markedly ($p<0.001$) mitigated arsenite-induced changes in these serum markers, oxidative stress indicators, NF-kB p65, and inflammatory cytokines. It also improved the structure of liver and kidney tissues, maintained cellular architecture, and reduced necrosis and inflammation. In conclusion, these results suggest that phyllanthin from *P. amarus* protects against arsenite-induced liver and kidney damage by inhibiting NF-κB activation, reducing inflammatory cytokine release, and decreasing oxidative and nitrosative stress, thereby enhancing overall antioxidant capacity. Therefore, *P. amarus* extract may be a promising treatment for pesticide-related liver and kidney injuries in rats.

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La filantina identificada en *Phyllanthus amarus* atenúa el daño hepático y renal inducido por arsenito: papel de la inhibición de la vía NF-Kb.

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Palabras clave: Antioxidante; Hepatotoxicidad; Nefrotoxicidad; NF-κb; Estrés oxidativo; *Phyllanthus amarus*; Arsenito de sodio.

Resumen. El arsenito de sodio es un compuesto inorgánico de arsénico, de prevalencia elevada y altamente tóxico, que causa toxicidad hepática y renal. *Phyllanthus amarus* está bien documentado por sus efectos hepatoprotectores y nefroprotectores. Este estudio tuvo como objetivo examinar los fitoconstituyentes activos del extracto metanólico de *P. amarus* (PAME) y sus mecanismos de acción sugeridos contra la toxicidad hepática y renal inducida por el arsenito en ratas experimentales. La estandarización del extracto de P. amarus se realizó mediante cromatografía líquida de alta resolución (HPLC). Ratas Wistar machos desarrollaron toxicidad hepática y renal tras la administración oral continua de arsenito de sodio (5 mg/kg) durante 4 semanas. A las ratas se les administró por vía oral coenzima Q10 (CoQ10; 10 mg/kg) o PAME (50, 100 y 200 mg/kg) de forma concomitante. En los resultados, el análisis de HPLC mostró la presencia de filantina con un tiempo de retención de 25,41 min y un área de 71,84%. La administración de arsenito dio lugar a un aumento significativo ($p<0,001$) de las enzimas hepáticas (ALT-alanina aminotransferasa), AST (aspartato aminotransferasa) y ALP (fosfatasa alcalina), de las enzimas renales (BUN (nitrógeno ureico en sangre), ácido úrico y creatinina) y de la bilirrubina directa y total en el suero. También elevó efectivamente ($p<0,001$) los niveles hepáticos y renales de malondialdehído, óxido nítrico, NF-κB (factor nuclear kappa de la cadena ligera de las células B activadas) p65, IL (interleucinas) y TNF-α (factor de necrosis tumoral alfa), y disminuyó las enzimas antioxidantes GSH (glutatión) y SOD (superóxido dismutasa), así como la capacidad antioxidante total hepática. Sin embargo, el extracto de *P. amarus* (200 mg/kg) atenuó notablemente ($p<0,001$) las alteraciones inducidas por el arsenito en estos marcadores séricos, los parámetros de estrés oxidativo, NF-κB p65 y los niveles de citoquinas inflamatorias. También mejoró la histología hepática y renal, preservó la arquitectura celular y redujo la necrosis e inflamación. En conclusión, estos hallazgos sugieren que la filantina de *P. amarus* ejerce efectos protectores contra la hepatotoxicidad y la nefrotoxicidad inducidas por el arsenito al inhibir la activación de NF-κB y disminuir la liberación de citoquinas inflamatorias y el estrés oxidativo-nitrosativo, mejorando así la capacidad antioxidante general. Por lo tanto, el extracto de *P. amarus* podría constituir un tratamiento eficaz para el daño hepático y renal inducido por pesticidas en ratas.

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INTRODUCTION

Sodium arsenite is a common, highly toxic inorganic arsenic compound. Environmental exposure to arsenic through drinking arsenic-contaminated groundwater can cause several health hazards, particularly affecting the liver and kidneys [1]. In more than 70 countries, approximately 140 million people drink water with arsenic concentrations exceeding the WHO provisional limit of 10 μg/L [2]. According to models, approximately 94–220 million people are at risk of exposure to high arsenic levels in groundwater[3]. When exposed, sodium arsenite induces inflammation and oxidative stress, primarily in the kidneys and liver, thereby disrupting homeostasis.

Disruption of this balance, along with the reactive nitrogen species (RNS) and reactive oxygen species (ROS)-mediated induction of oxidative stress, triggers hepatocellular injury [4]. Additionally, arsenite-induced hepatotoxicity and nephrotoxicity are characterized by mitochondrial damage and ATP depletion, which in turn lead to oxidative stress. This is evidenced by increased oxidative markers, such as lipid peroxidation (malondialdehyde, MDA) and nitrite/nitrate levels, as well as decreased antioxidant defenses, including glutathione (GSH), catalase, and superoxide dismutase (SOD) [5]. Sodium arsenite enhances pro-inflammatory signaling pathways and induces apoptosis in renal and hepatic tissues by upregulating proteins such as caspase-3 and tumor necrosis factor-alpha (TNF-α) [5,6]. Consequently, researchers aim to strengthen the antioxidant defense system to reduce the production of free radicals induced by arsenite toxicity.

Current therapeutic strategies for arsenite-induced toxicity involve chelation therapy and antioxidant treatment. Chelation therapy is a standard method, with 2,3-dimercaptosuccinic acid (DMSA) being the most commonly used chelating agent, which helps bind and remove arsenic from the bloodstream. However, DMSA alone is not enough to eliminate arsenic from intracellular compartments, leading to toxicity and cell damage. As a result, combined therapies are being investigated to improve their effectiveness [7]. A new approach is to add antioxidants alongside chelators. Coenzyme Q10 (CoQ10), which has antioxidant properties, protects against arsenic-induced intracellular damage and, when used with DMSA, offers enhanced protection against arsenite toxicity [7]. Dual therapy not only promotes the removal of arsenic from the extracellular space but also protects against intracellular arsenic toxicity, demonstrating broader pharmacological benefits in arsenic poisoning. However, these treatments are very costly, necessitating affordable alternatives. Plant-derived medicinal compounds present a promising, low-cost option for addressing arsenite-induced toxicity.

To counteract sodium arsenite toxicity, many studies have examined the protective effects of both natural and synthetic molecules. For example, naringin, hesperidin, and lipoic acid have been shown to reduce arsenic toxicity by restoring biochemical parameters, decreasing oxidative stress, and inhibiting inflammatory and apoptotic cascades[8,9]. These findings support the potential of such molecules to lessen sodium arsenite-induced hepatic and renal damage through their antioxidant and anti-inflammatory properties. *Phyllanthus amarus* Schum. and Thonn., also known as Bhuia amla, is a medicinal herb of great importance in the scientific field of Ayurvedic medicine [10]. It has been traditionally used for over 2000 years to treat secondary hepatitis and various liver injuries [10]. It has numerous traditional uses and is commonly employed to treat conditions such as jaundice, gonorrhea, heavy menstruation, and diabetes [11]. Qualitative analyses of the phytochemical composition of *P. amarus* have identified a wide range of compounds, including lignans (phyllanthin and hypophyllanthin), alkaloids, and bioflavonoids (e.g., quercetin). While it remains to be confirmed which of these possesses anti-

oxidant properties, scientific reports indicate that the herb exerts maximal effects on the liver and kidneys [10,12-14]. Such liver specificity is rooted in its traditional use for jaundice, as evidenced by the report by Santos et al. [15]. Studies have demonstrated the antioxidant effect of the ethanolic extract of *P. amarus*, indicating its protective role in experimental models of kidney and liver damage [11-14]. However, the exact mechanisms underlying the hepatoprotective and nephroprotective effects of these compounds against arsenite-mediated hepatic and renal toxicities still remain unknown. Therefore, this study aimed to investigate the biochemical mechanisms and phytoconstituents responsible for the hepatoprotective effects of *P. amarus* in an experimental model of arsenite-induced liver and kidney damage.

MATERIALS AND METHODS

P. amarus methanolic extract - preparation and identification

Air-dried powder from *P. amarus* aerial parts underwent maceration at ambient temperature using methanol (distilled). This process involved soaking and occasional agitation for 7 days, followed by filtration. The filtrate was dried in a tray dryer at 40°C, yielding a semi-solid *P. amarus* methanolic extract (PAME). Subsequently, colloidal silicon dioxide was incorporated, and the mixture was dried in a vacuum tube. Phytochemical analysis of PAME was conducted using high-performance liquid chromatography (HPLC) to quantify phyllanthin content. Analyses were conducted using an HPLC system (reverse-phase C_{18} column, 250 × 4.6 mm, flow rate 1.5 mL/min). For isolation and detection, a mobile phase comprising acetonitrile and buffer in a 40:60 volume ratio was employed. The buffer was prepared by dissolving potassium hydrogen phosphate (0.136 g) in o-phosphoric acid (0.5 mL). The optimal injection volume was 20 μL, and the detector wavelength was set to 230 nm. The autosampler temperature was maintained at 10°C, and the system operated at 1000 psi [16].

Animals

White male Wistar rats aged 8–10 weeks were obtained from the animal facility at Shandong First Medical University. The rats were kept in an environment with controlled temperature (24 ± 1°C), humidity (45-55%), and a normal light-dark cycle. During the study, the rats had free access to standard pellet feed and water. The Zhinanzhen Biology Ethics Committee approved the research protocol (approval number: A2024000414).

Experimental design

The rats were divided into six groups of 15 animals each and received the following treatments: Group 1: gum acacia (1% suspension, 10 mg/kg; Normal group), Group 2: gum acacia (1% suspension, 10 mg/kg) + Sodium arsenite (5 mg/kg) (vehicle control group), Group 3: Coenzyme Q10 (10 mg/kg, 1% suspension in gum acacia) + sodium arsenite (5 mg/kg) (CoQ10-treated group), and Groups 4 to 6: standardized extract of P. amarus (50 mg/kg, 100 mg/kg, or 200 mg/kg, 1% suspension in gum acacia) + sodium arsenite (5 mg/kg) (PA-treated groups), all administered orally for 28 days.

On the final day of the experiment (day 28), blood samples were collected from anesthetized rats via retro-orbital puncture, stored in glass tubes, and centrifuged for 10 minutes at 2,000 × g at 4°C. Serum levels of albumin, ALT (alanine transaminase), AST (aspartate transaminase), ALP (alkaline phosphatase), bilirubin (direct and total), BUN (Blood Urea Nitrogen), cholesterol, creatinine, LDL (Low-Density Lipoprotein), HDL (High-Density Lipoprotein), LDH (Lactate Dehydrogenase), triglycerides, and uric acid were measured using reagent kits according to the provided procedure (Accurex Biomedical Pvt. Ltd., Mumbai, India). The animals were then euthanized, and the

liver and kidneys were quickly removed and weighed with a balance at temperatures below 4°C. The tissues were divided into three sections and stored at -80°C. One section was used to assess oxidative and nitrosative stress markers (MDA (malondialdehyde), GSH (reduced glutathione), NO (nitric oxide), SOD (superoxide dismutase) activity) and total antioxidant capacity (TAC) following previously reported methods [16-22]. Another portion was analyzed to determine the concentrations of pro-inflammatory cytokines (IL-6, IL-1β, and TNF-α) and NF-κB p65 using a commercially available ELISA kit (Thermo Fisher Scientific, USA). The remaining tissue was examined histologically using hematoxylin and eosin (H&E) staining. Changes observed in the histological characteristics were classified according to a previously established grading system [23].

Statistical analysis

GraphPad Prism software (version 5.0; GraphPad, San Diego, USA) was used for statistical analysis. One-way analysis of variance (ANOVA) followed by Dunnett's post hoc test was performed. A two-sided Fisher's exact test was used to calculate the correlation coefficients. The results are presented as mean ± SEM, with statistical significance set at $p<0.05$.

RESULTS

Phyllanthin - Isolation and identification

PAME had a 59.12% w/w yield and contained glycosides, lignans, steroids, tannins, and phenols. HPLC column analysis lasted 40 minutes, during which phyllanthin was detected at 25.41 minutes, with a peak area of 71.84% (Fig. 1).

Body, liver, kidney, and spleen weights

Body weight was effectively decreased ($p<0.001$), while a significant ($p<0.001$) increase in spleen, kidney, and liver weights (both absolute and relative) was observed in vehicle control rats compared to normal rats. Rats treated with PA (200 mg/kg) showed a significant ($p<0.001$) reduction in the elevated weights of the spleen, kidney, and liver, along with a marked ($p<0.001$) increase in body weight compared to vehicle control rats. However, PA treatment at doses of 50 and 100 mg/kg did not produce any notable changes in the absolute or relative weights of the liver, spleen, and kidneys, nor in body weight, compared to the vehicle control group. CoQ (10 mg/kg) treatment effectively decreased spleen, kidney, and liver weights ($p<0.001$) and increased body weight ($p<0.001$) compared to the vehicle control group (Table 1).

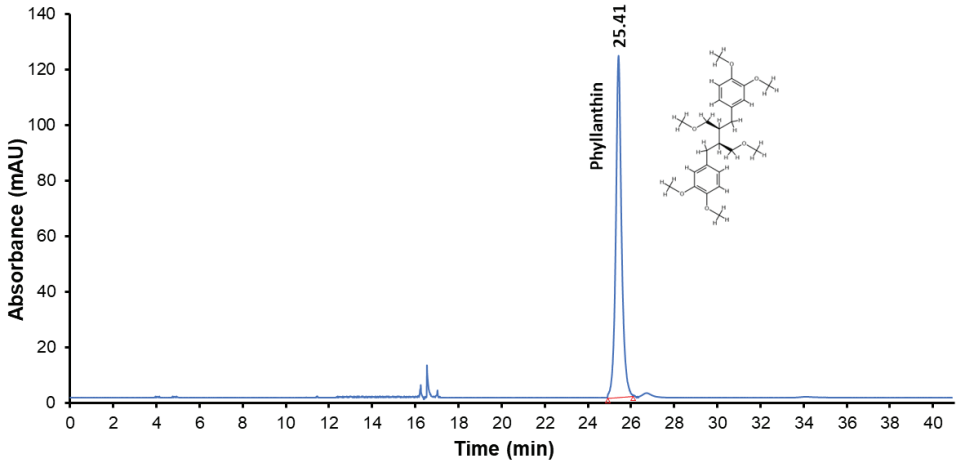

Fig. 1. HPLC chromatogram of the standardized *P. amarus* extract showing a phyllanthin peak at RT = 25.41 min. mAU, milli-absorbance units.

Table 1. Effect of *P. amarus* on body weight and organ weights.

Treatment	Normal	Vehicle control	CoQ (10)	PA (50)	PA (100)	PA (200)
Body weight (g)	239.70 ± 3.81	213.20 ± 3.47$^{###}$	234.70 ± 4.17***	211.70 ± 2.94	218.00 ± 1.39	223.50 ± 2.35***
Liver weight (g)	5.48 ± 0.22	7.34 ± 0.29$^{###}$	5.52 ± 0.33***	7.23 ± 0.30	7.31 ± 0.15	5.73 ± 0.29***
Liver weight / Body weight	22.90 ± 1.11	34.47 ± 1.54$^{###}$	23.65 ± 1.79***	34.20 ± 1.57	33.51 ± 0.60	25.69 ± 1.43***
Kidney weight (g)	1.20 ± 0.01	1.81 ± 0.01$^{###}$	1.29 ± 0.01***	1.77 ± 0.01	1.79 ± 0.01	1.30 ± 0.01***
Kidney weight / Body weight	5.01 ± 0.08	8.49 ± 0.16$^{###}$	5.50 ± 0.14***	8.39 ± 0.13	8.21 ± 0.06	5.81 ± 0.09***
Spleen weight (g)	0.19 ± 0.02	0.75 ± 0.02$^{###}$	0.36 ± 0.02***	0.74 ± 0.03	0.73 ± 0.02	0.52 ± 0.03**
Spleen weight / Body weight ($x10^{-3}$)	0.79 ± 0.08	3.53 ± 0.12$^{###}$	1.54 ± 0.11***	3.52 ± 0.19	3.33 ± 0.07	2.31 ± 0.15**

The results are presented as mean ± SEM, based on a sample size of 6. A one-way analysis of variance (ANOVA) was used for statistical analysis, and Dunnett's test was applied to each parameter individually. **p<0.01, ***p<0.001: vehicle control group, and $^{###}$p<0.001: normal group. CoQ (10), Coenzyme Q (10 mg/kg); PA, *Phyllanthus amarus*.

Serum parameters

Compared to the normal group, serum levels of BUN, uric acid, creatinine, direct and total bilirubin, LDH, ALP, AST, and ALT were significantly (p<0.001) elevated, while albumin levels were notably (p<0.001) decreased in the vehicle control group. These changes suggest substantial hepatic and renal injury caused by sodium arsenite, as these parameters are closely linked to liver and kidney functions. Treatment with PA (200 mg/kg) led to significant improvements, evidenced by a marked (p<0.001) reduction in serum BUN, ALT, AST, creatinine, uric acid, direct bilirubin, total bilirubin, LDH, and ALP levels, along with a significant (p<0.001) increase in albumin compared to the vehicle control group. Additionally, CoQ (10 mg/kg) significantly (p<0.001) inhibited arsenite-induced changes in serum creatinine, BUN, uric acid, albumin, bilirubin (direct and total), LDH, AST, ALT, and ALP levels compared to the control (Tabla 2).

Lipid profile

Following chronic sodium arsenite administration, a significant reduction (p<0.001) in serum HDL and a notable (p<0.001) increase in serum cholesterol, LDL, and triglyceride levels were observed in vehicle control rats compared to normal rats. These decreases in serum HDL and the elevations in cholesterol, LDL, and triglyceride levels were clearly (p<0.001) reduced with PA (200 mg/kg) treatment. Similarly, CoQ (10 mg/kg) administration resulted in significant improvements, increasing (p<0.001) HDL levels and decreasing (p<0.001) LDL, cholesterol, and triglyceride levels in the serum compared to vehicle control rats. However, no significant changes in serum cholesterol, HDL, LDL, or triglyceride levels were seen following PA (50 and 100 mg/kg) treatments (Table 3).

Hepatic and renal antioxidant parameters

Compared with normal rats, sodium arsenite administration significantly reduced hepatic total antioxidant capacity (TAC) in the vehicle control group. However, PA (200 mg/kg) effectively increased hepatic TAC (p<0.001) compared to the vehicle control group. Notably, CoQ (10 mg/kg) also significantly increased hepatic TAC (p<0.01) relative to the vehicle control group (Fig. 2A). Compared to the normal group, sodium arsenite markedly affected hepatic and renal antioxidant levels, as indicated by a significant (p<0.001) decrease in hepatic and renal GSH and SOD levels, followed by

a substantial ($p<0.001$) increase in nitric oxide and MDA levels in the hepatic and renal tissues of the vehicle control group. PA (200 mg/kg) effectively ($p<0.001$) restored GSH and SOD levels and significantly lowered MDA ($p<0.001$) and nitric oxide levels in hepatic and renal tissues compared to the vehicle control group. CoQ (10 mg/kg) administration also demonstrated strong hepatoprotective and nephroprotective effects by effectively increasing ($p<0.001$) hepatic and renal GSH and SOD levels and markedly reducing ($p<0.001$) hepatic and renal nitric oxide and MDA levels relative to the vehicle control group (Fig. 2B-2E).

Table 2. Effect of *P. amarus* on serum hepatic and renal biomarker levels.

Treatment	Normal	Vehicle control	CoQ (10)	PA (50)	PA (100)	PA (200)
BUN (mg/dL)	25.85 ± 1.47	50.72 ± 1.29$^{###}$	31.48 ± 1.26***	47.85 ± 0.89	46.91 ± 1.38	34.38 ± 1.26***
Creatinine (mg/dL)	0.63 ± 0.06	2.07 ± 0.11$^{###}$	0.92 ± 0.07***	2.12 ± 0.13	2.08 ± 0.12	1.57 ± 0.09***
Uric acid (mg/dL)	1.90 ± 0.12	4.41 ± 0.12$^{###}$	2.50 ± 0.06***	4.36 ± 0.11	4.40 ± 0.14	3.26 ± 0.13***
Albumin (mg %)	6.75 ± 0.56	2.39 ± 0.51$^{###}$	6.04 ± 0.41***	2.43 ± 0.50	2.61 ± 0.43	4.46 ± 0.40***
Direct bilirubin (mg %)	0.20 ± 0.01	0.67 ± 0.01$^{###}$	0.29 ± 0.02***	0.66 ± 0.02	0.62 ± 0.01	0.42 ± 0.01***
Total bilirubin (mg %)	0.12 ± 0.01	0.31 ± 0.02$^{###}$	0.16 ± 0.01***	0.31 ± 0.02	0.31 ± 0.01	0.23 ± 0.01***
ALP (IU/L)	50.47 ± 3.77	392.80 ± 3.81$^{###}$	94.35 ± 3.69***	383.80 ± 6.32	367.80 ± 6.12	257.90 ± 3.71***
AST (IU/L)	63.92 ± 13.33	284.30 ± 14.17$^{###}$	102.00 ± 11.65***	289.50 ± 12.34	264.50 ± 10.44	144.00 ± 11.42***
ALT (IU/L)	28.32 ± 7.65	146.50 ± 6.27$^{###}$	42.75 ± 10.28***	145.00 ± 9.97	140.00 ± 6.73	63.36 ± 8.47***
LDH (mg %)	482.00 ± 119.90	3418.00 ± 212.80$^{###}$	682.30 ± 152.20***	3399.00 ± 405.50	3239.00 ± 406.40	1456.00 ± 131.40***

The results are presented as mean ± SEM, based on a sample size of 6. A one-way analysis of variance (ANOVA) was performed for statistical analysis, followed by Dunnett's test applied individually to each parameter. ***$p<0.001$: vehicle control group and $^{###}p<0.001$: normal group. AST, Aspartate transaminase; ALP, Alkaline phosphatase; ALT, alanine transaminase; BUN, Blood Urea Nitrogen; CoQ (10), Coenzyme Q (10 mg/kg); LDH, Lactate Dehydrogenase; PA, *Phyllanthus amarus*.

Table 3. Effect of *P. amarus* on serum lipid profile.

Treatment	Normal	Vehicle control	CoQ (10)	PA (50)	PA (100)	PA (200)
Cholesterol (mg %)	18.71 ± 3.14	65.06 ± 8.72$^{###}$	35.70 ± 2.42***	61.54 ± 4.62	60.73 ± 5.07	29.74 ± 4.02***
HDL (mg %)	60.85 ± 1.09	22.26 ± 3.42$^{###}$	56.31 ± 3.68***	23.99 ± 2.07	21.94 ± 4.74	56.53 ± 3.15***
LDL (mg %)	2.10 ± 0.35	5.02 ± 0.51$^{###}$	2.12 ± 0.45***	5.14 ± 0.47	5.07 ± 0.47	2.34 ± 0.35***
Triglyceride (mg %)	60.33 ± 14.89	185.30 ± 19.41$^{###}$	72.25 ± 9.03***	167.30 ± 17.33	156.90 ± 13.88	122.4 ± 10.23***

The results are presented as mean ± SEM, based on a sample size of 6. A one-way analysis of variance (ANOVA) was conducted for statistical analysis, and Dunnett's test was subsequently applied to each parameter individually. ***$p<0.001$: vehicle control group and $^{###}p<0.001$: normal group. CoQ (10), Coenzyme Q (10 mg/kg); HDL, high-density lipoprotein; LDL, low-density lipoprotein; PA, *Phyllanthus amarus*.

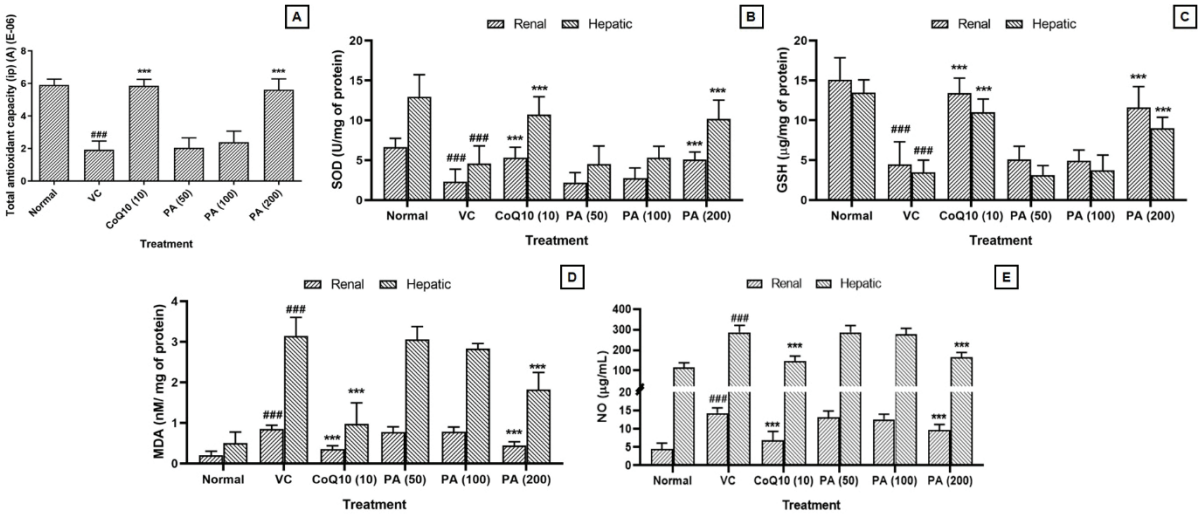

Fig. 2. *P. amarus* effects on hepatic and renal oxidative stress. A quantitative chart of the total antioxidant capacity of the liver (A). Quantitative measurements of SOD (B), GSH (C), MDA (D), and nitric oxide (E) levels in hepatic and renal tissues. Results are presented as mean values with SEM, based on a sample size of 6. One-way ANOVA was used for statistical analysis, with Dunnett's test applied to each parameter separately. ***p<0.001: vehicle control group and ###p<0.001: normal group. CoQ (10), Coenzyme Q (10 mg/kg); GSH, glutathione peroxidase; NO, nitric oxide; MDA, malondialdehyde; PA, Phyllanthus amarus; SOD, superoxide dismutase.

Protein expressions of hepatic and renal ILs, TNF-α, and NF-kB p65

Compared to the normal group, hepatic and renal ILs (IL-6 and IL-1β), TNF-α, and NF-kB p65 protein expression were significantly increased ($p<0.001$) in the vehicle control group. Administration of PA (200 mg/kg) effectively reduced hepatic and renal ILs, TNF-α, and NF-kB p65 protein levels ($p<0.001$) compared with the vehicle control group. Treatment with CoQ (10 mg/kg) demonstrated a substantial ($p<0.001$) protective effect against arsenite-induced liver and kidney damage, as shown by decreased hepatic and renal ILs, TNF-α, and NF-kB p65 protein expression compared to the vehicle control group (Table 4).

There was a strong, positive, and statistically significant correlation between serum ALT levels and hepatic TNF-α ($R2=0.737$ and $p<0.05$), IL-1β ($R2=0.8578$ and $p<0.01$), IL-6 ($R2=0.7493$ and $p<0.05$), and NF-κB p65 ($R2=0.8692$ and $p<0.01$) (Fig. 3A-3D). Serum levels of BUN were positively and significantly correlated with renal TNF-α ($R2=0.7693$ and $p<0.05$), IL-1β ($R2=0.7315$ and $p<0.05$), IL-6 ($R2=0.8323$ and $p<0.01$), and NF-κB p65 ($R2=0.8927$ and $p<0.01$) (Fig. 3E-3H).

Hepatic histopathology

The liver sections from the normal group showed well-preserved architectural features, including hepatocytes arranged in orderly cords radiating from the central vein, with uniform cellular dimensions and transparent cytoplasm. The nuclei were centrally positioned and exhibited normal morphology. No necrosis was observed; however, mild inflammation was noted (Fig. 4A). The histology of hepatic tissue from the vehicle control group showed histopathological changes, including significant ($p<0.001$) necrosis, distorted hepatocyte arrangement, and infiltration of inflammatory cells (Fig. 4B). Histological examination of liver sections from rats treated with CoQ (10 mg/kg) revealed a notable ($p<0.001$) improvement in morphology compared to the vehicle control group. Hepatocytes mostly maintained their normal structure, with mild necrosis

Table 4. Effect of *P. amarus* treatment in hepatic and renal Interleukins and NFkB-P65 levels.

Treatment	Normal	Vehicle control	CoQ (10)	PA (50)	PA (100)	PA (200)
Hepatic TNF-α (pg/mg)	11.23 ± 1.57	56.96 ± 2.54###	22.17 ± 3.18***	52.83 ± 4.32	51.09 ± 4.76	26.16 ± 4.50***
Hepatic IL-1β (pg/mg)	8.61 ± 0.88	111.50 ± 2.67###	24.17 ± 2.71***	109.00 ± 2.80	98.19 ± 5.61	55.69 ± 4.80***
Hepatic IL-6 (pg/mg)	29.19 ± 5.06	169.30 ± 8.19###	81.67 ± 7.37***	152.20 ± 6.82	152.20 ± 10.47	100.60 ± 9.31***
Hepatic NF-kB p65 (pg/mg)	141.50 ± 14.43	653.30 ± 7.00###	168.10 ± 8.93***	646.20 ± 13.25	559.30 ± 20.79	274.60 ± 16.08***
Renal TNF-α (pg/mg)	3.12 ± 0.67	14.13 ± 1.23###	4.42 ± 0.54***	12.46 ± 0.76	11.88 ± 1.00	5.58 ± 0.85***
Renal IL-1β (pg/mg)	11.39 ± 2.17	79.58 ± 5.70###	37.92 ± 4.17***	74.58 ± 4.82	71.94 ± 5.08	48.33 ± 3.71***
Renal IL-6 (pg/mg)	9.09 ± 1.56	97.02 ± 6.43###	22.78 ± 3.20***	94.39 ± 5.91	94.90 ± 1.97	24.70 ± 6.04***
Renal NF-kB p65 (pg/mg)	78.84 ± 1.69	214.70 ± 2.14###	98.41 ± 2.86***	213.10 ± 2.79	203.10 ± 2.48	119.90 ± 2.29***

The results are expressed as mean ± SEM, based on a sample size of 6. A one-way analysis of variance (ANOVA) was used for statistical analysis, and Dunnett's test was subsequently applied to each parameter individually. ***$p<0.001$: vehicle control group and ###$p<0.001$: normal group. CoQ (10), Coenzyme Q (10 mg/kg); ILs, Interleukins; NF-κB, nuclear factor kappa B; PA, *Phyllanthus amarus;* TNF-α, tumor necrosis factor-alpha.

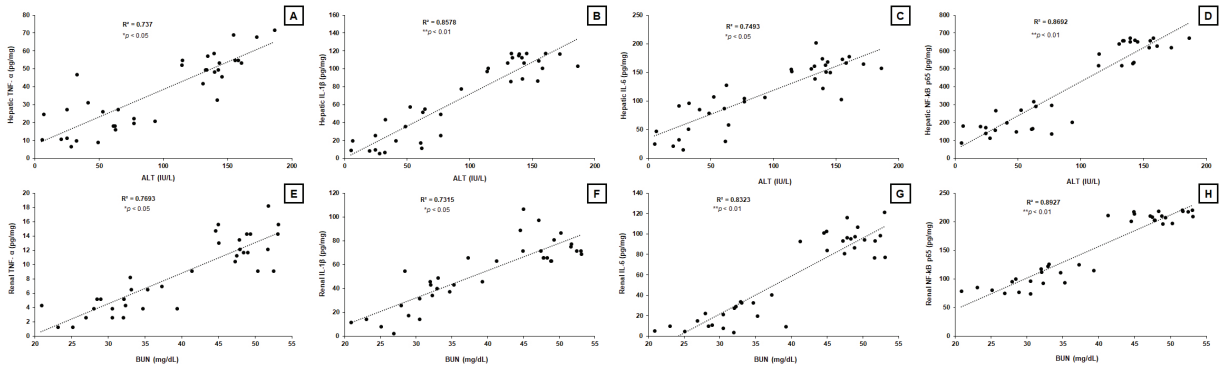

Fig. 3. Simple regression analysis of hepatic TNF-α (A), IL-1β (B), IL-6 (C), and NF-kB p65 (D) with ALT levels, and renal TNF-α (E), IL-1β (F), IL-6 (G), and NF-kB p65 (H) with BUN levels. A two-sided Fisher's test was used to calculate the correlation coefficients. ALT, alanine transaminase; BUN, blood urea nitrogen; ILs, interleukins; NF-κB, nuclear factor kappa B; TNF-α, tumor necrosis factor-alpha.

and inflammation, and their cytoplasm was less eosinophilic than in the vehicle control group (Fig. 4C). Histological analysis of liver tissue from the PA (50 and 100 mg/kg)-treated groups showed infiltration of inflammatory cells, vacuolation, and interstitial edema (Fig. 4D and 4E). Liver sections from the PA (200 mg/kg)-treated group showed mainly preserved hepatocytes, as evidenced by well-maintained cellular architecture and minimal necrotic changes. The cytoplasm showed reduced vacuolation, and inflammatory cell infiltration was significantly decreased ($p<0.001$) compared to the vehicle control group (Fig. 4F, Fig. 4M).

Renal histopathology

The renal sections of the normal group showed well-preserved kidney architecture. The glomeruli and renal tubules were clearly

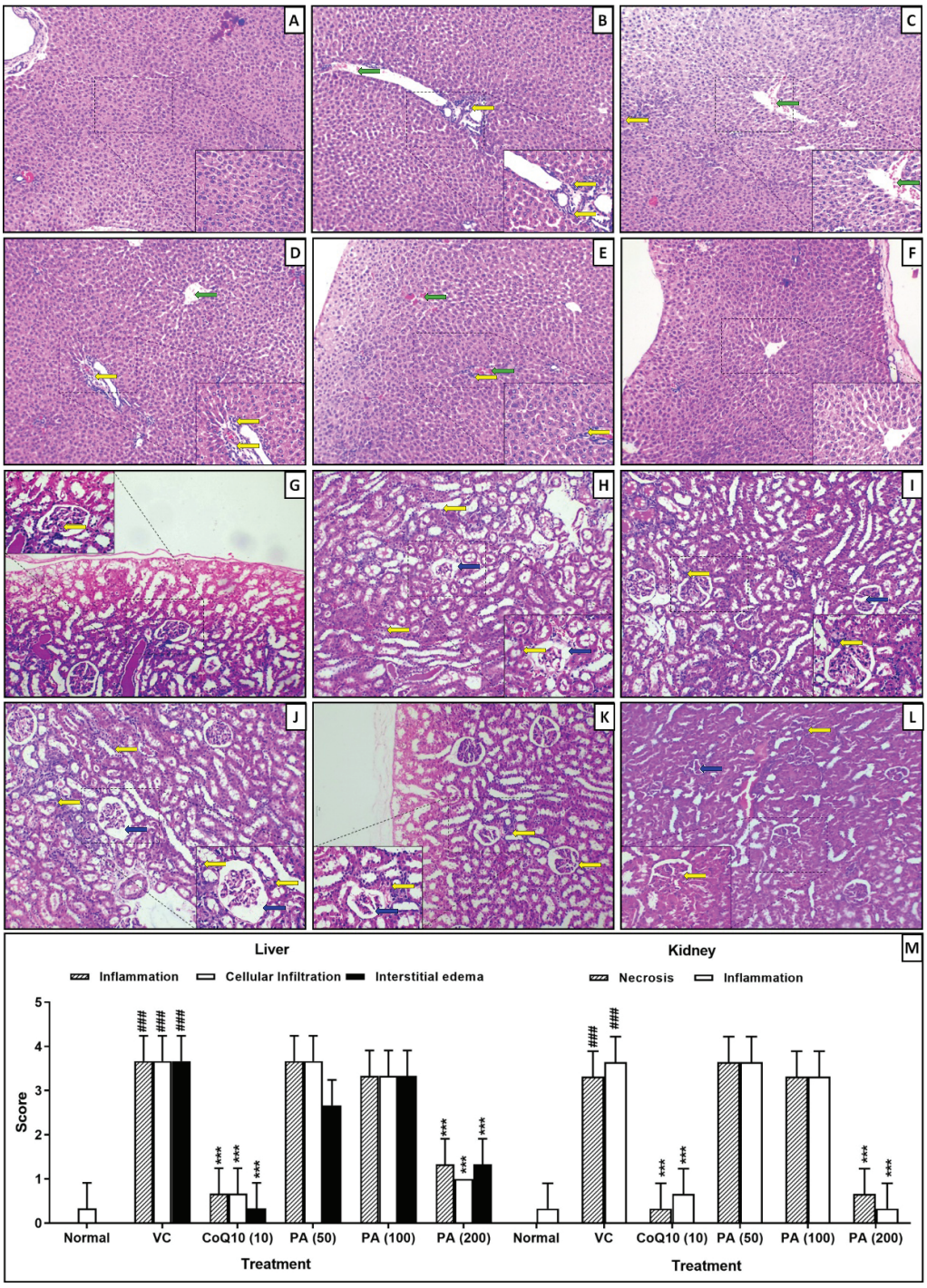

Fig. 4. *P. amarus* on rat hepatic and renal pathology.

Microscopic images of liver (A-F) and kidney (G-L) cross-sections from various rat groups, including normal (A and G), vehicle control (B and H), CoQ (10) treatment (C and I), PA treatment (50 mg/kg) (D and J), PA treatment (100 mg/kg) (E and K), and PA treatment (200 mg/kg) (F and L). H&E staining at 40X and 100X (inset). Quantitative data showing the effect of *P. amarus* treatment on rat hepatic and renal pathologies (M). The results are expressed as mean ± SEM, based on a sample size of 6. One-way ANOVA was used for statistical analysis, followed by Dunnett's test for each parameter. ***p<0.001: vehicle control group; ###p<0.001: normal group. CoQ (10), Coenzyme Q (10 mg/kg); PA, *Phyllanthus amarus*. Inflammatory infiltration (yellow arrow), cellular infiltration (green arrow), and necrosis (blue arrow) are indicated.

defined. No signs of inflammation, necrosis, or other cellular damage were observed (Fig. 4G). In contrast, renal tissue from the vehicle control group exhibited severe kidney damage, with prominent ($p<0.001$) tubular necrosis and infiltration of inflammatory cells (Fig. 4H). Kidney tissue from the CoQ10 (10) treated group demonstrated a significantly preserved renal architecture compared to the vehicle control group ($p<0.001$). Although mild inflammation was present, there was a notable reduction in tubular necrosis and overall structural damage (Fig. 4I). Groups treated with PA (50 and 100 mg/kg) showed extensive renal tissue damage similar to that in the vehicle control group, with clear evidence of inflammation and tubular necrosis (Fig. 4J and 4K). Treatment with PA (200 mg/kg) showed a marked ($p<0.001$) protective effect, comparable to the vehicle control, with kidney architecture largely preserved and only minimal signs of inflammation and reduced tubular damage (Fig. 4L and 4M).

DISCUSSION

Sodium arsenite causes significant toxicity in various biological systems, with effects ranging from genetic damage to organ harm. Studies have shown that the liver and kidneys are especially susceptible to arsenic-related toxicity because of their roles in detoxification and excretion [24,25]. Sodium arsenite triggers notable oxidative stress in both liver and kidney tissues, leading to apoptosis and inflammatory responses that impair their functions. As research advances, the use of antioxidants and other protective agents, such as hesperidin, lycopene, and bosentan, has demonstrated potential in reducing these toxic effects by lowering oxidative stress and inflammation and boosting cellular antioxidant defenses [9,26]. The current study examined the possible mechanisms by which *P. amarus* methanolic extract may protect against arsenite-induced liver and kidney damage in rats.

Chronic administration of sodium arsenite causes acute liver failure and hepatotoxicity, leading to fatal outcomes [25]. Researchers have noted that significant increases in AST, ALT, and ALP levels during arsenite- induced hepatotoxicity serve as indicators of liver function, along with histological changes [25]. Sodium arsenite is absorbed from the gut and detoxified through oxidative methylation in the liver. This process, driven by hepatic enzymes, converts inorganic arsenic into organic forms like monomethylarsonic acid and dimethylarsinic acid [27]. Paradoxically, this detoxification pathway depletes S- Adenosyl methionine and produces trivalent methylated metabolites that are more toxic than the original compound, sodium arsenite, causing hepatocellular damage[25]. Elevated ALT levels are key indicators of the severity of hepatocellular damage. Similar to ALT, AST also increases markedly in arsenite-induced liver injury; however, AST is less specific to the liver. In severe cases, AST levels can match or surpass ALT levels, especially in the later stages of liver necrosis. Therefore, increased ALT and AST levels, along with histological abnormalities during chronic sodium arsenite exposure, confirm its hepatotoxic effects [25]. Additionally, long-term exposure to sodium arsenite results in significant changes in serum biomarkers, such as BUN, uric acid, and creatinine, indicating renal dysfunction [28]. In this study, elevated levels of ALT, AST, ALP, uric acid, BUN, and creatinine were observed following arsenite administration. These increases in serum markers correlate with histological damage in the liver and kidneys, reflecting the hepatotoxic and nephrotoxic effects of sodium arsenite. Further histological analysis supported the protective potential of arsenite against arsenite-induced structural damage in hepatocytes, including irregular and indistinct central veins, cellular damage, tubular necrosis, and increased inflammatory cells, which were alleviated after treatment with *P. amarus*. Previous studies have also documented the hepatoprotective

and nephroprotective effects of *P. amarus* through the inhibition of carbon tetrachloride-induced elevation of hepatic biomarkers and high-salt diet-induced increases in kidney function markers [13,29]. The findings of this study reinforce those from earlier research [13, 29].

Reactive oxygen species (ROS), cytokines, chemokines, and hepatic macrophages are key contributors to liver and kidney damage [30]. The family of transcription factors called nuclear factor-κB (NF-κB) is evolutionarily conserved and remains inactive in the cytoplasm of various cell types. When activated, NF-κB translocates to the nucleus, where it plays a critical role in inflammatory processes, immune responses, and programmed cell death. ROS- induced inflammation is crucial for arsenite- related liver and kidney injury. Excessive production of free radicals triggers NF-κB activation at the inflammation site, leading to the expression of pro-inflammatory genes, including TNF-α and interleukins, ultimately raising cytokine levels. During the acute-phase response, pro-inflammatory cytokines are vital[31,32]. Elevated levels of TNF-α and IL-1β in the liver and kidneys serve as important indicators of hepatic and renal damage in rats [33]. Therefore, the transcriptional regulation of certain inducible inflammatory mediators is significantly affected by NF- κB [34]. Afolabi et al. reported that intestinal ischemia-reperfusion injury caused a significant increase in intestinal and hepatic IL-1β and TNF-α levels compared to the sham group [31]. However, administering the methanolic extract of *P. amarus* to rats with ischemia-reperfusion injury significantly inhibited hepatic IL-1β and TNF-α levels [31]. Furthermore, previous research demonstrated that *P. amarus* ethanolic extract suppresses NF-κB, a major regulator of inflammation, in RAW 264.7 cells [35]. Additionally, Phyllanthin from *P. amarus* has been shown to reduce elevated pro- inflammatory cytokine levels by inhibiting NF- κB activation in high- fat diet- induced fatty liver[36]. In this study, Phyllanthin from *P. amarus* also reduced arsenite-induced increases in pro-inflammatory cytokine production by inhibiting NF-κB. Therefore, the protective effects of phyllanthin from *P. amarus*, through its anti-inflammatory properties, align with earlier research [31]. Moreover, this investigation consistently showed that the extent of organ damage, as measured by serum markers ALT for the liver and BUN for the kidney, was strongly and significantly correlated with the local inflammatory response in these organs. Higher levels of organ injury were associated with increased tissue concentrations of proinflammatory cytokines and transcription factors. The high correlation coefficients ($R2 > 0.7$ for all plots) and statistical significance ($p<0.05$) across all analyses provide strong evidence supporting this relationship.

P. amarus has been extensively studied for treating chronic Hepatitis B infection. A randomized trial involving chronic Hepatitis B patients (n=60) who received *P. amarus* extract for 12 weeks showed a significant reduction in Hepatitis B virus (HBV) DNA levels [37,38]. Its antiviral effectiveness inhibits viral replication and raises liver enzymes (ALT and AST) [37,38]. Patients with chronic Hepatitis B (n=123) treated with *P. amarus* for 6 months experienced decreased HBV surface antigen (HBsAg) levels, supporting its antiviral and liver-protective properties[39]. Additionally, patients with type 2 diabetes treated with P. amarus for 10 weeks had significant reductions in fasting blood glucose and glycosylated hemoglobin levels [40]. Moreover, in individuals with non-alcoholic fatty liver disease, *P. niruri* supplementation lowered elevated oxidative stress markers such as malondialdehyde (MDA), resulting in an increased overall antioxidant capacity[41]. Safety assessments have also shown that *P. amarus* is well tolerated. Therefore, *P. amarus* appears to be a promising herbal treatment for pesticide-induced liver and kidney damage. However, further research is needed to determine its clinical effectiveness in these conditions.

CONCLUSION

The results of this study showed that *P. amarus* methanolic extract effectively protects against sodium arsenite-induced liver and kidney damage in rats. The protective effect of *P. amarus* is likely due to the presence of phyllanthin, which reduces NF-κB activation, thereby decreasing inflammation and oxidative-nitrosative stress, and boosting overall antioxidant capacity. Clinically, *P. amarus* standardized capsules or liquid extracts can be taken orally, with dosing carefully controlled based on safety and effectiveness data from preclinical studies. The dose should consider factors such as the severity of arsenic poisoning, the patient's body weight, and how long the exposure lasts, with treatment lasting long enough for detoxification and tissue healing. Using *P. amarus* extract as an additional therapy alongside traditional chelation could improve patient outcomes by lowering arsenic levels and reducing biochemical problems caused by arsenic toxicity.

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Conflict of interest

No conflict of interest.

Data availability

The raw data supporting this article will be provided to the corresponding author upon reasonable request.

Ethical statements

The research protocol was approved by the Institutional Animal Ethics Committee (IAEC) of the Zhinanzhen Biology Ethics Committee (approval number: A2024000414). This study was conducted following the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

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Authors contribution

Each author has contributed significantly to the development of this manuscript. JX and HK: conceived and designed the evaluation, performed parts of the statistical analysis, and drafted the manuscript; SS and YZ: conducted data collection and drafted the manuscript. All authors have read and approved the final version of the manuscript.

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