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RESEARCH ARTICLE

Biochemical Assessment of Boswellic Acid Enrich-Frankincense Extract and its Antioxidant, Antibacterial, Anticancer and Anti-inflammatory Potential in Ameliorating the Glycerol-Toxicity in Rats

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ABSTRACT

Therapeutic use of natural products is currently considered as a global trend, highlighting the need for further research to develop more effective natural alternatives. The Frankincense aquas extract (FAE) contained high levels of boswellic acids (43.2%) and volatile compounds, i.e., thunbergol (37.2%); therefore, FAE scavenged 92% of 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radicals, inhibited 84 and 86% of the viability of HePG-2 and MCF-7 cancer cell lines, the FAE (20-35µg/mL) inhibited the multidrug-resistant bacteria (MDRB). In this study, the Frankincense (5% w/v) aquas extract (FAE) was evaluated for its antioxidant, antibacterial, anticancer, and anti-inflammatory potential in glycerol-induced acute kidney injury (AKI) in 24 adult male Wistar rats. The experimental rats were randomly divided into four groups: (1) control, (2) AKI glycerol (50%, 10 mL/kg i.m.), (3) oral FAE (5% w/v), and (4) AKI glycerol+FAE two times daily through oral gavage for four weeks. Administrating FAE in AKI rats led to a significant improvement in renal function parameters (uric acid, urea, Neutrophil Gelatinase-Associated Lipocalin-NGAL), total proteins (TP), and cystatin C, with no effect on creatinine levels compared to the control group. Furthermore, it enhanced (p<0.05) GSH, SOD, and NO levels and decreased MDA levels, indicating that FAE improved the oxidative status of rats. Compared to the normal, FAE treatment also downregulated inflammatory markers, i.e., tumor necrosis factor-alpha (TNF-α) and Interleukin 6 (IL-6) in AKI rats. The kidney sections showed typical structure in FAE treated healthy rats as in control, and the AKI-FAE group showed a significant reduction in focal inflammatory cells. It was concluded that oral administration of FAE (5% w/v) for 28 days restored renal function in the AKI rat model by reducing inflammation and oxidative stress.

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INTRODUCTION

Plant bioactive compounds have different biological activities (Almuhayawi *et al.*, 2023; Alharbi *et al.*, 2024). The plants contain a diverse array of bioactive compounds, such as alkaloids, flavonoids, and terpenoids, which are responsible for their therapeutic effects. These compounds exhibit a wide range of biological activities, including antioxidant, anti-inflammatory, antimicrobial, antiviral and anticancer properties (El-Saadony *et al.*, 2023a,b). By harnessing the power of these natural compounds, medicinal plants offer a promising avenue for developing novel drugs and promoting overall well-being (El-Saadony *et al.*, 2024a,b; El-Sayed *et al.*, 2024). Frankincense is a yellowish-brown aloe gum resin of the Boswellia plant with numerous therapeutic properties, such as curcumin, and is used as a traditional treatment for different problems throughout the world (Van Vuuren *et al.*, 2010). Frankincense extracts and essential oils have been used as antiseptic, astringent, cicatrizing and soothing agents.

Frankincense is also an effective therapy for several diseases, such as blood diseases, cardiovascular diseases, jaundice, cough, asthma, hair loss, irregular menses, and vaginal discharges (Rashan *et al.*, 2019). It has exhibited antihyperlipidemic, anti-atherosclerotic,

antiarthritic, antifungal, anticancer, analgesic, anti-inflammatory and hepatoprotective effects (Ratnam and Bhakshu, 2023).

Frankincense hydrophilic resins include 60% terpenes; 6-30% gums comprise soluble polysaccharides mix and 5-9% essential oil. Pentacyclic triterpenes comprise the lipophilic resin, with boswellic acid as the principal active constituent. The gum component is rich in digestive enzymes and carbohydrates. The essential oil constituents are monoterpenes, diterpenes and sesquiterpenes (Al-Harrasi *et al.*, 2018).

The main components of boswellics belong to pentacyclic triterpenoids and their acetyl derivatives; these compounds can inhibit the biosynthesis of interleukins and other pro-inflammation mediators (Ragab *et al.*, 2024), where these substances maintain the inflammation in cells (Al-Harrasi *et al.*, 2018). Nuclear factor-kappa B (NF-κB), a transcription factor that serves as a central mediator of cytokines during inflammation, can be inhibited by natural inhibitor 3-O-acetyl-11-keto-β-boswellic acid (Cuaz-Pérolin *et al.*, 2008). Boswellic acid may also reduce oxidative stress and the production of tumour necrosis factor-alpha (TNF-α) and Interleukin (IL-1, -2, -4 and -6) pro-inflammatory cytokines (Ragab *et al.*, 2024).

Acute kidney injury (AKI) is an acute loss of renal function that can arise from various insults, including rhabdomyolysis (RM). The RM is characterized by the breakdown of muscle tissue and the release of myoglobin and other muscle proteins into the bloodstream (Petejova and Martinek, 2014). RM accounts for 15-30% of all cases of AKI (Cebi *et al.*, 2016).

Despite the reversibility of renal function impairment in the majority of survivors, AKI continues to have an alarmingly high mortality rate of over 50%, and there are currently limited therapeutic strategies available for its treatment (Ikizler *et al.*,2021). Therefore, there is a need to develop effective interventions to protect against and treat this condition.

Glycerol is commonly used to induce AKI in rats (Cebi *et al.*, 2016). Glycerol-induced AKI in animals is caused by renal hemorrhage and myoglobin nephrotoxicity, which regulate its pathogenesis (Eltahir *et al.*, 2023). In this type of AKI, the myoglobin heme endures a redox cycle that induces lipid peroxidation and oxidative stress in the proximal tubular cell. It stimulates the secretion of diverse mediators, such as chemokines and cytokines, which subsequently stimulate leukocyte activation and culminate in tubular necrosis (Homsi *et al.*, 2006). According to the current knowledge, the primary mechanisms of AKI are impaired renal blood flow, tubular obstruction, and direct cytotoxicity induced by myoglobin (Hebert *et al.*, 2022).

Previous studies have demonstrated that water extract of frankincense improved kidney function after 30 days in paracetamol and glycerol-induced AKI model (Abdulmumin *et al.*, 2014; Mohamed *et al.*, 2018), adenine-induced chronic renal failure model (Saad *et al.*, 2018) and thymol model (Wang *et al.*, 2023). The aim of the present study was to evaluate the therapeutic effects of boswellic acid enrich-frankincense extract in acute kidney injury using a glycerol-induced AKI rat model.

MATERIALS AND METHODS

Frankincense aqueous extract preparation: Frankincense was obtained from local herbalist shops in the Sultanate of Oman. The resin was ground to obtain a fine powder and then was stored in a dark plastic container at 5°C until needed. To prepare Frankincense aqueous extract (FAE), 5g of the resin powder was soaked in 100 mL of distilled water for 60 min and heated to 80°C for about 60 min. After cooling to room temperature, the solution was filtered to give (5% w/v) extract. The dose of 1 mL of extract was given by oral gavage two times daily (Zhou *et al.*, 2000).

Determination of boswellic acids: The FAE was filtered through a 0.2µm filter to remove any impurities. Subsequently, 1-3mL of the filtered extract was used for analysis. A Shimadzu HPLC system (LC-10AS, Japan) equipped with a C18 column, autosampler, quaternary pump, and solvent degasser was employed for phenolic acids analysis. The mobile phase (aqueous acetonitrile gradient) flowed at a 1.0mL/min rate, gradually increasing organic solvent content (5 to 60%). The autosampler (SIL-40CXS) automatically injected samples. Identification and quantification of phenolic acids were achieved using the UV detector at 210 and 260nm, and the retention times (Rt) were calculated (Saad *et al.*, 2021).

Determination of volatile organic compounds profile: The volatile organic compounds (VOCs) in FAE were analyzed using GC/MS analysis (Hewlett–Packard model 5890). The separation column was a fused silica capillary column DB-5 (60mm×0.32mm i.d. ×0.25µm) was conducted to isolate the volatiles. The oven temperature was raised gradually from 50 to 250°C. The carrying gas was Helium flowing at 1.1ml/min. The samples were injected at 2µl (220°C at 70eV); the mass spectrum scanning range was 39 to 400m/z. The collected data were recognized by comparing the isolated peaks to those of recognized chemicals and published data (Adams, 2017). The detected data from the mass spectra was compared with the NIST library.

Determination of phenolic compounds content: The total phenolic content (TPC) of the frankincense (5% w/v) aqueous extract (FAE) was quantified using the Folin-Ciocalteu colorimetric assay, with gallic acid as the standard (Singleton and Rossi, 1965). The total flavonoid content (TFC) was estimated by $AlCl_3$ colorimetric assay, as outlined by Chang *et al.* (2002). Quercetin was used as the standard for quantification.

Determination of therapeutic potential

Free radical-scavenging activity: The antioxidant activity of FAE was measured according to Jia *et al.* (2012). The FAE at concentrations of 0, 50, 100, and 200µg/mL was mixed with diphenyl picrylhydrazyl (DPPH) solution and incubated for 30 min at room temperature in the dark. The absorbance of the resultant mixture was quantified at 517nm. The following equation was used to estimate % antioxidant activity (AA):

$$\text{Scavenging activity (\%)} = \frac{\text{OD control} - \text{OD sample}}{\text{OD control}} \times 100$$

Bactericidal activity: The antimicrobial activity of FAE at 50, 100, and 200µg/mL concentration was assessed using the disc diffusion assay, following the methodology of Singh *et al.* (2016), with specific modifications for basil essential oils (EOs.) The 0.5 McFarland standard solution was made by mixing 0.05 mL of 1.175% barium chloride dihydrate, with 9.95 mL of 1% sulfuric acid to obtain a solution with equivalent turbidity to 1.5×10^8 (CFU per mL) cell density. Then 200 µL of each bacterial culture (*Staphylococcus aureus, Listeria monocytogenes, Bacillus subtilis, Escherichia coli, Salmonella typhimurium,* and *Campylobacter jejune*) was spread on Muller Hinton agar plates, and 6mm discs impregnated with varying FAE concentrations (0, 50, 100, and 200µg/ml) were added to plates. The antibacterial activity was expressed as the diameter of the inhibition zones after 24h of incubation at 37°C.

Cytotoxic effect on MCF-7 and HePG2 Cells: The viability of breast cancer cell lines (MCF-7) and liver cancer cell lines (HePG-2) were assessed by measuring their ability to convert the yellow dye 2,5-diphenyl-2H-tetrazolium bromide (MTT) to a purple formazan, a process dependent on healthy mitochondria (Mosmann, 1983). HePG-2 and MCF7 cells were cultured at 37°C in RPMI 1640 medium supplemented with a 1% L-glutamine solution and 1% antimycotic mix (10,000U/ml Potassium Penicillin, 10,000µg/ml Streptomycin sulphate and 25µg/ml Amphotericin B). After 10 days of batch culture, 10,000 cells were seeded per well in fresh medium on 96-well plates. Four hours later, 40ul of MTT salt (2.5µg/ml) was introduced to wells, and the plate was incubated at 37°C with 5% CO_2. After overnight incubation at 37°C, 200µl of 10% Sodium dodecyl sulfate (SDS) was applied to each well to terminate the reaction. The positive control was a 100µg/ml concentration of a known cytotoxic natural substance-induced complete cell death under identical experimental conditions (Alowaiesh *et al.*, 2023; Alsubhi *et al.*, 2023). The absorbance (595nm, reference 620nm) was measured on a Bio-Rad microplate reader to assess FAE effects on cell viability (final concentration <0.2%). Cell viability (%) was determined by using the following formula:

$$\text{Cell viability (\%)} = \frac{\text{Reading of MTT} - \text{Reading of extract}}{\text{Reading of MTT}} \times 100$$

Induction of acute renal injury: In this study, glycerol was used to induce AKI in the rat model, as described earlier (Al Asmari *et al*., 2017). Briefly, Wister rats were allowed to acclimatize for 1 week, after which they were deprived of water for 24h before the experiment. Then, these rats were treated with half of the dose of glycerol (50% v/v in sterile saline, 10mL/Kg body weight) in the muscles of each hind limb.

Animals and experimental design: In this study, 24 albino Wister rats with an average body weight of 150 to 200g were used. Rats were obtained from the Medical Scientific Foundation for Research, Pharmacology lab. They were confined in environment-controlled enclosures (24±1°C, 45±5% RH, and 12h light/dark cycle). The rats were provided with potable water and a commercially available diet for the experiment. One week was allowed as an acclimation period before the experiment. The experimental investigation received ethical approval from the Biomedical Research Ethics Committee of the King Abdulaziz University Faculty of Medicine in Jeddah, Saudi Arabia (ethical approval reference No. 256-22).

Rats were randomly divided into four groups (n=6 each). Group 1 served as a healthy control and received 1mL of normal saline twice daily by oral gavage for four weeks. Group 2 included rats with induced kidney injury (AKI) by glycerol, as described above. Group 3, a Frankincense-treated healthy group, was given a freshwater extract of frankincense (5% w/v) two times daily through oral gavage for four weeks. Group 4 consisted of AKI rats that received 5% w/v frankincense water extract via oral gavage twice daily for four weeks (Fig. 1). After four weeks, rats were weighed to determine the weight gain, and then three rats from each group were sacrificed by decapitation. The blood and kidneys were immediately removed. The serum samples were kept at -80°C for the subsequent biochemical analysis.

Preparation of tissue homogenates: Kidney specimens (300 and 500 mg) were washed in cold PBS (pH 7) to eliminate superfluous blood. The tissues were chopped and then homogenized in 0.5ml of cold PBS. Two freeze-thaw cycles (ultrasonication) were applied to the resulting suspension to disrupt the cell membranes further. The suspension was centrifuged at 1500×g for 15 min, and the supernatant (cytosolic fraction) was collected for further analysis.

Determination of serum biochemical parameters: Serum creatinine level was assessed colorimetrically according to the method of Larsen (1972), uric acid by the method of Bulger and Johns (1941), urea by the method of Talke and Schubert (1965), total proteins by the method of Lowry *et al.* (1951) and cystatin C by the protocol of Finney *et al.* (1997). Serum Neutrophil Gelatinase-Associated Lipocalin (NGAL) levels for experimental rats were measured using the Abcam ELISA kit. The determination of the serum sodium (Na^+), and potassium (K^+) concentrations was done using an ECLIA on a Dimension Vista System (Siemens 23 Healthcare Diagnostics, Tarrytown, New York, USA), using the V-LYTE Integrated Multisensors reagents (Siemens Healthcare Diagnostics, New York, USA).

Hematological parameters: For the analysis of complete blood count (CBC) parameters, a standard Coulter gram was performed using an animal 5-diff automatic hematology analyzer (Model: YSTE-DF50V).

Determination of oxidative stress indicators: Using kidney homogenate, SOD and MDA values were determined (Bio Diagnostic Company, Dokki, Giza, Egypt). The activity of GSH was measured in the homogenate, as described by Ellman (1959), and NO quantity was determined via a nitrite assay kit from Sigma-Aldrich according to manufacturer instructions (Cortas and Wakid, 1990).

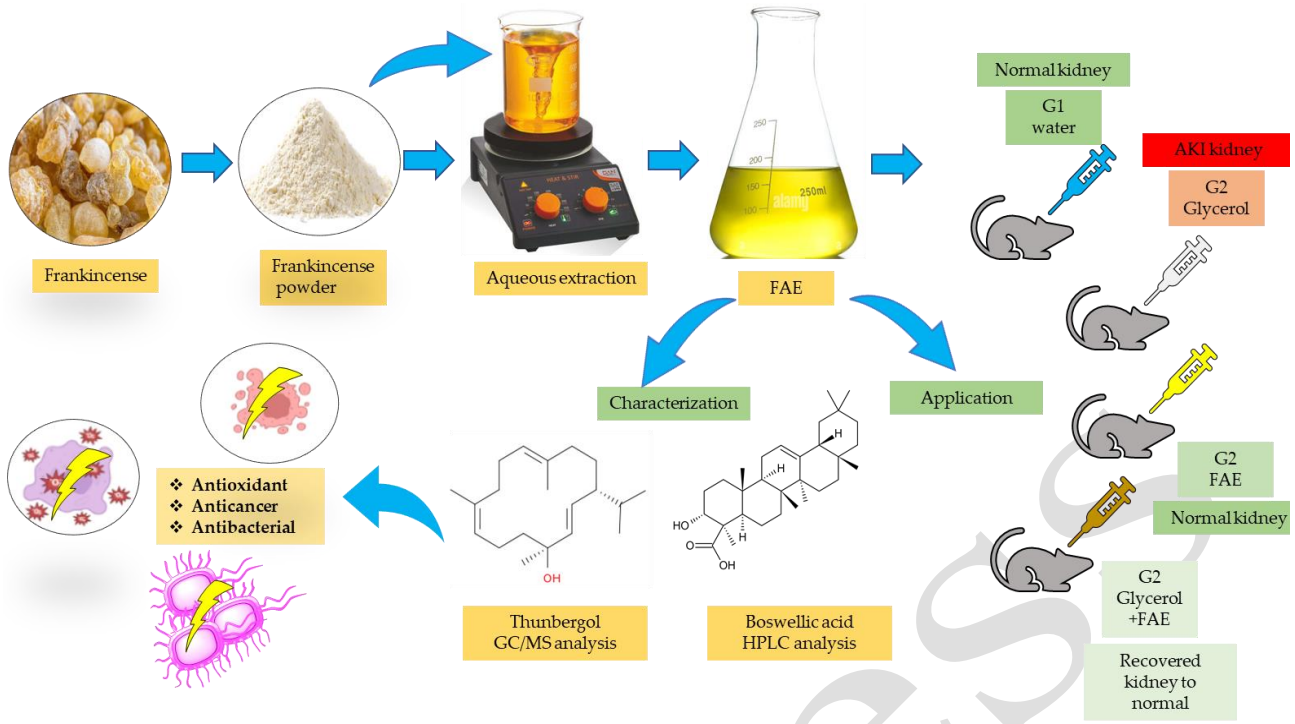

Fig. 1: Experimental layout for determination of biological activity of Frankincense aqueous extract in ameliorating the glycerol toxicity in rats.

Determination of the inflammatory markers: For the assessment of inflammation in the kidney, IL-6, C-reactive proteins (CRP), TNF-α, and fibrinogen levels were measured by using enzyme-linked immunosorbent assay (ELISA) kits (Ela Science, Houston, Texas, USA). The results were read on a Microplate Reader (Biotech Instruments, Inc., Winooski, VT, USA) at the King Fahd Medical Research Center, Jeddah, Saudi Arabia.

Histological analysis: The rat kidney tissues were first fixed in 4% paraformaldehyde, followed by dehydration, embedding in paraffin, and sectioning into 6µm thick sections. Following this, the biopsy specimens were stained with H&E, and examined under an invertedmicroscope for histopathological lesions and alterations. Six non-confidential microscopic domains were arbitrarily assessed per animal.

Data analysis: Mean values (±SE) were computed for various parameters. The analysis used the SPSS 23v program (IBM, Armonk, USA). Data were subjected to one-way ANOVA, followed by the LSD test, presuming that the variances of the groups were equal. However, the data on inhibition zone diameter for 3 FAE concentrations against 6 pathogenic bacteria were analyzed through two-way ANOVA. P-values less than 0.01 were deemed to indicate statistical significance.

RESULTS

Detection of boswellic acids in FAE: In the present study, the FAE was found to be rich in beswellic acids that were detected and identified by HPLC (Table 1). Boswellic acids (BAs) were of four primary triterpenes (11-keto-β-boswellic, α boswellic acid, β boswellic acid, and Acetyl β boswellic) with a total value of 35.4%, representing 82% of the detected boswellic acids. Moreover, two minors (acetyl 11-keto β- boswellic acid and acetyl-α- boswellic acid), with 18% value, were extracted from Frankincense aqueous extract. The RT of KBA (2.5 min) and β-BA (6.5 min) was assessed by HPLC UV detector.

Volatile compounds content in FAE: Table 2 shows the volatile compounds found in FAE using GC/MS analysis. The GC/MS profile of FAE showed mainly 12 volatile compounds. The main volatile compounds were Thunbergol (37.2±0.1%), followed by Isopropyl-1,5,9-trimethyl-15-oxa-bicyclo-pentadeca-5,9-dien-2-ol (33.3±0.9 %). Also, erolidol (7.5±0.3%), acetic acid, decyl ester (5.1±0.2%), and other volatile compounds were detected in low amounts (Table 2).

Biological activities of FAE

Antioxidant potential: Table 3 shows that FAE had considerable content of phenolic compounds where the total phenolic content was 35 mg/g and flavonoids content was 11mg/g in the treated G4 group (200µg/mL). This impacted the antioxidant activity that scavenged 92% of DPPH free radicals. The IC_{50} of FAE that scavenged the half DPPH content was 85µg/mL.

Table 1: HPLC profile of beswellic acids content in Frankincense aqueous extract

Peak No	Detected compound	Area (%)
1	11-keto-β-boswellic	7.2±0.3
2	Acetyl 11-keto-β-boswellic	3.6±0.1
3	α boswellic acid	8.8±0.9
4	β boswellic acid	12.9±0.5
5	Acetyl α boswellic	4.2±0.6
6	Acetyl β boswellic	6.5±0.8
	The sum of boswellic acids	43.2%

Sample size=3; data are presented as mean±SE.

Antibacterial activity: Concerning the antibacterial activity of FAE, results in Table 4 show that higher inhibition zones of FAE were against *Staphylococcus*

aureus, Bacillus subtilis, Listeria monocytogenes, Escherichia coli, Campylobacter jejune, Salmonella typhimurium, in the range of inhibition zones diameters of 21-29 mm at the concentration of 200µg/mL (G4) compared to other concentrations. The inhibition zone diameters increased significantly with an increase in FAE concentrations for all six bacteria (P<0.01). *Staphylococcus aureus* was the most vulnerable bacteria to FAE concentration; *Salmonella typhimurium* was the most resistant bacteria against the FAE concentrations. The MIC levels of FAE that inhibited the tested bacteria were in the range of 20-35µg/mL (Table 4), the lowest being against *Staphylococcus aureus*, while the highest against *Escherichia coli, Campylobacter jejune, Salmonella typhimurium* (P<0.01).

Table 2: Volatile compounds content in Frankincense aqueous extract

No	Volatile compounds	Area (%)
1	Acetic acid, decyl ester	5.1±0.2
2	Nerolidol	7.5±0.3
3	Geranyl-α-terpinene	0.5±0.1
4	Cembrene	1.3±0.6
5	Verticellol	1.72±0.1
6	Butyl 4,7,10,13,16,19-docosahexaenoate	0.46±0.2
7	Thunbergol	37.2±0.1
8	1,5,9-Tri-methyl-12-(propenyl) cyclo-tetra-deca-1,5,9-triene	4.7±0.4
9	8,12,15,15-Tetra-methyl-4-methylene-bi-cyclo-pentadeca-7,11- diene	4.8±0.8
10	1,3,6,10-Cyclo-tetra-decatetraene-3,7,11-tri-methyl-14-(1-methyl ethyl)	2.1±0.3
11	1-Isopropyl-4,8,12-tri-methyl-cyclotetradeca-3,7,11-trienol	3.89±0.7
12	Isopropyl-1,5,9-tri-methyl-15- oxa-bicyclo-pentadeca-5,9-dien-2-ol	33.3±0.9

Sample size=3; data are presented as mean±SE.

Table 3: Antioxidant potential of Frankincense aqueous extract

Antioxidant	Amount of extract
Total Polyphenols (mg/g)	35±0.9
Total Flavonoids (mg/g)	11±0.8
Scavenging Activity (%)	92±1.1
IC50 (µg/mL)	85±2.1

N=3; data are presented as mean±SE.

Table 4: Antibacterial activity shown by diameter of inhibition zone (mm) of different concentrations of FAE against pathogenic bacteria

Pathogenic bacteria	Concentration of FAE (µg/mL)			MIC
	50	100	200	(µg/mL)
Staphylococcus aureus	22±0.9aC	25±0.2aB	29±0.2aA	20c
Listeria monocytogenes	20±0.2bC	22±0.5bB	26±0.5bcA	30b
Bacillus subtilis	20±0.1bC	23±0.6bB	27±0.1bA	30b
Escherichia coli	18±0.5cC	21±0.1cB	23±0.6cA	35a
Salmonella typhimurium	16±0.3dC	19±0.4dB	21±0.2dA	35a
Campylobacter jejune	17±0.1cC	20±0.8cdB	23±0.2cA	35a

N=3; data are presented as mean±SE; Mean values with different lower-case letters within a column, and upper-case letters within a row differ significantly (P<0.01).

Anticancer activity: In this study, each FAE concentration exhibited cytotoxicity on liver (HePG-2) and breast (MFC-7) cancer cell lines (Fig. 2). The FAE at 200µg/mL concentration (G4) reduced the progress of HePG-2 and MCF-7 cancer cells by 84 and 86%, respectively, and overall, the results revealed that the treatment reduced the cancerous cell proliferation by 85%, which was comparable to that of DOX (Fig. 2C). These results correlated with microscopic images shown in Fig. 2A and Fig. 2B for breast cancer cell lines and liver cancer cell lines, respectively. On the other hand, the IC_{50} of treatments inversely correlated with the inhibition percentage, where the IC_{50} of FAE was lower (15µg/mL) compared to DOX (20µg/mL).

Results of in vivo experiments with FAE

Influence on body weight: In the present study, initial body weights of rats of the four groups G1 (control), G2 (AKI), G3 (FAE) and G4 (AKI-FAE) were 171.00±21.79, 179.33±21.69, 163.83±11.16 and 191.17±10.91g, respectively. At the end of the experiment, the increments in weights were 30, 8, 27, and 24% in the control, AKI, FAE, and AKI-FAE groups, respectively. Compared to the control group, intramuscular glycerol injection significantly decreased body weight (P=0.002) (group 2), while the final weight decreased by 16g. FAE administration for four weeks to glycerol-induced AKI rats (group 4) increased BWG (P=0.001) compared to the untreated control group (group 1). On the other hand, frankincense water extract administered to healthy rats (group 3) did not affect (P=0.506) body weight compared to the control (group 1).

Influence on biochemical parameters: In this study, a single intramuscular injection of glycerol (group 2) induced (P<0.01) an increase (P<0.01) in serum creatinine, uric acid, urea, NAGAL, total proteins, and cystatin C (Table 5), sodium (Fig. 3A) and potassium levels (Fig. 3B) compared to the control group (group 1). Administration of Frankincense to glycerol-induced AKI rats (group 4) significantly decreased all these parameters compared to the AKI group (group 2), except for creatinine. The creatinine levels were decreased by administering frankincense to kidney injury rats without statistical significance compared with control. Furthermore, compared to the healthy control group, these parameters did not differ significantly in FAE-treated healthy rats (group 3).

Influence on hematological parameters: The effect of frankincense on hematological parameters is shown in Table 6. There was a non-significant decrease in the number of red blood cells in AKI rats (group 2) compared to the controls (group 1). WBCs count and platelets increased, while Hb levels decreased (P=0.01), in AKI rats compared to control (group 1). Treatment of AKI rats with frankincense significantly increased RBCs and Hb and decreased WBCs compared to AKI rats. However, platelet levels decreased non-significantly in frankincense-treated AKI rats (group 4) compared to AKI rats (group 2). Red blood counts (RBCs) increased significantly in frankincense-treated healthy rats compared to healthy controls (group 1).

Table 5: Comparison of different treatments on kidney function tests in different studied groups

Parameters	Group 1	Group 2	Group 3	Group 4
Creatinine (mg/dL)	0.63±0.08	1.06±0.17***	0.63±0.06	0.90±0.24
Uric acid (mg/dL)	5.65±0.60	9.63±0.52***	5.31±0.47	7.03±0.57$$$
Urea (mg/dL)	13.98±1.51	57.17±1.83***	14.02±1.06	20.29±1.54$$$
NGAL (mg/mL)	26.47±2.49	103.83±4.36***	28.08±5.15	41.05±2.96$$$
Total proteins (g/dL)	6.53±0.42	9.77±0.22***	6.62±0.34	7.17±0.60$$$
Cystatin C (mg/L)	0.68±0.11	1.76±0.15***	0.64±0.15	0.84±0.10$$$

Data are presented as mean±SE);. *** significant differences vs the untreated group for each parameter (P<0.01); $$$ significant differences compared to AKI-group for each parameter (P<0.01).

Influence on oxidative status: The antioxidant potential of FAE in terms of oxidative stress markers in rats revealed that the activity of enzymatic and non-enzymatic antioxidants SOD, GSH, and NO decreased, while that of

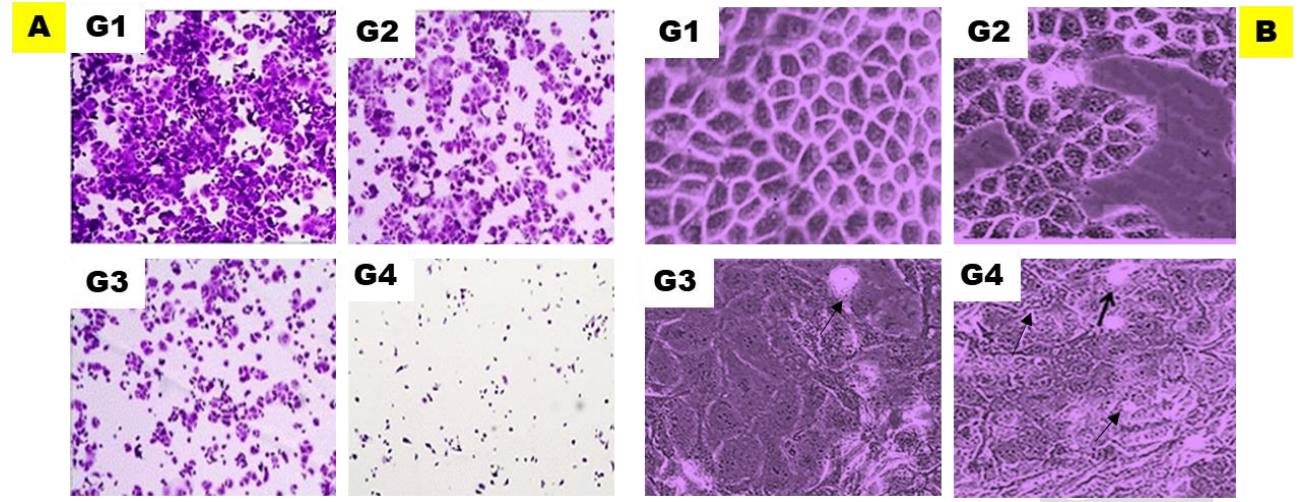

Fig. 2: (A) Microscopic images of the inhibition effect of FAE at different concentrations (G1=0, G2=50, G3=100, and G4=200µg/mL) against the progress of breast cancer MFC-7 cell lines, and (B) Microscopic images of the effect of FAE concentrations on liver cancer HePG-2 cell lines. (C) Histogram representing the inhibitory effect of FAE concentrations on the breast and liver cancer cell viability compared to Doxorubicin (DOX). Data are presented mean ±SE. Lowercase letters above bars indicate significant differences at p<0.05.

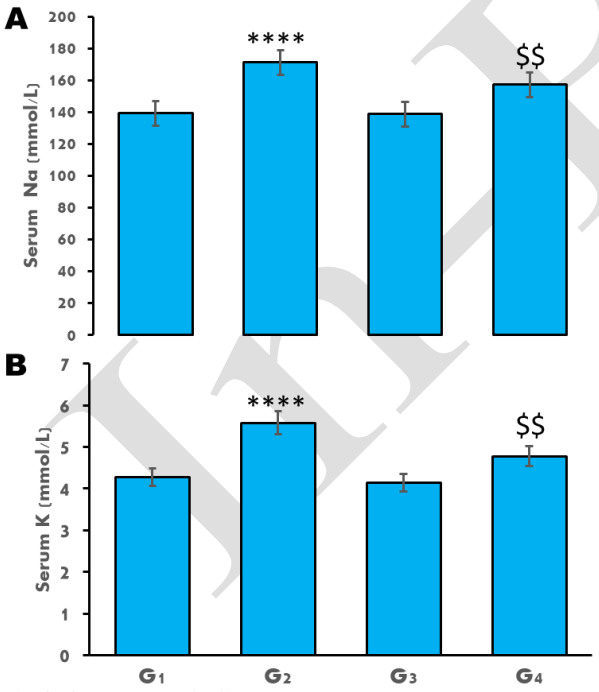

Fig. 3: Comparison of different treatments on serum electrolytes in rats of four groups. (A): Sodium (Na) concentration in rat serum and (B): Potassium (K) concentration in rat serum. Data are expressed as mean±SE. ***P<0.001 and ****P<0.0001 as compared with the control group 1 and $$ P<0.01 as compared with glycerol treated group 2.

MDA increased (P<0.01), significantly in AKI rats (group 2) compared with the healthy control rats (group 1). Treatment of AKI rats with FAE increased (P=0.001) GSH and NO concentrations and decreased MDA concentrations compared to the untreated AKI rats (group 2). However, Frankincense treatment of AKI rats increased SOD activity non-significantly (Table 7). Moreover, administration of Frankincense to normal rats (group 3) decreased GSH levels significantly but did not affect SOD, NO, and MDA levels compared to control rats (group 1).

Influence on immunological parameters: The pro-inflammation markers, IL-6, TNF-α, CRP and fibrinogen were analyzed to assess the impact of Frankincense on renal injury and inflammation. In the glycerol-induced AKI group, IL-6 and TNF-α levels, CRP and fibrinogen increased significantly (P<0.01) compared with the healthy control group (Table 8). The administration of Frankincense extract significantly reduced the concentrations of all pro-inflammatory cytokines compared to the AKI group (P<0.01).

Influence on renal histopathology: Fig. 4 shows the effect of Frankincense on histological changes in the kidneys of AKI and Frankincense-treated rats. The normal structure of the kidneys of control rats is shown in Fig. 4A. Kidney damage and necrosis were observed in AKI rats (group 2), and signs of inflammation were represented by the interstitial infiltration of the inflammatory cells (Fig. 4B). Treatment of AKI rats with Frankincense extract reversed glycerol-induced histopathological changes in the kidney. The kidney tissue of this group exhibited renal tubular epithelium cells and a standard kidney tissue structure with no specific pathological alterations (Fig. 4D). Frankincense extract treatment did not induce any major changes in the kidneys of healthy rats (Fig. 4C).

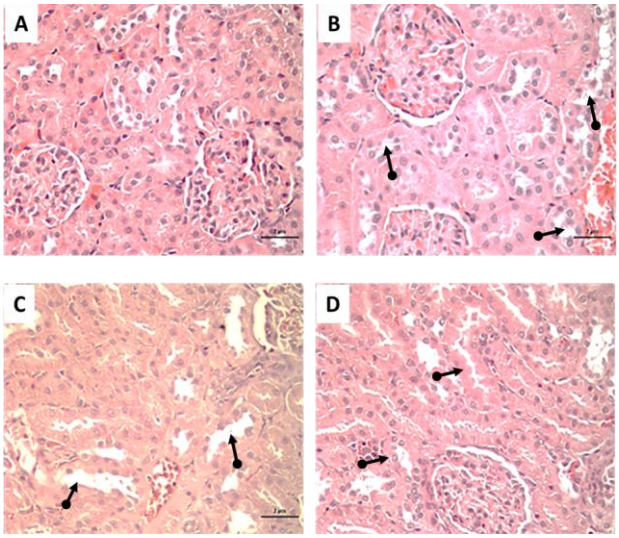

Fig. 4: Histological appearance of kidney in all study groups: Representative rat renal histopathology (H&E x40). A): The control kidney showing normal structure; B): Kidney section from group 2 treated with glycerol, showing focal inflammatory cells infiltration, congestion, and degeneration (arrows); C): The kidney section of Frankincense only treated group 3 showing mild focal inflammatory cell infiltration, congestion, and degeneration (arrows); and D): Kidney section of Frankincense and glycerol-treated group 4, showing improved focal inflammatory cell infiltration, congestion, and regeneration (arrows).

Table 6: Comparison of different treatments on complete blood count in treated and non-treated groups

Hematology	G1	G2	G3	G4
RBCs (106/µL)	5.07±6.35	4.12±1.07	6.56±7.25	7.458±8.60 $$$
WBCs (103/µL)	5.81±0.89	15.98±1.62***	7.51±0.39	7.34±1.76$$$
Hemoglobin g/dl)	13.80±0.86	9.05±0.51***	13.40±0.71	13.00±0.90$$$
Platelets (cells/mL3)	541.33±88.89	955.50±44.06***	636.00±166.25	796.33±65.48

Data are presented as mean±SE);. ***significant differences compared to the untreated group for the same hematological parameter (P<0.01); $$$significant differences compared to AKI-group for the same hematological parameter (P<0.01).

Table 7: Oxidative stress markers in treated and non-treated groups

Antioxidant	G1	G2	G3	G4
SOD (ng/mL)	176.50±12.28	75.00±4.10***	181.00±2.60	87.50±9.57
MDA (mmol/mL)	0.51±0.16	1.94±0.07***	0.52±0.14	0.62±0.11$$$
GSH (mg/L)	17.40±2.20	4.82±1.20***	13.10±1.97##	11.41±1.63$$$
NO (ppb)	8.30±0.81	2.15±0.39***	8.60±0.91	7.53±0.54$$$

Data are presented as mean±SE);. ***and ## significant differences versus the untreated group for the same antioxidant (P<0.01); $$$ significant differences compared to AKI-group for the same antioxidant (P<0.01).

Table 8: Comparison of different treatments on inflammatory markers in different studied groups

Markers	G1	G2	G3	G4
IL-6 (pg/mL)	5.07±0.77	27.25±1.25***	6.58±1.29	9.43±1.01$$$
TNF-a (pg/mL)	10.28±0.79	28.33±2.73***	11.97±1.28	14.78±0.80$$$
CRP (mg/L)	5.63±0.84	25.67±1.37***	5.41±0.49	8.27±1.02$$$
Fibrinogen (g/L)	3.08±0.61	7.96±0.54***	3.46±0.51	4.39±0.40$$$

Data are presented as mean±SE);. ***significant differences vs the untreated group for the same marker (P<0.01); $$$ significant differences compared to AKI-group for the same marker (P<0.01).

DISCUSSION

The search for natural alternatives to chemical compounds is a global trend (El-Saadony et al., 2020; Selim *et al.*, 2022; El-Kassas *et al.*, 2022; Abd El-Hack *et al.*, 2022a,b). Natural compounds offer a promising alternative to synthetic compounds with their rich history of traditional use. These natural compounds often contain bioactive compounds with different biological benefits (El-Tarabily *et al.*, 2021; Umair *et al*., 2022; Hegazy *et al.*, 2024). While they can relieve various ailments, it's crucial to approach their use cautiously and consult healthcare professionals. While some plant-based medicines have undergone rigorous scientific research and are considered safe and effective, others may lack sufficient evidence. It's essential to distinguish between scientifically validated herbal remedies and unproven folk practices (El-Saadony *et al.*, 2021a,b; Mueed *et al.*, 2024). This study investigated the potential of FAE in attenuating kidney failure in rats. The presence of active phenolic acids, boswellic acids (BA), and volatile compounds in FAE determined its activity, where Mannino *et al.* (2016) stated that Keto-BA and acetyl keto-BA are the two most active Bas. Their respective Rt values are consistent with those previously reported (2.6–3.4 and 7.5–8.6 minutes, respectively) by Borotová *et al*. (2023). In contrast, the Rt values for their acetyl derivatives were 7.9 and 3.7 minutes, respectively, confirming the previously reported values of 3.5–4.2 and 11.5–12.2 minutes, respectively (Borotová *et al*., 2023).

The retention time is typically observed to increase as compounds' molecular weight (MW) rises and is influenced by the quantity and nature of functional groups (Mathe *et al*., 2004). Earlier studies have shown that *Boswellia carterii* contains 5-9% essential oil, the composition of which varies with the biological source, and contains 65-85% terpenoids and about 20% soluble polysaccharides (Al-Yasiry *et al.*, 2016).

According to Al-Yasiry *et al.* (2016), Frankincense essential oil (FEO) has antioxidant properties that may be beneficial for treating diseases caused by oxidative stress. This is due to the presence of phytochemical substances such as terpenoids, flavonoids, cardiac glycosides, tannins, carbohydrates, and saponins. In fact, even at a low concentration of 62.5 µg/ml, FEO was able to inhibit the activity of DPPH by 43.84% in an antioxidant assay (Ezekiel *et al.*, 2020). Correlating with our study, Sultan (2020) showed that Frankincense had the highest polyphenol and flavonoid content in its aqueous extract; it reached a total polyphenol content (TPC) of 32.15µg/mL and total flavonoid content (TFC) of 20.29µg/mL.

Frankincense aqueous extract has high levels of phytochemicals, which might hinder bacteria through mechanisms like enzymatic inactivation and target site modifications. Correlating to our study, the administration of FAE-controlled antibiotic-resistant bacteria such as *S. aureus*, *E. coli*, *K. pneumonia*, and *B. subtilis* (Almutairi *et al*., 2022; Mueed *et al*., 2023). Also, Al-Kharousi *et al.* (2023) found that FEO had the smallest inhibition zone against E. *coli* (10.4 mm). The inhibition zones that were produced by the oils against *S. aureus* and *Bacillus* spp. were slightly larger than those produced against *E. coli* and *P. aeruginosa*.

In accordance with our study, Farahani *et al.* (2023) found that Frankincense extract showed antitumor properties in female breast cancer stem-like cells (CSLCs). In contrast to numerous anticancer compounds with restricted efficacy against tumor cells, Frankincense emerges as a viable alternative in the fight against breast cancer stem cells (CSCs). Also, Valente *et al.* (2024)

found that *Boswellia serrata* inhibited breast cancer proliferation and was well-tolerated in a Phase I, a window of opportunity trial.

In this study, the AKI rat model was induced by a single intramuscular injection of glycerol known to cause AKI (Petejova and Martinek, 2014). An unexpected failure in the kidney function shows acute kidney injury, which results in the buildup of hazardous nitrogen metabolites within the renal system. In this study, glycerol injection caused a serious renal injury represented by elevations in serum creatinine, uric acid, urea, NAGAL, cystatin C, total proteins, and serum electrolytes. Moreover, histopathological alterations in the kidney confirmed impairment of the renal function. Our results confirmed the induction of AKI by glycerol, as demonstrated by biochemical and histopathological findings consistent with those previously reported by other researchers (AlBasher *et al.*, 2020).

In this study, Frankincense extract administration (5% w/v) to glycerol-induced AKI rats for 28 days significantly lowered serum kidney function test parameters and reserved normal kidney tissues, indicating the ability of Frankincense to restore renal function. These findings agree with previous studies that reported the kidney restorative effect of Frankincense on acetaminophen-induced AKI (Abdulmumin *et al.*, 2014) or paracetamol and glycerol induced AKI in rats (Mohamed *et al.*, 2018). According to Al Asmari *et al.* (2017), Frankincense extract alleviated the reduction in body weight caused by glycerol injection. This effect may be due to the potential of Frankincense to interfere with protein catabolic pathways, which contribute to muscle protein damage.

In our study, serum creatinine levels increased following glycerol injection and decreased non-significantly following Frankincense extract treatment. This can be attributed to the influence of kidney secretion and other extrarenal factors on variations in creatinine levels that occur after renal injury recovery (Al-Marhoon *et al*., 2023). The present study revealed that Frankincense treatment significantly reduced cystatin C and NAGAL levels in rats with acute kidney injury, a marker of kidney damage that is more reliable than serum creatinine in determining glomerular filtration rate (Liu *et al.*, 2016). The NGAL is a protein produced by neutrophils in response to inflammation. It is rapidly upregulated and released into the urine and bloodstream in response to kidney injury, making it a sensitive and specific marker of AKI (Rossiter *et al.*, 2024).

Total protein levels were increased in the induced AKI model. The serum total proteins consist of albumin and globulin. Serum globulin, which is also referred to as gamma gap, includes all non-albumin proteins present in the blood, such as globulin, CRP, fibrinogen, interleukins, leukotrienes, and other regulatory and prothrombotic proteins (Peng *et al.*, 2020). This study revealed elevated CRP, fibrinogen, and IL-6 in AKI rats, indicating inflammation (Peng *et al.*, 2020).

In our study, blood hemoglobin levels significantly decreased in glycerol-induced AKI rats. This can be due to a decrease in the level of erythropoietin (EPO) hormone in the blood, which may be caused by kidney damage. The tubulo-interstitial renal cells are responsible for the secretion of EPO, a hormone that stimulates the production of red blood cells. Studies have shown that acute and chronic kidney injury can reduce EPO secretion from these cells (Yamashita *et al.*, 2016). Our data is consistent with a previous study, which reported decreased Hb levels in glycerol and paracetamol-induced AKI rat model (Mohamed *et al.*, 2018). The same study has also reported a reverse in Hb and RBC count after administration of Frankincense water extract (10% w/v) for 30 days.

Myoglobin can harm kidneys by causing oxidative stress and inflammation, leading to the conversion of ferrous-myoglobin to ferric-myoglobin by tubular cells, releasing reactive hydroxyl radicals, causing lipid peroxidation and membrane damage (Gwozdzinski *et al.*, 2021). The findings of our study agree with those of previous workers, who noted that glycerol significantly induced oxidation and limited the activity of antioxidant enzymes in the kidney (Adedapo *et al.*, 2020).

In the current study, the administration of Frankincense extract in rats with AKI demonstrated an antioxidant effect, as shown by a significant increase in reduced GSH and a decrease in renal MDA levels ($P<0.01$). According to the findings, the antioxidant properties of Frankincense are ascribed to its terpenoids, phenolic compounds and saponins (Biggs *et al.*, 2016). However, the increase in SOD activity was not statistically significant. A possible reason for this could be the use of a low concentration of FAE (5% w/v) in this study compared to the concentration (20% w/v) reported previously (Saad *et al.*, 2018).

Glycerol injection in rats reduced NO bioavailability, while Frankincense administration improved it. Myoglobin scavenging NO, a vasodilator, can lead to NO depletion, causing vasoconstriction and kidney damage with tubular necrosis. Our findings agree with those reported by Oyagbemi *et al.* (2021). The depletion of NO by myoglobin can also contribute to the development of oxidative stress and inflammation in the kidney tissue, which can aggravate AKI (Oyagbemi *et al.*, 2021). Frankincense administration in AKI rats increased NO levels and could exhibit a protective role against glycerol-induced nephrotoxicity caused by myoglobin.

In this study, glycerol-induced AKI resulted in significantly increased levels of CRP, fibrinogen and pro-inflammatory cytokines TNF- α and IL-6, platelets and WBCs count ($P<0.01$), indicating the presence of inflammation and immune activation. Platelets are essential for acute hemostasis and inflammation and contribute to the development of AKI (Su *et al.*, 2011). At locations of acute injury, platelets arrive first and interact with vascular cells and leukocytes (Jansen *et al.*, 2018). Additionally, they can induce the release of inflammation markers from monocytes, including tumor necrosis factor α and interleukins (Suzuki *et al.*, 2013). TNF-α and IL-6 are inflammation markers secreted by additional pro-inflammatory cytokines and mediators upon binding to sites of injury and inflammation (Lee *et al.*, 2015).

IL-6 and TNF-α have been linked to the progression of AKI and are utilized as prognostic indicators for mortality and clinical outcomes (Ramseyer and Garvin, 2013). CRP and fibrinogen are both indicators of inflammation (Luyendyk *et al.*, 2019). The findings of this study showed that inflammation markers, IL-6, CRP, TNF-α, fibrinogen, platelets, and WBCs were significantly reversed after

Frankincense treatment of AKI rats, suggesting an anti-inflammatory effect. The FAE is composed of active constituents that exhibit anti-inflammatory and antioxidant properties. Boswellic acids, isolated from Frankincense, have an anti-inflammatory effect against arthritis (Lim *et al.*, 2019). The most active component of Frankincense extract, 3-O-acetyl-11-keto-β-boswellic acid, may be responsible for its anti-inflammatory effect. This compound is a potent inhibitor of 5-lipoxygenase (5-LOX), a key enzyme in leukotrienes biosynthesis from arachidonic acid in the cellular inflammatory cascade. 3-O-acetyl-11-keto-β-boswellic acid prohibits the biosynthesis of leukotrienes, which are influential mediators of inflammation, by inhibiting 5-LOX (Sailer *et al.*, 1996).

Overall, no severe change in any studied parameters or histological morphology was found following Frankincense water extract (5%) treatment in healthy rats group 3). This extract did not cause hemolysis, as indicated by RBC count and Hb levels. This implies that Frankincense use is safe, and beneficial effects were confirmed in case of kidney damage. On the contrary, using a higher concentration (20% w/v) of Frankincense extract caused an increase in liver enzymes in healthy rats but did not show any histological changes in the liver (Saad *et al.*, 2018).

Conclusions: The results obtained from our research indicated that Frankincense aqueous extract (5% w/v) treatment for 28 days had renal function restorative effects by suppressing oxidative stress and inflammation in glycerol-induced AKI rat model. Based on these results, FAE may represent a viable therapeutic alternative in managing AKI.

Conflicts of interest: The authors do not have any conflict of interest to be declared.

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