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Ameliorative effects of frankincense oil on rats treated with a minimum toxic dose of paracetamol

Running title: Protective effect of frankincense oil against paracetamol toxicity

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Abstract

Paracetamol is a widely used analgesic and antipyretic drug, but its long-term usage has been associated with potential toxicity. The goal of this study was to investigate how frankincense oil prevents paracetamol toxicity. The study included twenty male albino rats divided into four groups: (G1) control, (G2) paracetamol, (G3) frankincense oil, and (G4) paracetamol + frankincense oil. For three days, 1000 mg/kg paracetamol was given orally, while frankincense oil was given orally at a dosage of 1000 mg/kg concurrently with paracetamol. Hematological parameters, lipid profile, cardiac markers, pancreatic function, blood antioxidant capacity, myeloperoxidase activity, blood, spleen, pancreas, heart, and lung histopathology were evaluated.

Paracetamol caused oxidative stress, hematotoxicity, dyslipidemia, myocardial damage, pancreatic dysfunction, spleen alterations, and lung tissue damage. However, coadministration of paracetamol and frankincense oil protected against these effects by boosting antioxidant capacity, improving hematological parameters, preserving pancreatic function, enhancing lipid profiles, preserving spleen morphology, and preventing lung damage, likely due to the antioxidant properties of frankincense oil.

In conclusion, this study highlights the potential of frankincense oil as a counteractive agent for paracetamol-induced organ toxicity. The findings suggest that frankincense oil supplementation may mitigate the adverse effects of paracetamol, offering a potential therapeutic approach to enhance overall health outcomes.

Keywords: Paracetamol; Toxicity; frankincense oil

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Introduction

Non-steroidal anti-inflammatory drugs, antivirals, antibiotics, steroids and, such as paracetamol, meloxicam, nimesulide, and ibuprofen, are frequently required to treat chronic illnesses and can have negative effects on the liver and kidneys (1). Paracetamol (also known as acetaminophen) is a commonly used over-the-counter painkiller and antipyretic medication (2). However, overdose or prolonged use of paracetamol can lead to liver and kidney damage (3).

Paracetamol toxicity occurs when the metabolism of paracetamol overwhelms the antioxidant capacity of glutathione (GSH), resulting in the production of excessive free radicals and oxidative stress. This oxidative stress can lead to cell death, organ dysfunction, and even death (4, 5). Paracetamol overdose prolongs its half-life, worsening its toxicity, and can lead to a three-stage manifestation of symptoms: mild within 24 hours, hepatic and renal failure within 72 hours, and severe symptoms including coagulation failure, hypoglycemia, multi-organ damage, and death within 96 hours (6).

N-acetylcysteine (NAC) is commonly used as an antidote for paracetamol overdose to replenish glutathione levels and reduce liver damage but may have compromised efficacy in patients with advanced liver disease (7). Therefore, alternative approaches to mitigating paracetamol toxicity are being explored.

Plant active components can scavenge ROS and enhance tissue antioxidant capacities, restoring balance in disrupted oxidative damage-defense systems (5). Natural antioxidants derived from alternative medical plants have shown promise in reducing paracetamol-induced oxidative stress (8). Frankincense oil, obtained from the bark of *Boswellia serrata*, has been utilized in traditional medicine and is known for its various health benefits (9, 10). Traditionally, frankincense oil is utilized to ease asthma symptoms, promote pancreatic enzyme secretion, improve memory and learning, and increase the digestibility of protein and energy (9, 10). It has been used to treat bronchial asthma, chronic pain, arthritis, digestive system diseases, diabetes, Alzheimer's, and cancer (11). Frankincense resin contains bioactive compounds such as boswellic acid, volatile oils, triterpene acids, and polysaccharides, which possess antioxidant, anti-inflammatory, antimicrobial, cardioprotective, and hepatoprotective properties (9, 12).

While previous studies have demonstrated the protective effects of frankincense extracts against hepatotoxicity caused by other factors, such as chronic alcohol consumption (13) or chemotherapy toxicity such as cyclophosphamide (14) and doxorubicin (15), there is a lack of research on its potential protective effect against paracetamol toxicity.

This study aims to fill that gap by comprehensively evaluating the potential protective effects of frankincense oil on multiple organs, including the spleen, lungs, heart, and pancreas, as well as assessing blood parameters and antioxidant levels. The findings of this research could provide valuable insights into the efficacy of natural interventions in mitigating the toxicity caused by paracetamol overdose and contribute to the development of alternative management strategies for paracetamol toxicity.

Materials and Methods

Chemicals and drugs

Paracetamol (Panadol®): 500 mg tablets purchased from El-Nasr Pharmaceutical Chemicals Co. in Egypt, grounded and dissolved in distilled water before administration. Frankincense oil of *Boswellia serrata* was purchased from a pure life company in Giza, Egypt.

Experimental Animals

Twenty 138-140g healthy adult male albino rats (*Rattus norvegicus albinus*), aged approximately 2-3 months old were included in this study. Those pathogen-free rats were sourced from the Animal House of the National Research Centre in Dokki, Giza, Egypt. Before the start of the experiment, the rats underwent a period of acclimatization in an animal holding room for a minimum of 2 weeks. The

room maintained natural conditions with a temperature of 25 ± 2 °C and natural daylight. The rats were housed in clean and spacious plastic cages (5 rats/cage), with unlimited access to food and water. The experimental procedures were performed in accordance with the approved guidelines for the care and use of laboratory animals by the Faculty of Science, Damanhur University, Egypt. The study received approval from the relevant ethical committee (Approval No. DMU-SCI-CSRE-230202).

Experimental design

The twenty male albino rats used in this study were separated into four experimental groups (5 rat/group) were assigned as follows:

1. Control group (G1): Rats in this group received no treatment.
2. Frankincense oil treated group (G2): Frankincense oil (1000 mg/kg) was given orally to rats in this group for three days (16).
3. Paracetamol-treated group (G3): Minimum toxic dosage of paracetamol (1000 mg/kg) was given orally to rats in this group for three days (17).
4. Paracetamol and Frankincense oil treated group (G4): paracetamol (1000 mg/kg) and Frankincense oil (1000 mg/kg) were given simultaneously to rats of this group for three days.

Collection of samples

Twenty-four hours after the last administration, that is on the 4th day, all animals were anesthetized by sodium pentobarbital and subsequently sacrificed. Blood samples were collected from the retroorbital plexus using a capillary tube and transferred into either EDTA tubes (Kemiko Vacutainer, Egypt) to determine hematological parameters or plain tubes and lift to clot, then centrifuge at 3000 rpm for 10 min to acquire clear sera which stored in – 20°C freezer to determine biochemical parameters. The tissues of the spleen, pancreas, heart, and lungs were collected in 10% formalin

for histopathological examination

Hematological Parameters

Blood collected in EDTA tubes was used to determine erythrogram and leukogram. Red blood cells count (RBCs), hemoglobin (Hb), hematocrit (PCV%), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC%), and platelets (PLT) count collectively known as erythrogram and total white blood cells (WBCs), and differential white blood cells count (leukogram) were measured using a hematology analyzer (HA-22/Vet Hematology Analyzer, Clindiag, Belgium). The blood smears were prepared and stained with Giemsa stain to assess the morphological changes in erythrocytes and leukocytes (18).

Biochemical parameters

Pancreatic Function and Glycemic Status:

Trinder (19) methods were applied to measure glucose levels using an enzymatic colorimetric kit (Cat. No. GL 13 20, Biodiagnostic, Egypt). Rat INS (Insulin) ELISA Kit (Cat. No: E-EL-R3034, Elabscience Biotechnology Inc., USA) was used to estimate the insulin levels following the manufacturer's protocols (20).

The following equations were used for calculating specific parameters:

1. Homeostatic Model Assessment-Insulin Resistance (HOMA-IR): HOMA-IR = (Fasting insulin [mU/l]) × (Fasting glucose [mg/dl]) / 405 (21).
2. Quantitative Insulin Sensitivity Check Index (QUICKI): QUICKI = 1 / (log [Fasting insulin (mU/l)] + log [Fasting glucose (mg/dl)]) (22).
3. Homeostasis Model Assessment of β-cells (HOMA-B): HOMA-B = (360 × Fasting insulin [mU/l]) / (Fasting glucose [mg/dl] − 63) **(23)**.

Lipid Profile parameters

The levels of serum total cholesterol (TC) were measured using Biodiagnostic, Egypt kits (Cat. No. CH 12 20), following the methods described by Richmond (24). Using Fassati and Prencipe (25) methods triglycerides (TG) were estimated using Biodiagnostic, Egypt kits (Cat. No. TR 20 30). The method of Lopes-Virella, Stone (26) was applied to

estimate High-density lipoprotein cholesterol (HDL-C) levels.

The following equations were used for calculating LDL-C and VLDL-C:

very low-density lipoprotein cholesterol (VLDL-C = TG / 5) (27)

low-density lipoprotein cholesterol (LDL-C = TC − [(vLDL-C) + (HDL-C)]) (28)

Heart Function: Assessment of Lactate Dehydrogenase (LDH) Levels and Atherogenic Indices

The levels of lactate dehydrogenase (LDH) in different samples were estimated using the colorimetric kits (Cat. No. E-BC-K046-S, Elabscience Biotechnology Inc., USA) based on the protocols described by Holbrook, Liljas (29).

The following Ali, Rumpa (30) equations were used for calculating atherogenic indices:

Cardiac risk ratio (CRR) = TC /HDL-C.

Atherogenic coefficient (AC) = (TC - HDL-C)/HDL-C.

Atherogenic index of plasma (AIP) = Log (TG/ HDL-C).

Castelli's risk index-2 (CRI-2) = LDL-C/ HDL-C

Total antioxidant capacity and Myeloperoxidase activity

The level of total antioxidant capacity (TAC) was determined using the colorimetric kit provided by Bio-diagnostic, Egypt (CAT. NO. TA 25 13)., following the methodology described by Koracevic, Koracevic (31). Using the MPO assay kit (E-BC-K074-M, (Elabscience, USA) myeloperoxidase (MPO) activity was detected.

Histological studies

The 48 hrs formalin-fixed spleen, pancreas, heart, and lung tissues were dehydrated in alcohol, cleared in xylene, and embedded in paraffin wax. Using rotary microtome (Leitz 1512, Leitz, Wetzlar, Germany) paraffin blocks were sectioned and stained with hematoxylin and eosin (32), then examined, and photographed using a microscope (Olympus CX41, Tokyo, Japan).

Statistical Analysis

Data were evaluated by using SPSS (Statistical Packages for Social Science, version 20) and One-Way ANOVA (Analysis of Variance) followed by Tukey's multiple comparison tests were employed and data were displayed as mean ± standard deviation (SD).

Results

Hematological results

Erythrogram

Table 1 presents the erythrogram data analysis, indicating no significant differences ($P < 0.05$) in RBC, MCV, MCH, MCHC%, PCV%, and platelet count between the rats treated with frankincense oil (G2) and the control group (G1). A significant elevation ($P < 0.05$) in Hb levels in the frankincense oil-treated group (G2) in contrast to the control group (G1). Paracetamol treated group (G3) exhibited a significant decline in RBCs, PCV%, MCV, MCH, Hb, and platelet count, and a significant elevation in MCHC%, compared to the control group (G1).

Simultaneous administration of frankincense oil and paracetamol resulted in a significant elevation in RBCs and an insignificant elevation in Hb, PCV%, MCV, and platelets, along with an insignificant elevation in MCH and an insignificant decline in MCHC compared to the paracetamol treated group (G3). However, there was a significant decline in PCV%, and Hb and insignificant changes in RBC, MCV, MCH, and MCHC levels when comparing the co-administration group (G4) to the control group (G1).

Leukogram

Leucogram data analysis is presented in Table 2. Oral administration of frankincense oil yielded similar results in WBC count and differential white blood cell count as the control group (G1). Paracetamol-treated group (G3) exhibited a significant elevation in total WBC count, neutrophil %, and monocyte %, as well as a significant decline in lymphocyte %, compared to the control group (G1). Simultaneous administration of frankincense oil and paracetamol (G4) caused a significant decline in total WBC count, along with an insignificant decline in neutrophil,

eosinophil, and monocyte %s, and an insignificant elevation in lymphocyte % compared to the paracetamol treated group (G3). However, there were no significant changes observed in WBC count, lymphocyte %, neutrophil %, eosinophil %, and monocyte % when comparing the co-administration group (G4) to the control group (G1).

Biochemical results

Pancreatic function and glycemic status

Pancreatic function and glycemic status parameters are displayed in Table.3. Oral administration of frankincense oil (G2) led to a non-significant elevation in glucose, insulin, and HOMA-IR value compared to the control group (G1). Administration of paracetamol (G3) caused a significant elevation in glucose and a significant decline in insulin level and HOMA-B value with an insignificant decline in HOMA-IR value and an insignificant elevation in QUICKI value compared to the control group. However, simultaneous administration of paracetamol and frankincense oil (G4) resulted in a significant decline in glucose, a significant elevation in insulin level and HOMA-B value, and no significant difference in HOMA-IR and QUICKI values compared to the paracetamol-treated group. Additionally, this group exhibited a significant elevation in glucose level and a significant decline in insulin level and HOMA-B value with an insignificant decline in HOMA-IR value and an insignificant elevation in QUICKI value compared to the control group (G1).

Lipid profile

The lipid profile parameters were analyzed and results are displayed in Table.4. Administration of frankincense oil (G2) caused insignificant changes in VLDL, LDL, TG, HDL, and cholesterol levels compared to the control group (G1). However, paracetamol administration (G3) led to a significant decline in HDL levels and a significant elevation in VLDL, LDL, TG, and cholesterol levels compared to the control group (G1). On the other hand, simultaneous administration of frankincense oil and paracetamol (G4) resulted in a significant elevation in HDL levels and a significant decline in VLDL, LDL, TG, and cholesterol levels compared to the paracetamol-treated group (G3). Additionally, this co-administrated group (G4) showed a significant decline in HDL levels and a significant elevation in VLDL, LDL, TG, and cholesterol levels compared to the control group (G1)

Heart function and atherogenic indices

Statistical analysis of LDH levels and atherogenic indices (CRR, AC, AIP, and CRI-2) values are displayed in Table.5. Oral administration of frankincense oil (G2) caused an insignificant change in LDH level and atherogenic indices (CRR, AC, AIP, and CRI-2) compared to the control group (G1). However, paracetamol administration (G3) caused a significant elevation in LDH level and atherogenic indices values compared to the control group (G1). Conversely, rats simultaneous administration of paracetamol and frankincense oil (G4) caused a significant decline in LDH level and atherogenic indices values compared to the paracetamol-treated group (G3) (Table 5). Additionally, this co-administrated group (G4) showed an insignificant increase in LDH level and atherogenic indices compared to the control group (G1).

Myeloperoxidase and total antioxidant capacity

The analysis of myeloperoxidase (MPO) and total antioxidant capacity (TAC) levels is summarized in Table 6. Oral administration of frankincense oil (G2) did not significantly affect MPO levels and TAC levels compared to the control group (G1). The administration of paracetamol (G3) significantly elevated MPO levels and significantly declined TAC levels compared to the control group (G1). However, the simultaneous administration of paracetamol and frankincense oil (G4) resulted in a significant decline in MPO levels and a significant elevation in TAC levels compared to the paracetamol group. Furthermore, this co-administration group (G4) exhibited insignificant changes in MPO levels and TAC levels compared to the control group (G1).

Table 1: Effect of different treatments on erythrogram parameters

Groups	**RBCS (10^6/µl)**	**Hb (g/dl)**	**PCV (%)**	**MCV (Fl)**	**MCH (Pg)**	**MCHC (%)**	**Plts (10^3/µl)**
Control group (G1)	8.2 ± 0.35^{b}	16.4 ± 0.84^{c}	50.3 ± 2.15^{c}	61.67 ± 2.8^{b}	20.04 ± 0.4^{b}	32.53 ± 3.9^{a}	678 ± 33^{b}
Frankincense oil group (G2)	8.4 ± 0.3^{b}	16 ± 0.53^{c}	50.3 ± 2.5^{c}	59.9 ± 0.84^{b}	$19.05\pm 0.23^{a,b}$	31.83 ± 0.7^{a}	679.33 ± 67^{b}
Paracetamol group (G3)	6.7 ± 0.15^{a}	11.73 ± 0.38^{a}	31.63 ± 1.26^{a}	47.4 ± 0.81^{a}	17.6 ± 0.28^{a}	37.1 ± 0.75^{b}	396.5 ± 96.5^{a}
Paracetamol and Frankincense oil group (G4)	$7.5\pm0.72^{a,b}$	14.23 ± 0.64^{b}	41.3 ± 1.56^{b}	55.32 ± 4.3^{b}	$19.04\pm 1.1^{a,b}$	$34.46\pm 1.47^{a,b}$	$494\pm 73^{a,b}$

Mean ± SD. Similar superscript letters ($p > 0.05$) indicate no significant difference and different superscript letters ($p < 0.05$) indicate significant differences.

Table 2: Effect of different on leukogram parameters

Groups	**WBCS (10^3/µl)**	**Differential leukocytic count**			
		Lymphocytes (10^3/µl)	**Neutrophil (10^3/µl)**	**Eosinophil (10^3/µl)**	**Monocytes (10^3/µl)**
Control group (G1)	6.02 ± 0.8^{a}	$73.2 \pm3.25^{b,c}$	15.5 ± 3.5^{a}	2.7 ± 0.6^{a}	5.5 ± 0.5^{a}
Frankincense oil group (G2)	8.17 ± 1.1^{a}	76.3 ± 3.1^{c}	15.5 ± 1.5^{a}	2.3 ± 0.58^{a}	4.7 ± 1.15^{a}
Paracetamol group (G3)	13.75 ± 1.35^{b}	62 ± 5^{a}	29.3 ± 5.03^{b}	4 ± 1^{a}	10.3 ± 2.1^{b}
Paracetamol and Frankincense oil group (G4)	5.97 ± 0.32^{a}	$64.2 \pm5.25^{a,b}$	$24. 3\pm 5.03^{a,b}$	3 ± 1^{a}	$7.5\pm 0.5^{a,b}$

Mean ± SD. Similar superscript letters ($p > 0.05$) indicate no significant difference and different superscript letters ($p < 0.05$) indicate significant differences.

Table 3: Effect of different treatments on pancreatic function and glycemic status

Groups	Glucose (mg/dl)	Insulin (mU/l)	Insulin resistance (HOMA IR)	Insulin sensitivity (QUICKI)	Pancreatic β-cell function (HOMA-B)
Control group (G1)	83.2±1.1[a]	2.4±0.1[c]	0.49±0.02[a,b]	0.43±0.002 [a]	42.9±4.1 [b]
Frankincense oil group (G2)	91.4±1.2[a]	2.75±0.35[c]	0.62±0.07[b]	0.42±0.008 [a]	35.1±6.8 [b]
Paracetamol group (G3)	232.6±7.1[c]	0.84±0.05[a]	0.48±0.04[a,b]	0.44±0.007 [a]	1.8±0.05 [a]
Paracetamol and Frankincense oil group (G4)	135.2±2.85[b]	1.45±0.25[b]	0.48±0.07[a]	0.44±0.013 [a]	7.3±1.5 [a]

Mean ± SD. Similar superscript letters ($p > 0.05$) indicate no significant difference and different superscript letters ($p < 0.05$) indicate significant differences.

Table 4: Effect of different treatments on lipid profile parameters

Groups	TG (mg/dL)	Cholesterol (mg / dL)	HDL (mg/dL)	VLDL	LDL (mg/dL)
Control group (G1)	58.4±0.7[a]	78.95±2.35[a]	55.2±1.15[c]	11.69±0.15[a]	12.01±3.4[a]
Frankincense oil group (G2)	60.7±1[a]	72.9±6.1[a]	51.07±1.78[C]	12.14±0.2 [a]	9.76±8[a]
Paracetamol group (G3)	176.5±2.9[c]	166.5±6.2[c]	26.85±0.95[a]	35.3±0.59 [c]	104.38±6.2[c]
Paracetamol and Frankincense oil group (G4)	70.6±5.2[b]	105.2±8.35[b]	33.1±3.01[b]	14.12±1.04 [b]	58±6.1[b]

Mean ± SD. Similar superscript letters ($p > 0.05$) indicate no significant difference and different superscript letters ($p < 0.05$) indicate significant differences.

Table 5: Effect of different treatments on heart function and atherogenic indices values.

Groups	**LDH (U/L)**	**CRR**	**AC**	**AIP**	**CRI-2**
Control group (G1)	201.17±9.5a	1.43±0.07[a]	0.43±0.07[a]	0.02±0.01[a]	0.22±0.07[a]
Frankincense oil group (G2)	206.7±7.64a	1.43±0.17[a]	0.43±0.17[a]	0.75±0.01[a]	0.19±0.16[a]
Paracetamol group (G3)	298.8 11.86b	6.21±0.36[c]	5.2±0.36[c]	0.82±0.15[c]	3.89±0.32[c]
Paracetamol and Frankincense oil group (G4)	213.5±4.5a	3.18±0.2[b]	2.18±0.2[b]	0.33±0.03[b]	1.75±0.2[b]

Mean ± SD. Similar superscript letters ($p > 0.05$) indicate no significant difference and different superscript letters ($p < 0.05$) indicate significant differences.

Table 6: Effect of different treatments on myeloperoxidase (**MPO**) and total antioxidant capacity (**TAC**)

Groups	**MPO (ng/ml)**	**TAC (mM / L)**
Control group (G1)	4.1±0.2[a]	2.2±0.1[c]
Frankincense oil group (G2)	4.55±0.45[a]	2.08±0.11[c]
Paracetamol group (G3)	8.45±0.65[c]	1.12±0.07[a]
Paracetamol and Frankincense oil group (G4)	6.45±0.55[b]	1.63±0.45[b]

Mean ± SD. Similar superscript letters ($p > 0.05$) indicate no significant difference and different superscript letters ($p < 0.05$) indicate significant differences.

Histopathological results of blood smears

Blood film slides prepared from the control group (G1) and the frankincense oil-treated group (G2) exhibited regular morphology of erythrocytes, leukocytes, and platelets (Figure 1A&B). However, administration of paracetamol (G3) led to various morphological abnormalities in erythrocytes, including anisocytosis (varying sizes and shapes), microcytic RBCs (small in size), dacrocytes (teardrop cells), and schistocytes (fragmented erythrocytes). Additionally, clumping of erythrocytes (agglutination) and aberrant neutrophil segmentation were observed (Figure 1C). In contrast, simultaneous administration of paracetamol and frankincense oil (G4) caused insignificant changes in the morphology of erythrocytes and leucocytes compared to the control groups (G1&G2) (Figure 1D).

Histopathological results of spleen tissue

Examination of spleen sections from the control group and frankincense oil-treated group (G1&G2) displayed normal spleen morphology, characterized by splenic parenchyma cells surrounded by a thin connective tissue capsule. Trabeculae extended within the organ, dividing it into lobules. The splenic parenchyma consisted of red pulp, with scattered white lymphatic follicles of white pulp (Figure.2A-D). The white pulp contained periarteriolar lymphoid sheaths surrounding central arterioles, follicles with germinal centers, and a marginal zone. The red pulp was composed of splenic cords located between blood sinuses (Figure.2B&D).

The paracetamol-treated group (G3) spleen tissue sections showed thickened splenic capsule was increased, and sub-capsular venous sinuses were dilated (Figure.2E) compared to the control groups (G1&G2). The lymphoid follicles within the white pulp exhibited lymphoid aggregate, atrophy, and a deteriorated appearance (Figure.2E&F). Increased tingible body macrophages in germinal centers containing ingested fragments of apoptotic cells were also observed (Figure.2F). The red pulp showed abundant histiocytes with foamy cytoplasm, and there was dilation and congestion of splenic sinuses (Figure.2F).

The spleen sections of rats co-administered paracetamol and frankincense oil (G4) exhibited normal capsule thickness and regular white pulp structure, with a red pulp ratio similar to the control groups (G1&G2) (Figure.2G). These sections also revealed a normal white pulp structure with clear periarteriolar lymphoid sheaths and activated germinal center numerous histiocytes in the red pulp and a few congested red pulp sinusoids (Figure.2H).

The spleen sections of rats administrated paracetamol and frankincense oil (G4) exhibited normal capsule thickness, regular white pulp structure, and red pulp ratio similar to control groups (G1&G2) (Figure.2G). Those sections revealed also a normal white pulp structure with histiocytes in the red pulp and a few congested red pulp sinusoids (Figure.2H).

Histopathological results of pancreas tissue

Inspection of the pancreas sections in the control group and frankincense oil-treated group (G1&G2) displayed normal pancreatic tissue architecture, consisting of lobules of varying sizes and shapes disconnected by thin interlobular septa (Figure.3A-D). The lobules exhibited densely packed pale stained acini lined with large pyramidal cells and rounded or oval scattered dark stained islets of Langerhans (Figure.3B&D).

Pancreas sections from the paracetamol-treated group (G3) showed normal pancreatic acini along with hypertrophied islets of Langerhans and dilated, congested blood vessels (Figure.3E&F). Interestingly, sections from rats treated simultaneously with paracetamol and frankincense oil (G4) appeared close to normal, except for the presence of congested interlobular ducts (Figure.3G&H).

Histopathological results of heart tissue

The heart sections in the control group (G1) exhibited a regular arrangement of cardiomyocytes which were separated by delicate interstitial connective tissue and a relatively sparse distribution of capillaries (Figure 4A). The cardiomyocytes exhibited a long and branched morphology with centrally located nuclei, and prominent cytoplasmic striations (Figure 4A).

Similar findings were observed in the frankincense oil-treated group (G2) (Figure.4B).

Histological sections of the paracetamol-treated group (G3) revealed early signs of cardiotoxicity, characterized by disorganized cardiac muscle fiber striations, nuclear pyknosis in cardiomyocytes, and increased congested interstitial space with dilation of blood capillaries (Figure.4C). Conversely, simultaneous administration of paracetamol and frankincense oil (G4) resulted in normal cardiomyocytes with limited congestion in the interstitial space and dilation of blood capillaries (Figure.4D).

Histopathological results of lung tissue

Examination of lung sections of the control group (G1) and the frankincense oil-treated group (G2) revealed regular lung tissue architecture characterized by a spongy structure with different-sized polygonal alveoli separated by thin interalveolar septa. Alveolar sacs and clear bronchioles were observed, mingling with the alveoli (Figure.5A-D). The bronchioles were lined by columnar epithelium with goblet cells, forming folds surrounded by a layer of smooth muscle cells and peribronchial cells (Figure.5A&C). The alveolar epithelial lining consisted of squamous alveolar cells (Figure.5B&D).

In contrast, lung tissues from the paracetamol-treated group (G3) exhibited significant pulmonary tissue injury, characterized by a decrease in the number of alveoli, expansion of alveolar cavities (emphysema), thickened interalveolar septa, bronchi stenosis, congestion, and necrosis of peribronchial cells (Figure.5E&F). Interestingly, simultaneous administration of paracetamol and frankincense oil (G4) markedly prevented lung damage. The lung sections of these rats displayed a structure resembling that of the control groups (G1&G2), with only a few thickenings of interalveolar septa in some regions and less alveolar emphysema compared to the paracetamol-treated group (Figure.5G&H).

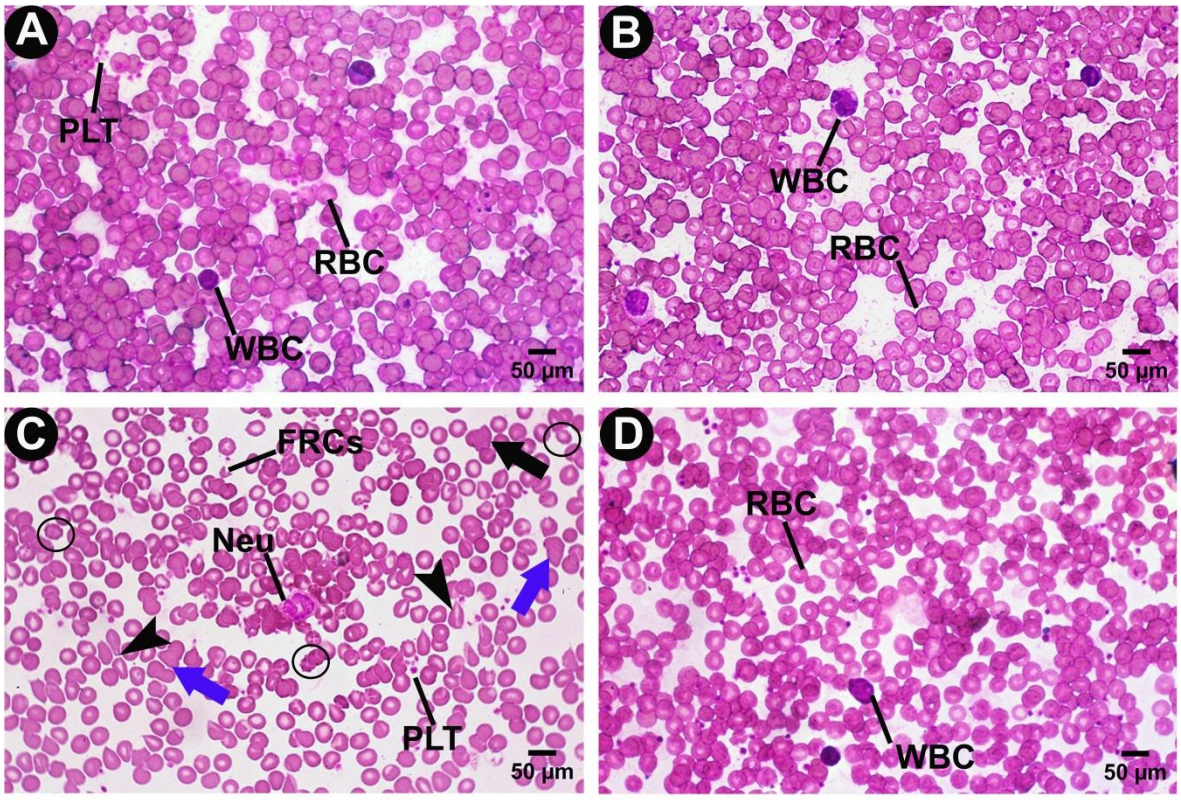

Figure 1: Blood film slides of different treatment groups (H&E Stained; X1000). The blood film of the control group (A) and frankincense oil-treated group (B) displayed normal morphology of red blood cells, white blood cells, and platelets. C: blood film of paracetamol treated group noticed microcytic RBCs (Black circle), RBCs agglutination (Blue Arrows), dacrocytes (Arrowhead), fragmented red blood cells (FRCs), neutrophil (Neu). D: Blood film of paracetamol and frankincense oil-treated group showing normal red and white blood cell morphology.

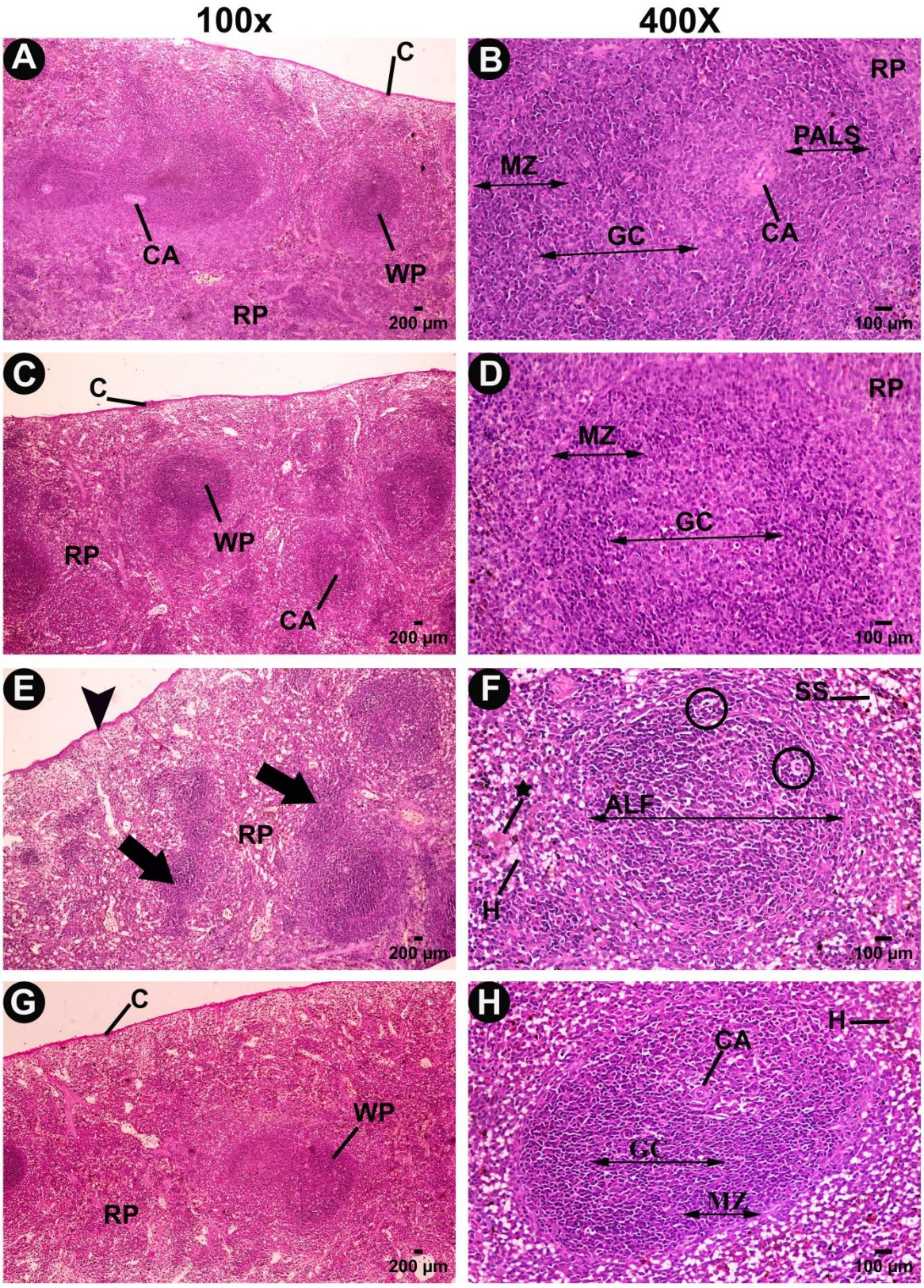

Figure 2: Spleen sections of different experimental groups (H&E Stained). Spleen sections of control (A&B) and frankincense oil-treated group (C&D) showing capsule (C), white pulp (WP), red pulp(RP), central arteriole (CA), periarteriolar lymphoid sheath (PALS), germinal center (GC) and marginal zone (MZ). E-F: spleen sections of paracetamol treated group notice thickened capsule (Head arrow), deteriorated white pulp (arrows), atrophic lymphoid follicle (ALF), tingle bodies (black circles), dilated splenic sinuses (SS), congested sinuses (Asterisk), histiocytes (H). H-I: spleen section of paracetamol and frankincense oil-treated group showing normal capsule thickness and regular in size oval in shape lymphoid follicle (LF) with peripheral central arteriole (CA) increased number of histiocytes (H).

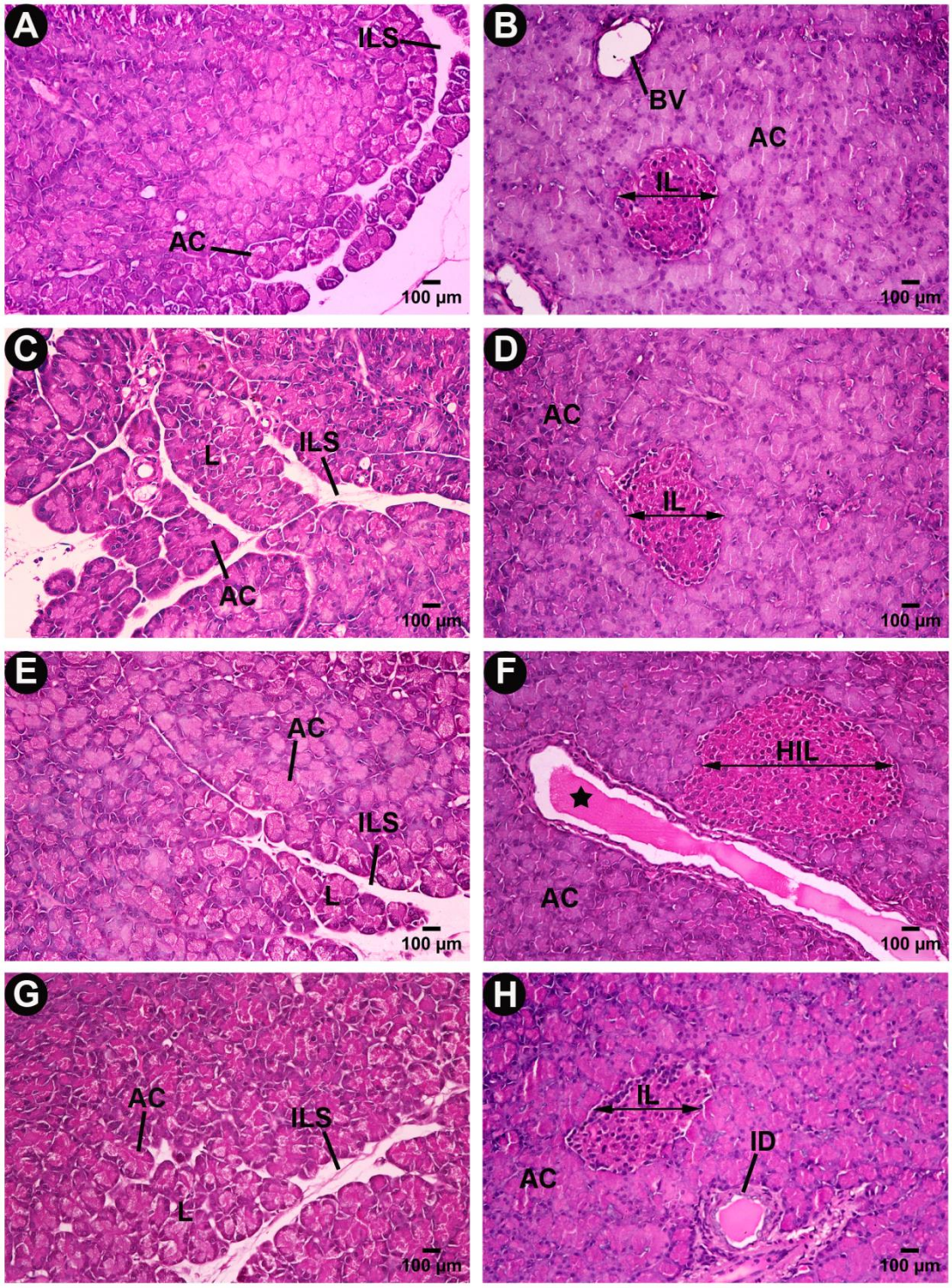

Figure 3: pancreatic sections of different experimental groups (H&E Stained; X400). Pancreatic section of the control group (A-B) and frankincense oil-treated group (C-D) showing normal architecture of the pancreas separated into lobules (L) with thin interlobular septa (ILS), pancreatic acini (AC), islets of Langerhans (IL), blood vessel (BV). E-F: Pancreatic section of paracetamol treated group showing normal acini (AC), dilated congested blood vessel (Asterisk), and hypertrophoid islets of Langerhans (HIL). G-H: Pancreatic section of paracetamol and frankincense oil-treated group showing normal pancreatic acini (AC), islets of Langerhans (IL), and congested interlobular duct (ID).

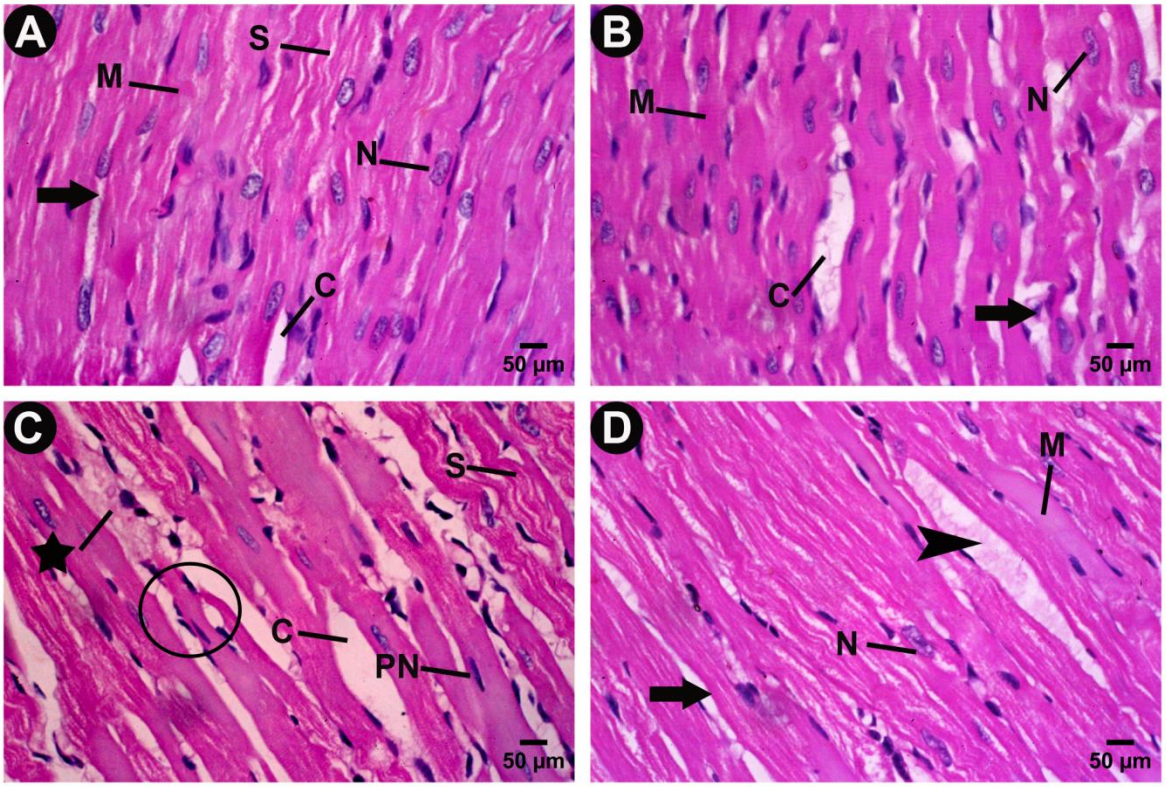

Figure 4: Heart sections of all experimental groups (H&E Stained; X1000). Heart sections of the control group **(A) and** frankincense oil-treated group (B) showed cardiac myocyte (M), centrally located oval nuclei **(N),** striations **(S),** interstitial space between the muscle fibers **(arrow),** few capillaries (C). C: Heart section of paracetamol treated group showing disorganization and fragmentation of myocyte (circle), pyknotic nucleus (PN), increased interstitial space with congestion (Asterisk), and dilated blood capillaries (C). D: Heart section of paracetamol and frankincense oil-treated group showing almost normal histological appearance of cardiac myocyte (M), dilated congestion capillary (Head arrow), interstitial space (arrow).

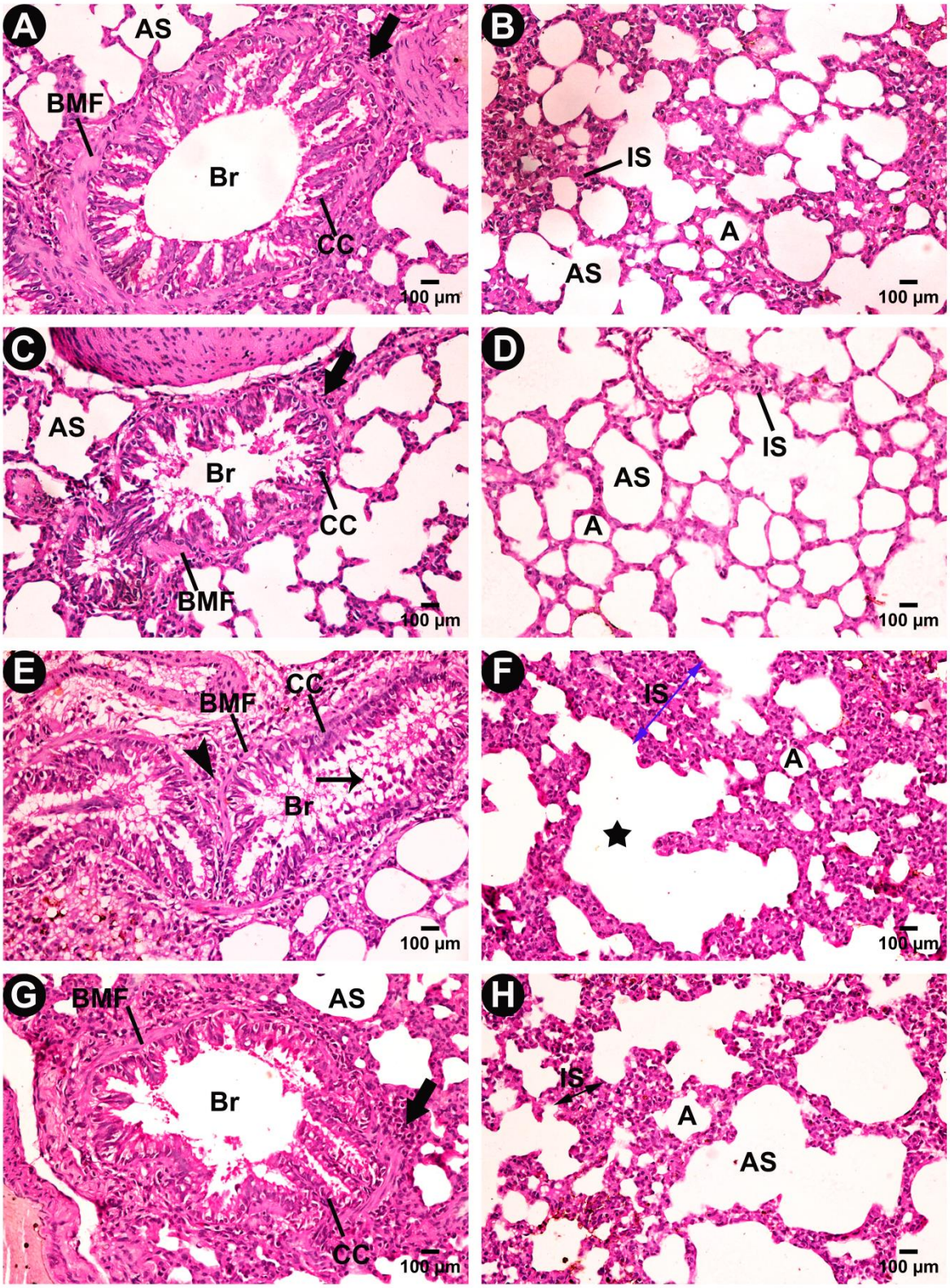

Figure 5: Lung sections of different experimental groups (H&E Stained; X400). Lung sections of control (A&B) and frankincense oil-treated group (C&D) showing bronchiole (Br) lined with columnar cells (CC) and surrounded by bronchiole muscles fibers (BMF), peribronchiolar tissue (black arrow), alveolar sacs (AS), alveoli (A), interalveolar septa (IS). E-F: Lung sections of paracetamol treated group showing, congestion of bronchiole (thin arrow), hypertrophoid peribronchiolar tissue (Head arrow), thickened interalveolar septa (blue double head arrow), emphysema (Asterisk). G-H: Lung sections of the paracetamol and frankincense oil-treated group showed mild dilated alveolar sacs (AS), and normal interalveolar septa (IS).

Discussion

Paracetamol, an over-the-counter analgesic and antipyretic drug, is widely used for pain relief, headache management, and fever reduction. However, its long-term usage has been associated with potential toxicity (33). Paracetamol-induced toxicity arises from its ability to reduce glutathione levels, leading to the creation of reactive oxygen species (ROS) and disrupting the delicate balance between ROS production and antioxidant capacity, resulting in cellular and tissue damage (3, 34, 35).

To counteract the adverse effects of paracetamol, the use of antioxidants has been suggested (36). Frankincense oil, in particular, contains interactive constituents that have shown the ability to absorb, and neutralize free radicals, augment antioxidant capacities, and restore balance in disrupted oxidative damage-defense systems (16).

The findings of this study highlight the detrimental effects of paracetamol-induced oxidative stress, as evidenced by the increased release of myeloperoxidase (MPO) and decreased total antioxidant capacity (TAC). These results indicate the presence of inflammation, infiltration of neutrophils, production of ROS, tissue damage, and impaired antioxidant defense mechanisms resulting from paracetamol intoxication (37, 38). These observations align with previous studies that have reported similar outcomes, including increased oxidative stress markers, reduced TAC levels (3, 5, 34), and enhanced lipid peroxidation, leading to tissue damage and compromised antioxidant defense (39).

In contrast, the coadministration of paracetamol and Frankincense oil in this study demonstrated a reduction in oxidative and inflammatory injuries induced by paracetamol. This was evident through the increased TAC levels and decreased MPO levels observed. These beneficial effects can be attributed to the presence of active ingredients in Frankincense oil, such as boswellic acid (40), limonene, α-pinene (11), phenols, flavonoids (41), and triterpenes (42), which possess antioxidant properties, act as free radical scavengers, inhibit lipid peroxidation, and exhibit anti-inflammatory activities.

Paracetamol intoxication caused hematotoxicity, reflected in abnormal blood cell counts and impaired function. It resulted in decreased RBC counts, hemoglobin concentration, hematocrit, MCH, MCV, and platelet count, and increased MCHC indicates the detrimental impact of paracetamol on hematopoietic parameters. These findings align with previous studies that have linked paracetamol toxicity to oxidative stress-induced damage in immune and hematopoietic organs (43). Hemolytic anemia and thrombocytopenia were also reported as consequences of paracetamol-induced inhibition of RBC synthesis (2, 5, 35, 44, 45). Reduced RBC size, hampered heme production in the bone marrow, or slowed RBC generation are possible causes of the decreased hemoglobin level (46).

In the current study, microcytic cells, tear drop cells, and fragmented erythrocytes observed in blood smears of the paracetamol group further support the presence of iron deficiency, bone marrow dysfunction, and hemolysis (47, 48). These morphological changes in RBCs provide additional evidence of the hematological abnormalities associated with paracetamol intoxication.

In terms of white blood cell (WBC) counts the administration of paracetamol led to a decrease in lymphocytes and an increase in total WBC count, neutrophils, and monocytes. These alterations suggest potential impairments in immune response and antibody production (2). The liver damage induced by paracetamol overdose and the subsequent release of toxic metabolites is known to trigger an innate immune response, leading to the activation of leukocytes and dysregulation of immune cell activity (5, 6, 46). Lymphocytopenia, indicative of a weakened immune system, has also been reported in animals exposed to paracetamol (48).

In this study, simultaneous administration of paracetamol and frankincense oil restored blood cell counts and hemoglobin levels to normal compared to the paracetamol-treated group. This indicates that frankincense oil may protect against hepatic injury

and associated blood cell abnormalities (49). Moreover, frankincense oil effectively prevented or reversed thrombocytopenia induced by paracetamol, suggesting its potential to enhance platelet production. These results align with a study by Ismail, Abdelnour (50), who reported an increase in platelet count in animals fed a frankincense-enriched diet. Overall, frankincense oil exhibits beneficial effects on blood cell parameters, offering potential protective and therapeutic benefits.

The spleen, an important organ in the immune system, plays a role in blood filtration, recycling old RBCs, and retaining WBCs and platelets (44). In this study, the administration of paracetamol led to distortion and shrinkage of the spleen's white pulp. Similar findings were reported by Abbasi, David (6), who noticed lymphocyte population decline, white pulp shrinkage, and cellular deformation due to toxic metabolites from paracetamol overdose. Additionally, fenpropathrin intoxication in rats resulted in splenic and thymus atrophy, characterized by significant lymphocyte depletion, necrosis, apoptosis, and the presence of intra-cytoplasmic fragments of apoptotic lymphocytes in the white pulp (51). These findings highlight the detrimental effects of paracetamol on the spleen and its immune-related functions.

Simultaneous administration of paracetamol and frankincense oil prevented spleen damage in this study. The group receiving both substances showed stimulated lymphoid follicular activation and sinus histiocytosis in the spleen. Previous studies have reported similar findings, where natural extracts, such as cashew extract (44), have shown the ability to reverse paracetamol toxicity by stimulating lymphocyte and histiocyte production, enhancing the immune system's defense capabilities. Frankincense supplementation has been associated with increased activation of B- lymphocytes, and T-lymphocytes and the production of antibodies (IgG and IgM) (9).

Paracetamol administration in this study led to hyperglycemia, reduced insulin levels, and impaired pancreatic cell function marked by decreased HOMAB values. It also resulted in islets of Langerhans hyperplasia. The decrease in pancreatic cell function (HOMA-B) is related to the development of diabetes (52). These findings are consistent with the observed increase in serum glucose levels and reduced pancreatic cell function in paracetamol-intoxicated animals (5). Previous studies have shown that oxidative damage caused by monosodium glutamate can lead to reduced antioxidant enzymes, pancreatic cell damage, islet hyperplasia, elevated blood insulin levels, and glucose leakage from tissues (53). These effects can be attributed to oxidative stress-induced injuries to pancreatic islet β-cells, overproduction of mitochondrial ROS, and insufficient antioxidant enzymes in the β-cells (54).

In the present study, simultaneous administration of frankincense oil and paracetamol preserved normal pancreatic tissue structure and function to some extent, as indicated by normal islet size, lower glucose levels, higher insulin levels, and HOMA-B value. The antioxidant effects of frankincense may be responsible for this protection, which restores the oxidation state, regenerate liver cells, and improve the architecture of the islets of Langerhans (41). Frankincense administration has been shown to reduce fasting glucose levels by inhibiting carbohydrate digestion, glucose absorption, and glucose release from the liver (55). Treatment with frankincense has also been found to significantly decrease fasting glucose levels and increase insulin secretion in diabetic patients (56). These findings imply that frankincense supplementation may represent a promising treatment strategy for managing glucose metabolism and insulin resistance in diseases like diabetes.

The lipid profile blood tests conducted in this study served to identify lipid abnormalities and assess the risk of cardiovascular diseases using atherogenic indices including ACC, CRR, CRI-2, and RIP. Elevated levels of LDL, TG, and atherogenic indices along with decreased levels of HDL, were observed in the paracetamol-treated group, indicating the presence of dyslipidemia, which represent a substantial risk factor for numerous heart conditions.

These findings were consistent with previous studies that reported similar changes in lipid levels due to paracetamol toxicity conditions (3, 5, 37, 39). The increase in atherogenic indices indicated an augmented hazard of heart-related diseases (30, 57).

Additionally, in the present study, myocardial damage, interstitial space congestion, and blood capillary dilation were observed in association with elevated LDH levels in the paracetamol-treated group. The elevation of LDH activity, which is indicative of myocardial damage, was also reported in previous research (30). These results are in line with the previous research carried out by Latif, Assar (35) who reported the paracetamol-intoxicated group displayed deterioration and vacuolation in myocytes with significantly congested cardiac capillaries. This is attributed to the accumulation of intracellular Ca2+ leading to the activation of phosphofructokinase and anaerobic glycolysis that results in the production of lactate, with oxidative damage causing a loss of Ca2+ homeostasis, which marks an irreversible late stage of cell death as a result of paracetamol toxicity (46). These results highlight the detrimental effects of paracetamol on lipid metabolism and cardiac health, emphasizing the need for monitoring and intervention to mitigate the risk of cardiovascular diseases in individuals exposed to paracetamol toxicity.

In the present study, simultaneous administration of paracetamol and frankincense oil resulted in HDL levels significantly elevated while TG, cholesterol, LDL, and atherogenic indices significantly declined compared to the paracetamol-treated group, indicating the hypo-lipidemic and cardioprotective effects of frankincense oil against paracetamol toxicity. This effect on the blood lipid profile may be attributed to the ability of plant fibers to reduce cholesterol absorption, increase TG and LDL clearance, and enhance HDL release (58). The supplementation with frankincense has also been shown to significantly decrease cholesterol, TG, and LDL levels, possibly through the support of enzymes involved in cholesterol conversion to bile acids, leading to a decrease in cholesterol levels (50). Previous studies have reported the hypolipidemic effect of frankincense, which can be attributed to its antioxidant capacity, improvement of β-cell function, stimulation of insulin secretion, and steroid-like properties that lower lipid profiles (40, 41, 55).

The administration of paracetamol during pregnancy can lead to toxic damage in both maternal and fetal lung organs, increasing the risk factor for asthma (59). In the present study, administration of paracetamol resulted in the thickening of interalveolar walls, the collapse of alveoli, and decreased aeration area, accompanied by reduced total antioxidant capacity and elevated levels of myeloperoxidase (MPO). This pulmonary damage is attributed to increased lipid peroxidation, which is mediated by free oxygen radicals and a decrease in antioxidant factors. Similar findings were reported in a study on valproic acid-induced pulmonary toxicity, characterized by the destruction of alveolar walls, alveolar ducts, and the presence of red blood cells in the lumen, along with increased MPO levels (60). Previous research on pulmonary toxicity induced by substances such as amiodarone and nicotine also revealed distorted lung morphology, thickened interalveolar septa, emphysema, leukocyte infiltration, interstitial tissue proliferation, and vascular congestion (61, 62).

In contrast, simultaneous administration of paracetamol and frankincense oil in the present study prevented paracetamol-induced pulmonary toxicity, as evidenced by the presence of normal alveolar sacs and spaces, and mild thickening of thin-walled alveoli. Similar protective effects on lung architecture were observed with frankincense extract treatment in rats exposed to benzo(a)pyrene, where normal alveolar sacs and spaces, and alveoli with thin walls were observed, with only a few collapsed pulmonary alveoli with thick walls (10, 12). These findings suggest the potential of frankincense oil and its main component, boswellic acid, in improving lung architecture and protecting against pulmonary toxicity induced by substances such as paracetamol and benzo(a)pyrene. Further research is needed to clarify the basic mechanisms and explore the

therapeutic applications of frankincense in lung-related disorders.

Conclusion

This research highlights the detrimental effects of paracetamol toxicity on various organs, including antioxidant, hematological, lipid, cardiovascular, pancreatic, pulmonary, blood, and spleen. Paracetamol caused oxidative stress, hematotoxicity, dyslipidemia, myocardial damage, impaired pancreatic function, pulmonary toxicity, and spleen dysfunction. Interestingly, the coadministration of paracetamol and frankincense oil showed promising results in mitigating paracetamol toxicity. Frankincense oil exhibited antioxidant properties, protecting against oxidative stress and inflammation. It restored normal hematological parameters, preserved pancreatic structure and function, improved lipid profiles, and prevented heart, spleen, and lung damage. These findings suggest the potential of frankincense oil as a therapeutic intervention for paracetamol-induced toxicity.

Overall, this research emphasizes the need for caution in paracetamol use to minimize organ damage. It suggests exploring natural compounds like frankincense oil as adjunctive therapies to counteract paracetamol's toxic effects and enhance health outcomes. Further studies should focus on understanding the underlying mechanisms and conducting clinical trials to evaluate frankincense oil's efficacy and safety in humans.

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