DOI:10.21608/pssrj.2025.342926.1327

أظهر تأثيرات مضادة للأكسدة وكسحية ومضادة) Boswellia serrataاللبان المر(
للالتهابات : تطبيق على التهاب المفاصل الروماتويدى فى الفئران

Frankincense (*Boswellia serrata*) exhibits antioxidant, scavenging and anti-inflammatory effects: Application on rheumatoid arthritis in rat model

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المستخلص

التهاب المفاصل الروماتويدي هو حالة التهابية تؤثر بشكل رئيسي على بطانة المفاصل الزليلية، مما يؤدي إلى تآكل العظام، وفقدان الغضاريف بشكل لا رجعة فيه،وأحيانًا يؤدي إلى إعاقة دائمة. تناولت هذه الدراسة الأنشطة الحيوية والمحتوي الكيميائي للبان المر (Boswellia serrata) وتأثيره على الفئران المصابة بالتهاب المفاصل الروماتويدي. أُجريت الدراسة على ثلاثين فأرًا بالغًا تم تقسيمهم إلى خمس مجموعات متساوية. كانت المجموعة الأولى هي المجموعة الضابطة الطبيعية (عدد: 6) بينما تم معالجة المجموعات الأربع الأخرى (6 فئران في كل منها) بـ 0.1 مل من مادة مساعد فرويند الكامل التى تم حقنها في المفصل الأيمن لكل فأر، وتم تكرار الحقن بعد ذلك بسبعة أيام ولم تظهر علامات الالتهاب إلا بعد 14 يوم من الحقن. تم تغذية المجموعة الضابطة الطبيعية والمجموعة الضابطة المصابة على الوجبة القياسية في حين تم تغذية المجموعات الثلاث المتبقية على نفس الوجبة مع إضافة تركيزات مختلفة من اللبان المر وهى 2.5% و5% و7.5% لمدة 28 يوم. تم تقدير كلا من الفينولات ، الفلافونويد ، التربينويد ، التراي تربينويد ،المواد المضادة للأكسدة الموجودة فى اللبان المر. بعد انتهاء التجربة تم تقدير كلا من الجلوبولين المناعي، المالوندالدهيد، الأجسام المضادة النووية، الأجسام المضادة للببتيد السيتروسي الحلقي، عامل الروماتويد، بروتين C التفاعلي، معدل ترسيب كريات الدم الحمراء، كرياتين فوسفو كينيز، إنزيم الجلوتاثيون بيروكسيديز، والكتاليز. بينت النتائج ان اللبان المر غني بكلا من الفينولات ، الفلافونويد ، التربينويد ، التراي تربينويد ،المواد المضادة للأكسدة . كما أظهرت النتائج أن نسبة كلا من الجلوبيولين المناعى (IgG) وبروتين C التفاعلي (CRP) فى الفئران المصابة قلت بنسبة 11.28، 46.42% عند تركيز 7.5% من اللبان المر بينما زادت نسبة الجلوتاثيون بيروكسيديز (GPX) بنسبة 85.71% عند نفس التركيزوذلك مقارنة بالمجموعة الضابطة المصابة. كما أشارت هذه النتائج إلى أن للبان المر تأثيرات مضادة للأكسدة وكسحية ومضادة للالتهابات فى الفئران المصابة بالتهاب المفاصل الروماتويدى.

الكلمات المفتاحية:

بروتين سي التفاعلي، الكتاليز، الأمراض المناعية الذاتية، عامل الروماتويد، جلوتاثيون بيروكسيد، معدل ترسيب كريات الدم الحمراء، الجلوبيولين المناعى.

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Abstract

Rheumatoid arthritis is an inflammatory condition that mainly damages the synovial joint lining, resulting in bone erosion, irreversible cartilage loss, and occasionally permanent disability. This study looked at the biological activities and bioactive chemical content of frankincense powder (*Boswellia serrata*) and how it affected rats with rheumatoid arthritis. The study was conducted on thirty adult rats were split up into five equal groups. The first group was the normal control group (n: 6), and the remaining four groups n=24 (6 rats in each group) was injected with 0.1 ml of total adjuvant into the right tarsal joint for each animal, which was repeated 7 days later and became. Rheumatoid arthritis signs appeared after 14 days. The normal control group and the model control group obtained a base diet, while the other three rheumatoid arthritis groups obtained a base diet with 2.5, 5, and 7.5% frankincense powder daily for 28 days. Total phenolics, flavonoids, terpenoids, triterpenoids, and antioxidants activity of frankincense powder were determined. At the conclusion of the trial, Immunoglobulin, malondialdehyde, antinuclear antibodies, anti-cyclic citrullinated peptide antibodies, rheumatoid factor, C-reactive protein, erythrocyte sedimentation rate, creatine phosphokinase, glutathione peroxidase, and catalase were measured. The results of the study indicated that the frankincense powder was rich in total phenolics, flavonoids, terpenoids, triterpenoids and antioxidant. Frankincense powder treatment at 7.5% resulted in a significant ($p \leq 0.05$) reduction in Immunoglobulin (IGG) and C-reactive protein (CRP) by 11.28% and 46.42%, respectively compared to the model control group. However, Glutathione peroxidase (GPX) was significantly ($p \leq 0.05$) increased in rheumatoid arthritis rats treated with 7.5% of frankincense powder by 85.71% compared to the model control group. These results suggested that frankincense exhibits antioxidant, scavenging and anti-inflammatory effects on rheumatoid arthritis in rats.

Keywords:

C-reactive protein، Catalase, Autoimmune disease, Rheumatoid factor, glutathione peroxidase, erythrocyte sedimentation rate, immunoglobulin

Introduction

Rheumatoid arthritis (RA) is an inflammatory disease characterized by severe inflammation of the joints and tissues Guo *et al*., (2018). Autoantibodies against the fragment crystallizable region (Fc region) of immunoglobulin IgG, rheumatoid factor, and anticitrullinated protein antibodies (ACPA) are produced in this inflammatory disease and are used to categorize RA patients as either seropositive or seronegative according to Guo, (2018). Although its precise etiology is still unknown, a few variables may raise the chance of developing RA. Thus, it is essential to reduce the incidence and consequences of arthritis in order to plan for future clinical and public health requirements according to Hootman *et al*., (2016). Poorly managed RA causes severe morbidity and mortality due to permanent bone and joint destruction, as well as extra-articular symptoms such rheumatoid nodules, cardiovascular problems, pericarditis/pleuritis, and vasculitis according to Figus, (2021). Non-steroidal anti-inflammatory drugs (NSAIDs), corticosteroids, disease-modifying antirheumatic medications (DMARDs), and biological response modifiers are among the medications frequently used to treat RA, however they are linked to a number of negative side effects according to Kumar*et al*.,(2019). Side effects connected to DMARDs are stomatitis, liver, kidney, gastrointestinal tract malfunctioning, and bone marrow suppression as mentioned by Pirotta, (2010). Therefore, there is an need to explore other alternative and complementary anti inflammatory therapies that traditional medicines, medicinal herbs and their phytochemicals provide, but have less adverse impact on human health, which needs to be emphasized, as noted by Gandhi *et al*., (2022). Empty *et al*., (2024) note that people suffering from gout, osteoarthritis, sciatica, and RA can benefit from the use of frankincense, a herbal treatment that has a pain relieving and inflammation reducing effect. Among the bioactive components of Folwer's frankincense, boswellic acid and acetyl-11-keto-ß-boswellic acid stand out as the most potent 5-lipoxygenase (5-LOX) synthesis inhibitors. Considering that 5-LOX is one of the central enzymes in the leukotriene biosynthetic pathway and is overexpressed in RA synovium, the 5-LOX signaling pathway is crucially involved in inflammatory processes. Thus, the reduction of 5-LOX activity by boswellic acid could reduce overall inflammatory symptoms in arthritis patients, according to Gandhi *et al*., (2022). Finding out how well frankincense powder worked as an adjuvant with cortisone to reduce pain in rats with RA was the goal of this investigation. Additionally, the determination of bioactive compounds content and biological activities in frankincense will be in the scope of this investigation.

2. Materials and methods

2.1. Materials

2.1.1.Frankincense sample

Frankincense was identified by the Department of Botanical Taxonomy, Faculty of Agriculture, Menoufia University, after being purchased at a local market in Shebin El Kom, Menoufia Governorate, Egypt.

2.1.2.Chemicals and reagents

The infectious substance used in the experiment, known as Complete Freund's Adjuvant, was purchased from Sigma Chemicals in USA. The Morgan Company for Chemicals in Cairo, Egypt, supplied casein, the main protein source used to make the rats' diet. We obtained vitamins, salt mixtures, cellulose, choline chloride and L-methionine from the EL-Ghomhorya Company for Trading Drugs, Chemicals, and Medical Requirements in Cairo, Egypt. Alkan Medical Company in St. El-Dokki, Giza, Egypt, provided all biochemical test kits and reagents used in this study. All chemicals and reagents used in this study were of analytical grade or as pure as commercially available.

2.1.3. Animals

The laboratory animal department of the College of Veterinary Medicine at Cairo College in Egypt provided thirty adult male white albino rats of the Sprague-Dawley strain weighing 150 ±10 g and aged 10 weeks. The individual rats were kept in stainless steel cages in a well-ventilated environment with regulated standard settings such as a temperature of 20–23 °C, a humidity of 50–60% and a 12-hour light-dark cycle. Before the tests began, the animals were given one week to acclimatize.

2.2. Methods

2.2.1. Frankincense powder preparation

The frankincense was obtained in dried form, then milled to obtain powder form, packed in polyethylene bags ,and stored until used

2.2.2.Basal diet

Protein (10 %), corn oil (10 %), vitamin mix (1 %), mineral mix (4 %), choline chloride (0.2 %), methionine (0.3 %), cellulose (5 %) and the remainder is corn starch (69.5 %), which was developed in accordance with the AIN-93 diet by Reeves *et al*., (1993). The mixture of salts and vitamins was prepared according to Drury and Wallington, (1980).

2.2.3. Rheumatoid arthritis induction

Each rat was given an injection of 0.1 ml of a complete adjuvant into the right tarsal joint and this procedure was repeated seven days later. According to Taksande *et al*., (2017), signs of RA appeared after 14 days.
On day 14, the same individual used photographs of the afflicted rear limbs to blindly assess each rat's arthritis score. Kinne *et al*., (1995) utilized the images to assess the severity of each paw's arthritis based on the amount of erythema and edema in the periarticular tissue

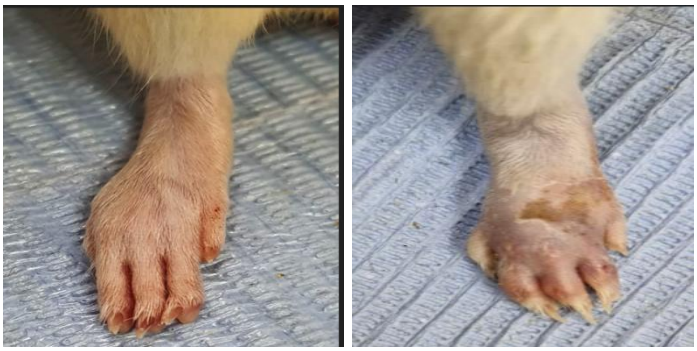


Photo 1: The changes in CFA-induced rat model of RA. A representative regular image of the RA rat's hind paw, showing obvious swelling 14 days after injection of CFA.

2.2.4. Experimental design

Thirty adult male white albino rats of the Sprague-Dawley strain, aged 10 weeks and weighing 150-160 g, were used for this experiment. All rats were fed a basal diet prepared according to the AIN-93 guidelines of Reeves *et al*., (1993) for 7 days. After the acclimatization period, the rats were divided into five equal groups. The first group was the normal control group and the other four groups were injected with 0.1 ml of total adjuvant into the right tarsal joint of each animal and this was repeated 7 days later. The normal control group and the RA model control group received a basal diet, while the remaining three RA groups received a basal diet in which the starch was replaced with 2.5, 5 and 7.5% frankincense powder, respectively.

2.2.5. Blood sampling

After fasting for 12 hours, the rats were anesthetized with diethyl ether at the end of the 28-day experiment. Blood samples were then taken and the serum was

separated by centrifugation. The serum was frozen and stored at 20°C for further analysis.

2.2.6. Estimated chemical composition of frankincense

Following the proper procedures specified in AOAC,(2006), the nutritional composition of the frankincense sample was examined using established techniques to ascertain moisture (oven drying), protein (total nitrogen), fat (Soxhlet), ash (Muffle furnace), and fiber (enzymatic gravimetric, AOAC 991.43). Total carbs were calculated using the difference method: Total carbohydrates (%) = 100 -(%moisture + %protein + %fat + %fiber + %ash). The accessible carbs were determined by subtracting crude fiber from total carbohydrates.

2.2.7. Total phenolic content (TPC)

Singleton and Rossi, (1965) used the Folin-Ciocalteu reagent to measure total phenolics. Gallic and equivalents (GAE) are used to express the results.

2.2.8. Total flavonoid content (TFC)

The amount of flavonoids in the powdered samples was calculated using a colorimetric test that was developed by Zhisen *et al*., (1999). Catechin and equivalents (CE) are used to express the results.

2.2.9. Total terpenoid and triterpenoid content

The Adrian *et al*., (2022) method was used to extract and measure total terpenoids. The Schneider *et al*., (2009) method was used to extract and measure the total triterpenoids.

2.2.10.Determination of total antioxidant activity by AA, DPPH,FRAP and ABT

The standard (α-tocopherol and BHT; Sigma Chemical Co., St. Louis, Mo) and sample powder extracts' antioxidant activity (AA) was assessed using the α-carotene bleaching (BCB) method, which follows Marco's, (1968) protocol. Al-Saikhan *et al*., (1995) used the following formula to determine antioxidant activity (AA) as a percentage of inhibition compared to control. The Katalinic *et al*., (2006) method was used to measure the frankincense's antioxidant activity against DPPH. Milligram equivalents of quercetin per milligram of dry weight were used to express the results.The FRAP assay is used to evaluate an antioxidant's ability to change ferric ions into ferrous ions. This assay determines the antioxidant capability of a sample by measuring its reduction potential. According to Munteanu and Apetrei, (2021), an increase in antioxidant activity is correlated

with an increase in reduction potential. Trolox milligram equivalents per gram of dry weight were used to express the results. According to Chen *et al*., (2004), the (ABTS/H2O2/HRP) technique for ABTS+ radical production was used to determine the total antioxidant activity. Trolox milligram equivalents per gram of dry weight were used to express the results.

2.2.11. Biochemical Analysis

In order to measure serum C-reactive protein (CRP), an enzyme-linked immunosorbent test was used. Scheld and Nathan, (2002). Engvall and Perlman, (1971) used the enzyme-linked immunosorbent assay (ELISA) technology to measure rheumatoid factors (RF).The erythrocyte sedimentation rate (ESR1-2) was measured using the procedures outlined by Sykes, (1948). An enzyme-linked immunosorbent test was used to measure serum anti-cyclic citrullinated peptide (anti-CCP) Avčin *et al*., (2002). Autoantibodies against IgG are known as RF.Since their discovery by Soltys *et al*., (1997), they are most likely the most researched antibodies. Glutathione peroxidase (GPx), malondialdehyde (MDA), and catalase (CAT) were measured in accordance with Jentezch *et al*., (1996), Hu, (1994), and Erel, (2004), respectively. The Burrels and Wells, (1977) and Granfors, (1979) protocols were used for the analysis of immunoglobulin M (IgM) and immunoglobulin G(IgG).

2.2.12. Paw volume

On days 0, 5, and 15, the injected limb's paw volume was measured by immersing it vertically in the plethymometer up to the lateral malleolus level. Using the formula, percentage inhibition = (Vc − Vt) × 100/Vc, where Vc is the mean change in paw volume of the arthritic control group and Vt is the mean change in paw volume of the treatment group, the mean change in paw volume and percentage inhibition of paw edema were determined according to Kumar *et al*.,(2019).

2.2.13. Histological examinations of the heart

Immediately after, cardiac muscle fibers were removed and preserved in 10% neutral formalin. The histological and histochemical investigations were processed using paraffin slices, which had a thickness of 5 µm. Sections were stained with Harris' haematoxylin and eosin for general histology Bancroft and Gamble, (2002). Drury and Wallington, (1980) used Schiff's periodic acid reagent (PAS) to identify polysaccharides, and Pears, (1977) used Mallory's trichrome stain to stain collagen fibers.

2.2.14. Histological examinations of joints

Excision of the right paw tissue and ankle joints was followed by their fixation in 10% formalin, decalcification in 5% nitric acid, and paraffin embedding. After being sliced to a thickness of 3 mm, the sections were flattened and affixed to the slides. They were examined for histopathological alterations using a light microscope after being stained with hematoxylin and eosin Renju *et al*., (2013).

2.2.15. X-ray

Using a standardized Larsen score, the rheumatologist who analyzed the radiographs assessed the joint injury's progression at the time of evaluation while being blind to the clinical and laboratory data. Common reference films were used to calculate the Larsen score Larsen *et al*., (1977). Radiograph analysis to determine prognosis is a recognized standard practice in RA Solymossy *et al*., (1999).

2.2.16. Ethical approval

In compliance with Egyptian animal welfare regulations, the ethical standards for the handling and care of animals were closely adhered to. The Institutional Animal Care and Use Committee (IACUC) of Menoufia University granted ethical approval for this investigation (Reg. No. MUFHE/F/NFS/37/24).

2.2.17 Statistical analysis

The Statistical Analysis System SAS, (2000) was used to perform analysis of variance (ANOVA) for a completely randomized design, and the results were presented as mean ± SD. The experimental groups were compared using a one-way analysis of variance (ANOVA) and Duncan's multiple-range test. P-values were considered statistically significant if they were less than 0.05.

Results and discussion

Estimated chemical composition of frankincense

Table (1) displayed the frankincense sample's approximate chemical composition. The results showed that frankincense is a good source of protein (2.45±0.24 g), carbs (90.9±7.2 g), calcium (41.2±0.7 mg), K (3.05±0.01 mg), and p (3.87±0.06 mg) per 100 g of the sample.The contents of fat, ash, fiber, and water were 0.47±0.05 , 0.7±0.07, 0.48±0.05, and 5±0.5%, respectively.

Table (1): Estimated chemical composition of frankincense

Components	Mean ± SD
Moisture (g/100g)	5±0.5
Protein (g/100g)	2.45±0.24
Fat (g/100g)	0.47±0.05
Ash (g/100g)	0.7±0.07
Carbohydrate (g/100g)	90.9±7.2
Fiber (g/100g)	0.48±0.05
K (mg/100g)	3.05±0.01
P (mg/100g)	3.87±0.06
Ca (mg/100g)	41.2±0.7

Bioactive compounds and biological activities of frankincense

Frankincense powder's biological activity and active compounds are shown in table (2). According to the data, frankincense powder has a moderate amount of total phenolics, a high content of terpenoids and triterpenoids, and a low amount of flavonoids in comparison to phenolics.A common assay for determining antioxidant capacity is DPPH. This suggests that frankincense powder has strong antioxidant action. According to the FRAP assay, which gauges antioxidant strength by measuring the decrease of ferric ions, frankincense powder exhibits moderate antioxidant activity.Another common antioxidant assay is ABTS, which uses frankincense powder to demonstrate comparatively high antioxidant activity. Furthermore, compared to the well-known antioxidant α-tocopherol (vitamin E), frankincense powder exhibits extremely strong antioxidant activity. These findings are consistent with those of Nwachukwu, (2020), who demonstrated that frankincense has been found to include a variety of phytochemicals, including triterpenoids, non-terpenoids, and boswellic acid. Using animal models, frankincense and its phytochemicals have demonstrated positive effects on inflammation-related disorders Governa *et al*., (2018). The essential oils and other bioactive components of frankincense have been linked to biological activity; among these, the boswellic acids have drawn the most interest Byler, (2018). The

pentacyclic triterpenoid compounds known as boswellic acids are thought to be the most pharmacologically active ingredients in frankincense Siemoneit, (2011). many studies have demonstrated that nonspecific inflammation and elevated free radical production are key factors in the development of RA Hadjigogos, (2003). The imbalance between prooxidants and antioxidants in RA may lead to the insufficiency of antioxidant defense mechanisms and the accelerated oxidative responses. It has been demonstrated that RA patients had twice as much protein damage from free radicals in their synovial fluid El- Karn *et al*., (2008).

Table (2) Bioactive compounds and biological activities of frankincense

Compounds	Amount	
	Range	Mean±SD
Total phenolics (mg GAE/100g, d.b.)	48.45 – 51.67	49.96±1.6
Flavonoids (mg CE/100g, d.b.)	0.82- 0.98	0.89±0.08
Terpenoids (mg linalool/100g^{-1} d.b.)	984.1 – 998.45	991.63±7.2
Triterpenoids (mg ursolic acid 100g^{-1}d.b.)	476.67 – 583.89	535.57±54.3
DPPH µ mol TE/g	1.42- 1.64	1.55±0.1
FRAP µ mol TE/g	3.24- 3.67	3.42±0.2
ABTS µ mol TE/g	7.43- 8.09	7.71±0.3
AA (%) α -tocopherol (50mg /ml)	91.27-93.72	92.45±1.2

Values are expressed as means± SD. GAE: Galic acid equivalents, CE: catechin equivalents. DPPH:2,2-Diphenyl-1-picrylhydrazyl. FRAP:Ferric Reducing Antioxidant Power ABST: 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid. AA: Antioxidant activities.

Impact of frankincense powder on immunoglobulin in normal and RA groups

Table (3) showed how different frankincense powder (FP) concentrations affected different immunoglobulin (Ig) levels in normal, model, and experimental groups that received FP at 2.5, 5, and 7.5% concentrations. RA rats were induced with Complete Freund's Adjuvant produced significantly increased ($p \leq 0.05$) levels of IG compared with the normal conrtol group indicating immune system activation. The model control group had higher levels of IG, which implies a dysregulation of the immune system in RA. These findings are entirely consistent with those of Millauer *et al*., (1999), who showed that RA patients had significantly higher levels of total IgE, IgG, and IgA than the normal control group.By promoting the synthesis of cellular mediators and specific immunoglobulins, the antigenic-complete Freund's adjuvant (CFA) activates the immune system according to Mythilypriya *et al*., (2008). Tumor necrosis factor (TNF-α) is said to be a key player in the alternation of cytokine production that occurs in RA, an autoimmune

inflammatory disease. Morever, increased nuclear factor kappa beta (NF-κβ) transcription by TNF-α is an important step that leads to the synthesis of more pro-inflammatory cytokines according to Abd-El- Moneim *et al.*, (2022). In contrast, FP treatment of the RA rat's diet significantly ($p \leq 0.05$) decreased the levels of (IGA, IGE, IGM, and IGG) in comparison to the model control group. According to Al-Harrasi, (2008) and Zhang *et al.*, (2013) who found that frankincense and its boswellic acid content is suspected of being responsible for the anti-inflammatory effects of frankincense. Additionally, it has been demonstrated that frankincense can block pro-inflammatory cytokines Kumar *et al.*, (2019). At the same table, it was observed that the RA rat's diet treated with 5 and 7.5% of FP was more effective ($P \leq 0.05$) in lowering immunoglobulin (Ig) levels than that treated with 2.5% of FP. For IgG and IgM the present data showed significantly reduced in RA rats treated with 2.5, 5 and 7.5% of FP by 4.61, 11.89 and 11.28% and by 3.28, 7.21, 8.89%, respectively compared to the model control group. From the previous results, it can be considered that the highest reduction in (IGA, IGE, IGM, and IGG) values was observed in the rats treated with 5 and 7.5% of FP, respectively. Overall, the data suggested that frankincense has immunomodulatory properties, helping to reduce the elevated IG levels associated with RA.

Table (3): Impact of frankincense powder on immunoglobulin in normal and RA groups

parameters	Normal control	Rheumatoid arthritis groups			
		Model control	FP (2.5%)	FP (5%)	FP (7.5%)
IGA (mg/dl)	$44.22^{d} \pm 0.77$	$51.64^{a} \pm 1.24$	$49.41^{b} \pm 1.29$	$47.3^{c} \pm 2.1^{c}$	$46.09^{c} \pm 1.67$
IGE (iu/ml)	$15.62^{c} \pm 1.69$	$29.33^{a} \pm 2.93$	$24.62^{b} \pm 2.75$	$18.51^{c} \pm 0.6^{c}$	$18.25^{c} \pm 1.69$
IGM (mg/dl)	$52.79^{c} \pm 1.84$	$60.6^{a} \pm 3.2$	$58.61^{ab} \pm 1.56$	$56.23^{bc} \pm 1.62^{bc}$	$55.21^{bc} \pm 1.53$
IGG (mg/dl)	$272.53^{d} \pm 1.55$	$331.1^{a} \pm 5.3$	$315.83^{b} \pm 4.65$	$291.7^{c} \pm 1.8^{c}$	$293.73^{c} \pm 2.35$

Values are expressed as means± SD; means in the same raw with different letters are significantly different ($P < 0.05$). **FP**: Frankincense powder, **IGA**: immunoglobulin A, **IGE** : immunoglobulin E , **IGM** : immunoglobulin M and **IGG** : immunoglobulin G.

Impact of frankincense powder on malondialdehyde, glutathione peroxidase, and catalase in normal and RA groups

Table (4) illustrated how various frankincense powder (FP) concentrations affect the levels of malondialdehyde (MDA), glutathione peroxidase (GPX), and catalase in the normal and RA groups.The levels of CAT and GPX in model control group were significantly decreased after (CFA) injection for RA while MDA،a biomarker of lipid peroxidation had the opposite trend. The decrease in the levels of CAT, and GPX, and the increase in MDA may be due to the cause of the oxidative stress resulting from exposure to (CFA). The results were in line with those of Umara *et*

***al*., (2014) who discovered that activity of CAT decreased significantly in model control group in the joints as compared to normal control group . The model control group showed a significant increase in MDA compared with the normal control group, indicating high oxidative damage. It's interesting to note that the current study showed that the MDA was significantly (p≤0.05) reduced in RA rats treated with 2.5, 5, and 7.5% of FP by 32.06, 54.31 and 51.09%, respectively compared to the model control group. The highest reduction in MDA value was observed in RA rats treated with 5 and 7.5% FP. these results come in harmony with Ata, (2022) who indicated that FP reduced MDA levels in rats, suggesting its protective role against oxidative damage. On the other hand CAT and GPX were significantly (p≤0.05) increased in RA rats treated with 2.5, 5, and 7.5% of FP by 49.12, 150.88, and 138.59%, and by 35.16, 93.4, and 85.71%, respectively compared to the model control group. Morever, it was observed that treating RA rat's diet with 5 and 7.5% FP was more effective (P≤ 0.05) in increasing CAT and GPX levels than that treated with 2.5% FP. From the previous results, it can be considered that frankincense protects against oxidative stress in RA.**

Table (4): Impact of frankincense powder on malondialdehyde, glutathione peroxidase, and catalase in normal and rheumatoid arthritis groups

parameters	Normal control	Rheumatoid arthritis groups			
		Model control	FP (2.5%)	FP (5%)	FP (7.5%)
MDA (nmol/ml)	$11.13^{d}\pm1.85$	$36.4^{a}\pm1.8$	$24.73^{b}\pm1.25$	$16.63^{c}\pm1.45$	$17.8^{c}\pm1.1$
GPX (mu/ml)	$112.33^{a}\pm1.53$	$45.5^{d}\pm3.5$	$61.5^{c}\pm1.5$	$88^{b}\pm3$	$84.5^{b}\pm2.5$
CAT(u/l)	$1.91^{a}\pm0.02$	$0.57^{d}\pm0.06$	$0.85^{c}\pm0.02$	$1.43^{b}\pm0.13$	$1.36^{b}\pm0.13$

Values are expressed as means± SD; means in the same raw with different letters are significantly different ($P < 0.05$). **FP**: Frankincense powder, **MDA**: malondialdehyde, **GPX**: glutathione peroxidase, and **CAT**: catalase

Impact of frankincense powder on Anti-nuclear antibody, Anti-Cyclic Citrullinated Peptide Antibody, Rheumatoid factor, and C- reactive protein in normal and RA groups

Data in Table (5) displayed that the effect of Frankincense powder (FP) on autoimmune markers of normal and model groups. According to the data, the model control group had significantly (p≤0.05) higher levels of anti-nuclear antibody (ANA), anti-cyclic citrullinated peptide antibody (Antic-cp), rheumatoid factor (RF), and C-reactive protein (CRP) than the normal and treated groups.These results are in accordance with Hussein *et al.*, (2024) who found that patients with RA had markedly elevated serum levels of CRP, RF, and Anti-CCP when compared to the normal control group. RF, CRP, and anti-CCP antibody levels significantly increased after receiving CFA injections in comparison to the normal group Mansour *et al.*, (2022). In addition to other symptoms like swollen

and painful joints, the autoimmune disease also causes considerable amounts of inflammation, which causes CRP levels to rise Geyer *et al.*, (2021). One of the criteria for classifying RA was a high RF titer, and for a long period, RF was the only serum antibody useful for diagnosis.However, compared to autoantibodies such antibodies against citrullinated proteins, RF's sensitivity and specificity for identifying RA patients were very low. Nevertheless, it has been proposed that RF could be a pathogen and a crucial component in controlling the illness Wernhoff, (2003). In contrast, the ANA, Anticcp, RF, and CRP levels were significantly ($p \leq 0.05$) reduced in RA rats treated with FP at concentrations of 2.5, 5, and 7.5%. For CRP, Anticcp and RF the present data showed significantly reduction in RA rats treated with 2.5, 5 and 7.5% of FP by 35.36, 54.23, and 46.42%, by 22.82, 44.39,and 41.08%, and by 44.13, 76.92, and 76.11%, respectively compared to the model control group. The highest reduction in ANA, Anticcp, RF, and CRP values was observed in the rats treated with 5 and 7.5% FP . Overall the data suggest that frankincense has a beneficial effect in reducing autoimmune and inflammatory markers associated with RA.

Table (5): Impact of frankincense powder on Anti-nuclear antibody, Anti-Cyclic Citrullinated Peptide Antibody, Rheumatoid factor, and C- reactive protein in normal and rheumatoid arthritis groups

parameters	Normal control	Rheumatoid arthritis groups			
		Model control	FP (2.5%)	FP (5%)	FP (7.5%)
ANA(Au/ml)	$0.14^{c}\pm0.01$	$0.46^{a}\pm0.01$	$0.34^{b}\pm0.01$	$0.33^{b}\pm0.02$	$0.3^{b}\pm0.02$
Anticcp(u/ml)	$5.5^{d}\pm0.3$	$12.05^{a}\pm0.85$	$9.3^{b}\pm0.5$	$6.7^{c}\pm0.4$	$7.1^{c}\pm0.3$
RF(Iu/ml)	$0.18^{c}\pm0.02$	$2.47^{a}\pm0.37$	$1.38^{b}\pm0.14$	$0.57^{c}\pm0.09$	$0.59^{c}\pm0.08$
CRP (mg/dl)	$1.74^{c}\pm0.03$	$4.61^{a}\pm0.09$	$2.98^{b}\pm0.15$	$2.11^{d}\pm0.08$	$2.47^{c}\pm0.22$

Values are expressed as means± SD, means in the same raw with different letters are significantly different ($P < 0.05$).**FP**: Frankincense powder, **ANA**: Antinuclear antibody, **Anticcp**: Anti-Cyclic Citrullinated Peptide Antibody, **RF**: Rheumatoid factor, and **CRP**: C- reactive protein.

Impact of frankincense powder on erythrocyte sedimentation rate in normal and RA groups

Table (6) shows the effect of frankincense powder (FP) on erythrocyte sedimentation rate for normal and RA groups. When rats were treated with Complete Freund's adjuvant, their levels of ESR1 and ESR2 were significantly higher ($p \leq 0.05$) than those of the normal control group, indicating the presence of higher inflammation. Hussein *et al.*,(2024) appear to support these findings in their report which noted that the ESR levels of RA patients were significantly greater than those of the normal control group. However, FP treating at 5 and 7.5% had significant ($p \leq 0.05$) suppressive effects on erythrocyte sedimentation rate when compared to the model control group. For ESR1 and ESR2 the present data

showed significantly decrease in RA rats treated with 2.5, 5 and 7.5% of FP by 20.12, 60.06, and 80.18%, and by 27.83, 61.17, and 72.33%, respectively compared to the model control group. The data in the same table showed that the highest reduction in ESR1 and ESR2 values was observed in the rats treated with FP at 7.5%. Evidence suggests that frankincense is useful in decreasing elevated ESR levels resulting from RA because it has anti-inflammatory characteristics. Both FP concentrations of 5% and 7.5% are quite successful at reducing ESR 1 and ESR 2, however, the 7.5% concentration has the most impact in bringing inflammation down to near-normal levels.

Table (6): Impact of frankincense powder on erythrocyte sedimentation rate in normal and RA groups

parameters	Normal control	Rheumatoid arthritis groups			
		Model control	FP (2.5%)	FP (5%)	FP (7.5%)
ESR 1 (mm /h)	0.33^{d}±0.58	3.33^{a}±1.15	2.66^{a}±1.15	1.33^{b}±0.58	0.66^{c}±0.58
ESR 2 (mm /h)	0.66^{c}±0.58	6^{a}±1	4.33^{a}±1.53	2.33^{b}±0.58	1.66^{b}±0.58

Values are expressed as means±SD; means in the same raw with different letters are significantly different (P<0.05). **FP**: Frankincense powder and **ESR**: erythrocyte sedimentation rate.

Impact of frankincense powder on creatine phosphokinase in normal and RA groups

Table (7) illustrated how frankincense powder (FP) affects the levels of creatine phosphokinase (CPK) in the normal and RA groups. CPK levels in the model control group are significantly higher (p≤0.05) than in the normal control group, indicating increased inflammation or muscle injury that is commonly associated with RA. In contrast, rats treated with FP at 2.5, 5, and 7.5% substantially had lower levels of CPK than the model control group. For CPK the present data showed significantly decrease in RA rats treated with 2.5, 5 and 7.5% of FP by 9.52, 25.85, and 28.57%, respectively compared to the model control group. These findings also demonstrated that rats given FP at 5 and 7.5% had the largest decrease in CPK value.According to the data lower CPK levels indicated that frankincense protects against inflammation or muscle injury in RA. Boswellic acids, which are included in frankincense, inhibit pro-inflammatory enzymes to reduce inflammation in a different situations according to Viswanad *et al.*, (2014).

Table (7): Impact of frankincense powder on Creatine phospho Kinase in normal and RA group

parameter	Normal control	Rheumatoid arthritis groups			
		Model control	FP (2.5%)	FP (5%)	FP (7.5%)
CPK(u/l)	134.5^{d}±2.5	220.5^{a}±8.5	199.5^{b}±3.5	163.5^{c}±11.5	157.5^{c}±5.5

Values are expressed as means± SD; means in the same raw with different letters are significantly different (P < 0.05). **FP**: Frankincense powder and **CPK**: Creatine phospho Kinase

Impact of frankincense powder on paw volume in normal and RA groups

The differences between the frankincense powder concentrations influence on the paw volumes of both the control and RA groups was illustrated in Table (8). After the CFA injection, the model control group showed a remarkable increase ($p \leq 0.05$) in their paw volume, which was significantly larger than that of the normal control group. This increase may suggest the edema or swelling that is seen with inflammatory processes in rheumatoid arthritis.These findings concur with those of Kumar, *et al.*, (2019) who reported that all CFA-induced arthritic groups had significantly higher paw volume values. Complete Freund's adjuvant injection causes edematous inflammation, increased vascularity owing to vasodilation, and a noticeable infiltration of inflammatory cells in comparison to the normal group according to Saleem *et al*., (2020). Articular cartilage deformation is caused by RA, an inflammatory systemic disease that involves immunological dysregulation and inflammation.Multiple joints are affected, resulting in noticeable disabilities Abd-El-Moneim *et al.*, (2022). In contrast, rats treated with FP at 2.5, 5, and 7.5% had significant ($p \leq 0.05$) decreased paw volume compared to the model control group. Paw volume was significantly ($p \leq 0.05$) decreased in RA rats treated with 2.5, 5, and 7.5% of FP by 22.87, 35.32, and 52.6%, respectively compared to the model control group. These findings follow the same pattern as those of Fan *et al*., (2005) who demonstrated that frankincense decreased paw volume and arthritic scores in rats.The rats given 7.5% FP had the largest decrease in paw volume, according to the data in the same table. Overall, frankincense helps to reduce inflammation-related swelling in RA.

Table (8): Impact of frankincense powder on paw volume in normal and rheumatoid arthritis groups

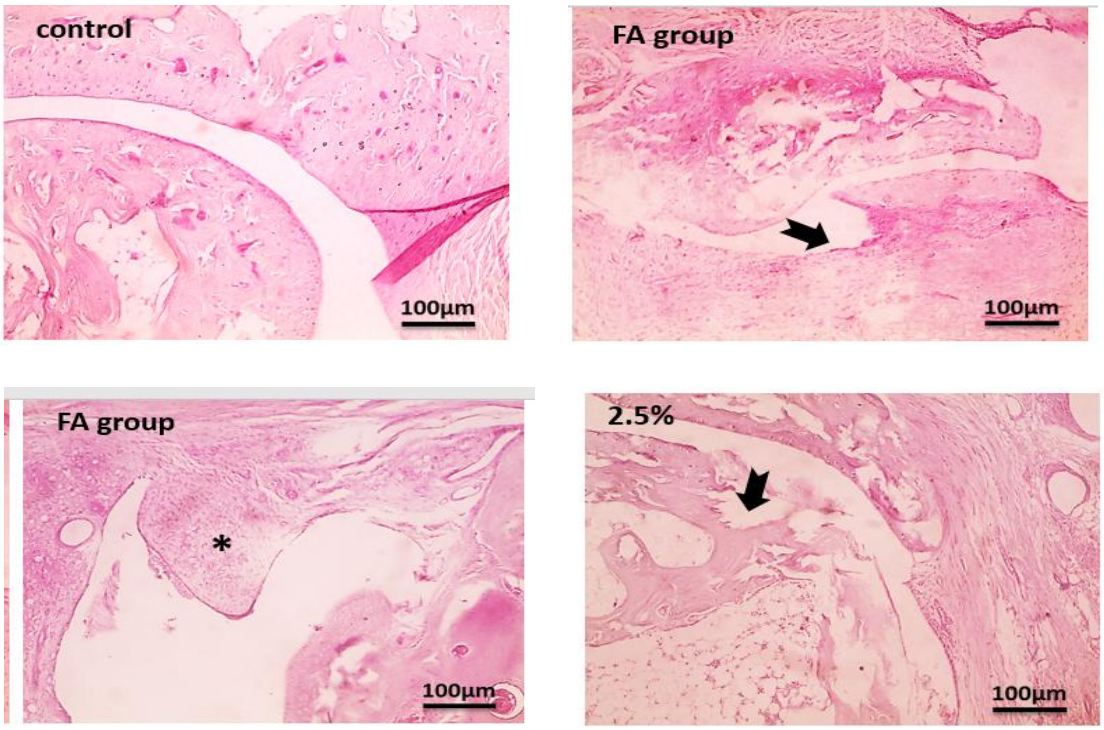
parameter	Normal control	Rheumatoid arthritis groups			
		Model control	FP (2.5%)	FP (5%)	FP (7.5%)
paw volume	$3.48^{e}\pm0.03$	$7.87^{a}\pm0.04$	$6.07^{b}\pm0.12$	$5.09^{c}\pm0.03$	$3.73^{d}\pm0.06$

Values are expressed as means± SD; means in the same raw with different letters are significantly different ($P < 0.05$). **FP**: Frankincense powder.

Impact of frankincense powder on histological examination of paw tissues of normal and RA rats

The impact of frankincense powder on histological examination of the paw tissues of both normal and RA rats was demonstrated in Figure (1). There are no indications of inflammation in the peri-articular tissues, and the paw tissues in the normal group display normal articulating surfaces. Paw tissues in the model group exhibit severe inflammation and synovial membrane hyperplasia together with

notable articular cartilage loss and ulceration (thick black arrow). These findings are entirely consistent with those of Abd-El- Moneim *et al*., (2022) who showed that Proliferation of synovial membrane fibroblasts is caused by prolonged production of TNF-α in the joint and serum. Rheumatoid arthritis (RA) is a chronic autoimmune inflammatory disease that is common around the world. It is characterized by a hyperplastic synovial membrane that can destroy nearby bone and articular cartilage in joints according to Feldmann *et al*.,(2001). In the 2.5% FP treated group, paw tissues exhibit focal articular cartilage degradation and ulceration (thick black arrow) with moderate hyperplasia of synovial membrane (thin black arrow). Paw tissues In the 5% FP treated group exhibit a mild hyperplasia of the synovial membrane (thin black arrow). Finally, Paw tissues are showing normal articulating surfaces with no signs of inflammation in peri-articular tissues in treated group with 7.5% FP. ɑ- and β-boswellic acid, as well as other pentacyclic triterpenic acids, give frankincense its anti-inflammatory properties. Boswellic acid is involved in the inhibition of inflammation induced by factors such as histamine,leukotriene, 5-lipooxygenase, human leukocyte elastase,prostaglandins, tumor necrosis factor,cytokines, and free oxygen radicals Almeida-da-Silva *et al*., (2022).



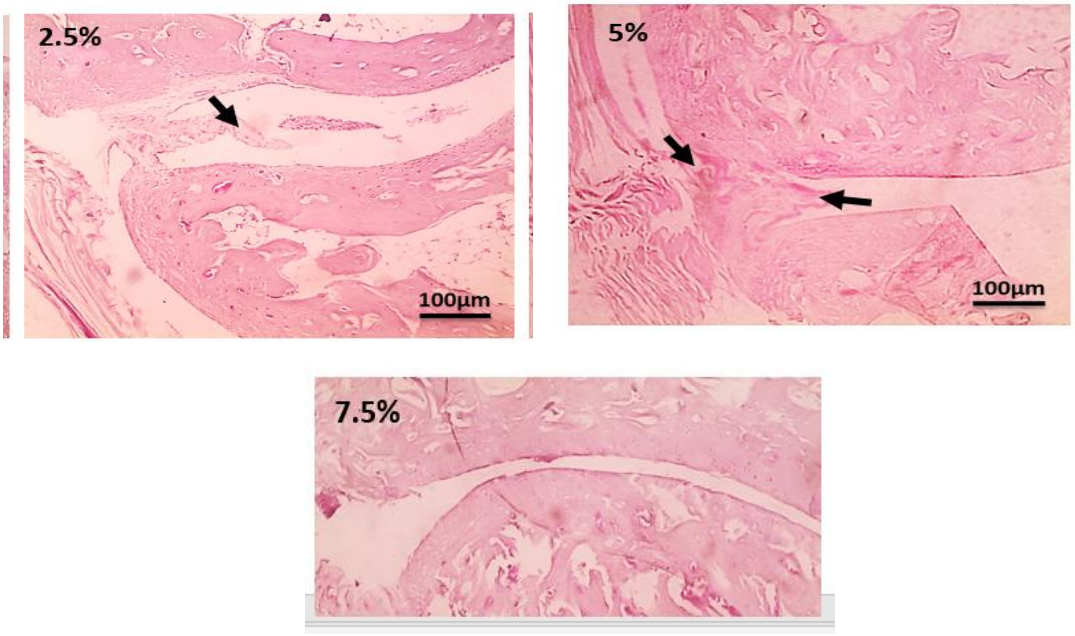
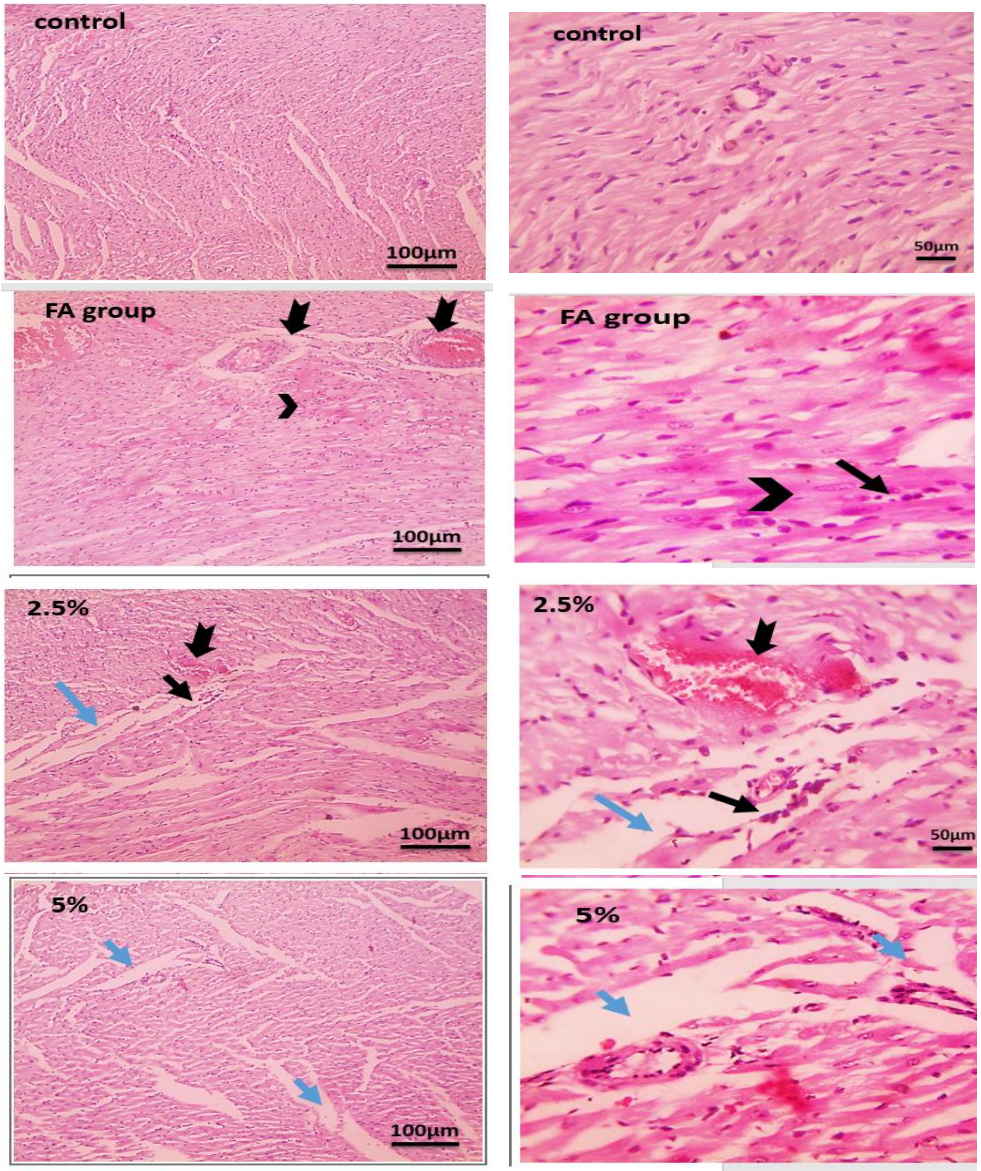


Figure (1): Paw tissues of all experimental groups
Control group :normal group, **FA group**:model group, **2.5% group:** RA rats treated with 2.5% frankincense powder, **5% group**::RA rats treated with 5% frankincense powder, and **7.5% group**: RA rats treated with 7.5% frankincense powder

Impact of frankincense powder on histological examination of heart of normal and RA rats

Figure (2) demonstrated how frankincense powder (FP) affected the histological examination of the hearts of both normal and RA rats. Heart sections are showing normal muscle fibers, and blood vessels with little interstitial tissue in the normal control group. Cardiac sections showing vascular dilation and congestion with perivascular edema (thick black arrow), hyalinization of muscle fibers (arrowhead) with the presence of few leukocytic cells in interstitial tissue (thin black arrow) in the model control group. These results were consistent with Kitas *et al*.,(2001) who found that histopathology of the heart showed chronic inflammation and fibrosis. Therefore, the commonest cardiac complication of RA is pericarditis and peripheral oedema. In the treated group with 2.5% FP cardiac sections showing less vascular dilation& congestion (thick black arrow), interstitial edema (blue arrow) with the presence of few leukocytic cells in interstitial tissue (thin black arrow). In addition, cardiac sections of the treated group with 5% FP are showing interstitial edema (blue arrow). Finally, cardiac sections in the treated

group with 7.5% FP show improved histology. These results were consistent with (Talaat *et al*.,2023) who noted that the oil in frankincense produced normal cardiomyocytes with some congestion in the intersititious space and blood capillary expansion.



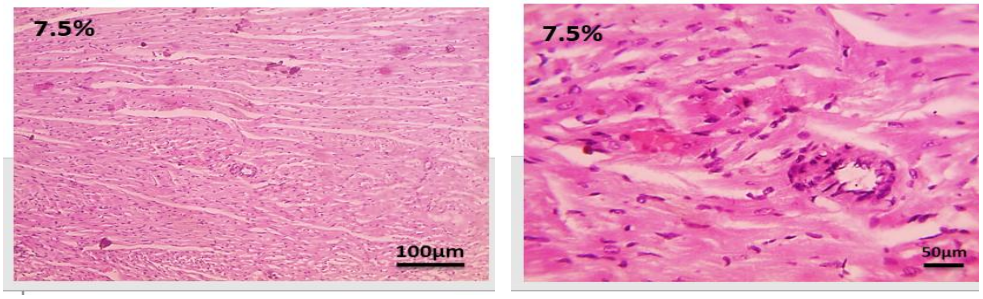
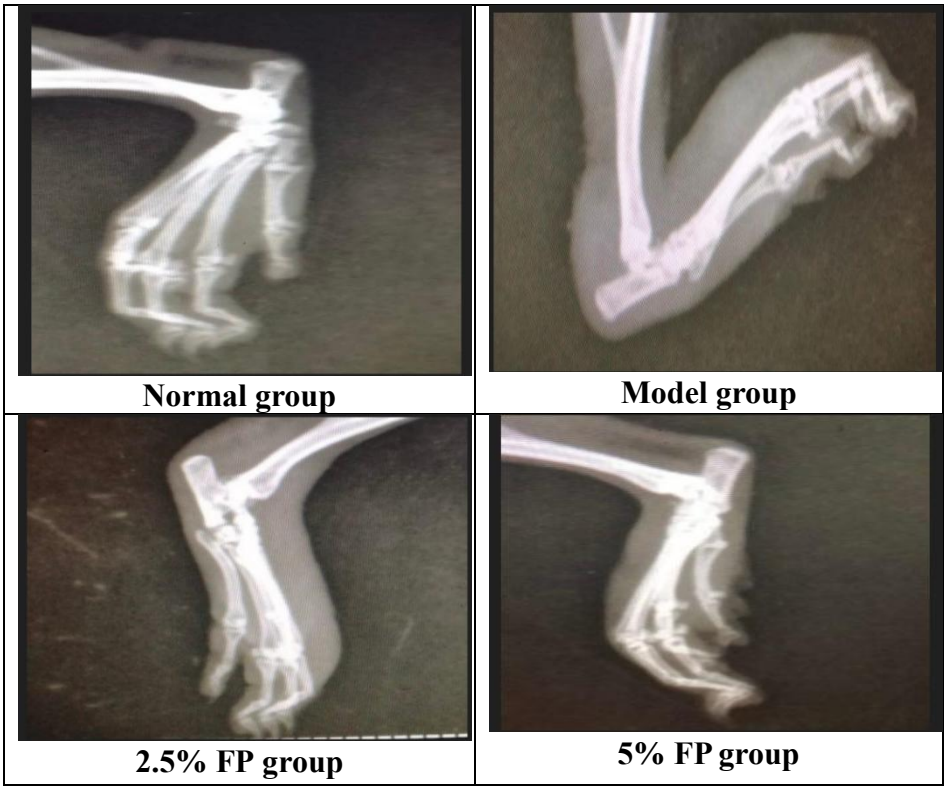


Figure (2): Heart sections of all experimental groups
Control group :normal group, **FA group**:model group, **2.5% group:**RA rats treated with 2.5% frankincense powder, **5% group**::RA rats treated with 5% frankincense powder, and **7.5% group**: RA rats treated with 7.5% frankincense powder

Impact of frankincense powder on X-ray radiography of ankle joints of normal and rheumatoid arthritis group

X-ray (1,2,3,4,5) show ankle joints radiography of normal and RA rats. In the normal control group the ankle joints of rats displayed no visible soft tissue swelling, edema, joint abnormalities, and normal joint spacing with no signs of erosion or inflammation (x-ray1) . In contrast, the ankle joints of rats from the model control group showed massive edema and soft tissue inflammation around bone and joints (x-ray 2). This is probably the result of immune-mediated joint destruction. On the other hand, the ankle joints of rats from group3 and group4 (2.5 and 5% FP) exhibited decrease in edema of soft tissues and the surrounding structures and joint space seem less inflamed (x-rays 3 and 4). Additionally, ankle joints of rats from group 5 (7.5% FP) showed Subside edema، or no visible soft tissue swelling, Joint structures appear more defined, closer to those of the healthy group. This indicated that a dose-dependent response, with better outcomes at higher concentrations (x-ray 5). These results were consistent with Fan *et al.*, (2005) who found that the extract of frankincense decreased arthritic scores, reduced paw oedema in rats.



X-ray radiography of ankle joints of normal and rheumatoid arthritis group

Conclusion

Rheumatoid arthritis is a chronic inflammatory disease which leads to destruction of joints. Current RA therapy approaches either alter the disease process (DMARDs) or provide symptomatic relief (NSAIDs). Despite their effectiveness, their side effects also restrict their use. This has led to a renewed interest in well-tolerated alternative anti-inflammatory treatments. Our objective was to assess frankincense's anti-arthritic and antioxidant properties. This study shown that the experimental diets with frankincense powder at 2.5, 5, and 7.5% have anti-inflammatory, scavenging, and antioxidant properties. These effects might be explained by the phytochemicals of FP such as phenolics , flavonoids, terpenoids , triterpenoids and antioxidant. β- boswellic acid, a triterpene derived from frankincense is extensively known for its anti-inflammatory properties.

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