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Study the Effects of Turmeric Extract Nanoparticles Gel on the Healing of Achilles Tendon Injury in Rats

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Abstract:

This study investigates the healing effects of Turmeric extract nanoparticles gel extracted from Turmeric root on Achilles tendon injury in Wistar rats, comparing its efficacy with control group that received no treatment. A total of 20 Wistar rats were randomly divided into two groups, with each group consisting of 10 animals. The first group was control group that dropped with 0.2 ml of phosphate buffer saline (PBS) and the second group treated with (0.44mg /kg) 0.13 ml of turmeric extract nanoparticles gel. The treatment was applied topically once after making tendon injury induced by exposing the tendon to cutting and anastomosis. The clinical evaluations were done daily until 28 days post operation. Histopathological examination was achieved on 14 days as well as 28 days post operation to evaluate healing process. The animals were housed in metal-topped plastic cages under standard laboratory conditions, with unrestricted access to food and water. The histopathological observations showed the presence of thick and regular arranged collagen fibers in the Turmeric-treated group (G2), numerous dilated and engorged blood vessels are evident, as well as new blood vessel formation at site of injury, these effects promote collagen production and tenogenesis. Animals' appetite and movement levels rapidly returned to normal. The results of clinical evaluation at 28 days post operation displayed that all variables of the injured tendons were significantly differed ($P< 0.05$) from the control group. In conclusion, the current study demonstrated that the Turmeric extract group accelerated and improved healing of the injured Achilles tendon based on clinical and histopathological findings. These findings corroborated the indicators of improvement as well as the Turmeric group was the superior due to the synergistic effects obtained on advantage in quality and quantity of healing tendon formatting in a period of 28 days postoperatively, and this demonstrated both mechanical and histological support to the regeneration of the injured tendon. The study aimed to the persistent challenges in improving tendon recovery and restoring its normal function, this study aimed to: Evaluating the efficacy of Turmeric Extract Nanoparticles gel on healing and regeneration of Achilles Tendon injury through: 1-Clinical evaluation. 2-Histopathological examination.

Keyword: Turmeric extract, Nanoparticles gel, Achilles tendon, Injury, Rat.

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Introduction:

Tendons are dense connective tissues that connect bones and skeletal muscles, playing a critical role in maintaining body posture as well as the integrity and function of the musculoskeletal system by buffering and transmitting forces (Xu *et al*., 2024). It is formed mainly from collagen, especially collagen type 1, and a number of other minor collagen types and proteoglycans, the central collagen structure with interposition of tenocytes, encircling by a non-collagen molecules network and covered by a thin membrane called epitenon. The blood and nerve supply of the tendon provided mostly from the endotenon and epitenon (D'Addona *et al*., 2017; Taye *et al*., 2020).

The Achilles tendon is the thickest, powerful and biggest tendon in the body (Maffulli *et al*., 2020). It is an important connective tissue that maintains the natural movement and stability of the joints. Self-healing of Achilles tendon is weak due to lack of vascularization and consequently lack of nutrients (Zhu *et al*., 2022). In addition, problems associated with motor disorders such discomfort, instability in the joint, damage to the cartilage, and eventually osteoarthritis can result from an Achilles tendon injury. These issues can seriously impairing limb and joint function (Russo *et al*., 2022). Standard treatments for Achilles tendon injuries include surgical, un surgical and rehabilitation techniques. The degree of the Achilles tendon injury determines the course of treatment. For example, surgery is frequently recommended to treat complete or open Achilles tendon ruptures. Allograft, xenograft, and direct suture are examples of surgical intervention. If there is no functional disability and the Achilles tendon is partially ruptured, conservative treatment may be selected. Furthermore, poor healing outcomes and joint stiffness can result from surgery. Re-rupture afterward is typical due to the difficulty of fully restoring a damaged tendon to its pre-injury state. (Dale *et al*., 2018; Novakova *et al*., 2018).

Curcuma longa, commonly known as Turmeric, is a rhizomatous herbaceous perennial plant belonging to the Zingiberaceae family (Tung *et al*., 2019). It originated in India and is widely cultivated in China, Sri Lanka, West and East Africa and other tropical countries. It is used in Chinese Traditional Medicine for the treatment, prevention and management of various illnesses such as cancer, coughs, diabetes, Arthritis, diarrhea, inflammation, psoriasis, hepatobiliary diseases, skin disorders, gastric ulcers and peptic ulcers (EL-Kenawy *et al*., 2019).

Curcumin is one of the active ingredients in turmeric makes up between 3% to 10% of the turmeric powder which can be extracted. (Tung *et al*., 2019). It supports blood flow, elimination stagnation, relieve depression, and have powerful natural flavoring effect on the color, flavor, and texture of food. The major components of turmeric are curcumin and demethoxycurcumin, bisdemethoxycurcumin and turmeric essential oil (Kocaadam and anlier, 2017; Kotha and Luthria, 2019). Numerous effects of curcumin have been confirmed by studies, including anti-inflammatory,

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antioxidant, anticarcinogenic, antidiabetic, antibacterial, antiprotozoal, antiviral, antifibrotic, immunomodulatory, and antifungal properties (Abd El-Hack *et al*., 2021).

Materials and Methods:

Twenty adults male Wistar rats weighing 280±20gm and ages between 10 to 14 weeks were used. Animals were housed in laboratory animal house / College of Science / University of Al-Qadisiyah, and randomly divided into main two groups (n=10). All animals submitted to complete tenotomy of Achilles tendon and sutured by modified Kessler technique. All animals submitted to complete tenotomy of Achilles tendon and sutured by modified Kessler technique.

- First group (G1): Control group.
- Second group (G2): Turmeric extract nanoparticles gel group.

Clinical evaluation was performed daily, and the specimens were collected from the injured tendon on days 14 and 28 post-operation for histopathological examination to evaluate the progression of the tendon healing process.

Turmeric collection

The Turmeric root was bought from the local market. The turmeric extraction was obtained by Soxhlet device. Nanoparticles production from the turmeric extract were obtained by Ultrasonic device. Many tests were achieved to evaluate the extract and confirm its quality, including the Field Emission Scanning Electron Microscopy (FESEM), X-Ray Diffraction (XRD), UV-Visible Absorption Spectroscopy, Fourier Transform Infrared (FTIR) spectroscopy and Gas Chromatography/Mass Spectrometry (GC/MS).

Turmeric Extraction by Soxhlet Apparatus

Fresh and dry turmeric rhizomes (*C. longa*) were bought from Diyala market. They were taken in a clean sterile bag and processed within few hours after sampling. For twenty minutes, rhizomes were ground into tiny particles within a dry grinder. After that, the powder was run through a 212-micron mesh size sieve to almost entirely eliminate any coarse fibers. For turmeric extraction by Soxhlet apparatus (Fig. 1), 200 grams of turmeric powder that had been sieved were used. Each time, 50 g were occupied and put in the extraction thimble, which acts as filter paper, so the solvent could come into touch with them. A total of 500 milliliters of 99% ethanol were added to the round-bottom distillation flask as a solvent. The heated ethanol in the flask evaporated and moved into the condenser, where it changed into a fluid that enter the extraction chamber that holds the powdered turmeric that been sieved. The chamber of extraction was designed to overflow and leak back in the flask that is boiling when the solvent level surrounding the sample above a certain level. For eight hours, turmeric rhizome powder was extracted at 60 degrees Celsius. After the extraction process is complete, the

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material is put into Petri plates and kept there until the alcohol evaporates. The substance was scraped and obtained (Patil *et al*., 2019; Jiang *et al*., 2021).

Tendon healing assessment

Both macroscopic and histopathological studies, along with clinical data, were used to evaluate tendon recovery. The macroscopic grading method outlined in (Table 1) was used to evaluate the gross morphology of the reconstructed tendon at the rupture location (Abdel-Wahab *et al*., 2025).

A healthy tendon should be smooth, white, reflective, and free of swelling or adhesions to the surrounding tissue.

Table (1) System of macroscopic scoring for healing of Achilles tendon with modification, (Abdel-Wahab *et al*., 2025).

Variables	Description	Scores
Inflammation	Not exist Exist (edema, redness, swelling)	1 0
Achilles tendon adhesions	No adhesion Adhesion	1 0
Connection to skin	Not connected connected	1 0
The color	Red Bride white	1 0
Tendon surface at injuries area	Intact uneven	1 0
Tendon shape	Normal Thickened intensely	1 0
Level of injury	At level of tendon surface Above the level of tendon surface	1 0

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Preparation of Turmeric Extract Nanoparticles Using Ultrasonic Method

Ten grams (10g) of Turmeric extract were dissolved in 100 milliliters of (ethanol 99%) in order to create nanoparticles using the ultrasonic method (Sahne *et al*., 2016; Rajalakshmi and Dhivya, 2018). The filtered solution was treated with ultrasonic waves by means of Ultrasonic Cell Disruptor device (Ultrasonic Cell Disruptor: UCD-150; Ultrasonic Power 150 W, Probe 6mm) (Faithful Intelligent Ultrasonic Processor) in College of Veterinary medicine / University of Al-Qadisiyah (Fig.2), The turmeric substance in the solution become to a size of 62 nm after 30 minutes of 50-watt wave energy (Rai *et al*., 2015; Aldulemy and Abdul-Razak, 2021). The treated solution (Turmeric extract) was put in a Petri dish until dried and then scraped .

Characterization of Turmeric Extract Nanoparticles

To evaluate the structural analysis of the turmeric extract and turmeric extract nanoparticles, a number of tests were carried out (the shape, size, morphology, and surface area) including Field Emission Scanning Electron Microscopy (FESEM), X-Ray Diffraction (XRD), UV Visible Absorption Spectroscopy, Fourier Transform Infrared (FTIR) spectroscopy and Gas Chromatography/Mass Spectrometry (GC/MS) (Kotha and Luthria, 2019; Begum *et al*., 2022). The UV-visible absorption spectroscopy, FTIR assessment and GC/MS test were done in chemistry department / College of Science / University of Al Qadisiyah. The (FESEM) test and (XRD) was performed in Baghdad/ Al-yarmouk/ Al-Khora Examinations Laboratory.

Field Emission Scanning Electron Microscopy (FESEM)

FESEM analysis (FEI Company, USA): Field Emission Scanning Electron Microscope (FESEM) is a microscope that works with electrons (particles with negative charge) instead of light. These electrons are liberated by a field emission source. Electrons scan the object in a zigzag pattern. At low accelerating voltages and short working distances, the Field Emission Scanning Electron Microscope (FESEM) offers ultra-high imaging. It allows for the examination of the top surface of nanopowders, nanofilm, and nanofiber in a variety of application including mineralogy ceramics, polymers, metallurgy, electronic devices, chemistry, physics, and life sciences. The image resolution is as low as 0.6 nm at 15KV and 1.2 nm at 1KV. This system has many detectors to identify different signals, such as a back-scattered electron (BSE) detector for contrast in material composition and a secondary electron (SE) detector for topographic data. Clear, less electrostatically distorted pictures with spatial resolution down to 11/2 nanometers are produced by field emission (FESEM), Which is three to six times better (Prabhu *et al*., 2021).

X-Ray Diffraction (XRD)

The X-Ray Diffraction analysis was carried out using an Aeris Research device (Malvern panalytical/UK) in order to investigate the crystallographic structure of the formulation of turmeric extract nanoparticles. It's a powerful analytical device to analyze the purity and crystalline phases of

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sample particles. XRD provides the first information about the materials phases, crystalline structure, average crystallite size, micro and macro strain, orientation parameter, texture coefficient, degree of crystallinity, crystal defects (Pandey *et al*., 2021).

UV-Visible Absorption Spectroscopy

UV-Visible spectroscopy is measured as the greatest significant spectrophotometric procedure that is most frequently applied to the examination of a wide range of substances. This process is based on measuring how electromagnetic radioactivity (EMR) interacts through substance at a definite wavelength. This analytical technique, which measures light intensity in the UV (10–400 nm) and VIS (400–800 nm) ranges as a purpose of wavelength, is known as spectrophotometry. The standard unit of measurement for UV and VIS radiation wavelengths is nanometers (nm). Only UV and VIS light are absorbed by the analyte, and the amount of radiation that the analyte absorbs is measured. (Akash *et al*.,2020).

Fourier Transform Infrared (FTIR) Spectroscopy

One of the most effective methods for identifying the kinds of chemical bonds (functional groups) in compounds is the Fourier transform infrared spectrophotometer (FTIR) (Tensor, Bruker, USA). (FTIR) is a very efficient and accurate analytical method commonly employed in biomolecular research due to its ability to provide detailed structural and functional insights into biomolecules such as proteins, carbohydrates, nucleic acids, and lipids. This no-invasive method operates by analyzing the vibrational modes of molecules, offering a unique spectral "fingerprint" that enables the description and identification of compound biological examples. The adaptability of FTIR spectroscopy allows it to examine biomolecules in a variety of states and circumstances, supporting a wide range of applications such as protein analysis, biofuel production, cancer research, and medical diagnostics (Jain *et al*., 2025).

Gas Chromatography/Mass Spectrometry (GC/MS)

GC-MS technique (GCMS-TQ8040, Shimadzu) Chromatography is an analytical method that uses the physicochemical interactions of the analytes entrained by a mobile phase to separate complicated mixtures. In the case of GC, the separation takes place in a capillary column containing a stationary phase and an inert gas. The retention time is the amount of time that the analytes elute and separate differently. They then proceed to the detector for analysis. As for it, mass spectrometry is an analysis method known as mass spectrometry makes it possible to ascertain a substance's molecular dispersion based on its mass. By separating the substances' ions according to their mass/charge ratio, the mass spectrometer is a tool that enables highly accurate analysis of the composition of various chemical elements and atomic isotopes (Varela Martínez *et al*., 2022).

Turmeric Extract Nanoparticles Gel Preparation

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The required quantity of Carbopol (934) (2gm) was obtained from local market and spread in a beaker containing 50 ml of distilled water. The beaker was set aside for 15 minutes to allow the Carbopol to swell. Then, a weighted quantity of turmeric extract (2gm) was dissolved in (ethanol 99%) and added to the beaker and stirred for one hour in 40 °C, after that adequate amount of distilled water was added to obtain a Turmeric gel (Fig.3). (Nikunjana *et al*., 2009).

Surgical Procedure

The rats have been anesthetized with combination of ketamine (50 mg/kg. B.w.) and xylazine (10 mg/kg. B.W.), given by intramuscular shot (Navarro *et al*., 2021; Bennett and Lewis, 2022). Achilles tendon injury (tenotomy and tenorrhaphy) is induced on the posterior aspect of the left hindlimb in each animal. The operation site was prepared aseptically. Complete transverse cutting of Achilles tendon 1 cm above the hock joint is achieved with scalpel in (20) rats (Control, Turmeric extract nanoparticles gel groups). Afterward creation 2 cm length longitudinal skin opening on the posterior aspect of the left hindlimb (Fig.4). Tenorrhaphy by Modified Kessler technique where done (Rawson *et al*., 2013) (Fig.5), using Polydioxanone (PDO) Absorbable Monofilament Surgical Suture size (5-0) USP. Then the skin opening is closed with simple continuous suturing with Polypropylene Non-absorbable Monofilament Surgical Suture size (4-0) USP (Fig.6).

Group One (G1) /Control Group

Control group (G1) 10 male rats, after complete Achilles tendon tenotomy and tenorrhaphy by Polydioxanone absorbable suture material, the tendon dropped with 0.2 ml of phosphate buffer saline. Daily clinical examination and specimens were taken post operation in 14 and 28 days for histopathological examination to evaluate healing process.

Group Two (G2) /Turmeric Extract Nanoparticles Gel Group

Turmeric extract nanoparticles gel group (G2) 10 male rats, after complete Achilles tendon tenotomy and tenorrhaphy by Polydioxanone absorbable suture material the Turmeric extract nanoparticles gel (0.44 mg /kg) 0.13 ml (Zhang *et al*., 2016) was applied topically on the site of tendon injury Figure (fig.7). Clinical examination and specimens were taken post operation in 14 and 28 days for histopathological examination to evaluate healing process.

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Figure 1: Soxhlet apparatus used for turmeric extraction.

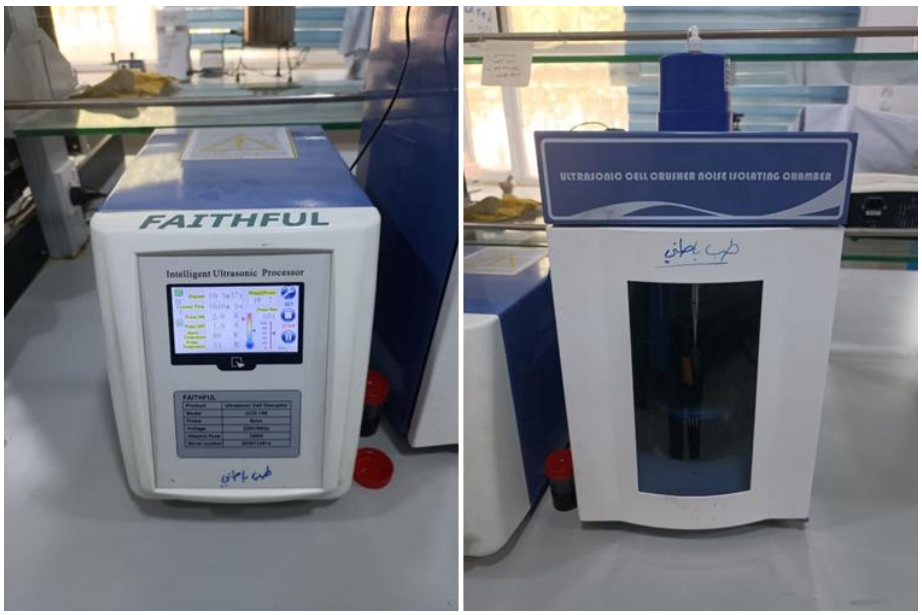

Figure 2: Sonicator device. Faithful Intelligent Ultrasonic Processor: Ultrasonic Cell Disruptor UCD-150 Ultrasonic Power 150 W; Probe 6mm.

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Figure 3: Turmeric extract nanoparticles gel preparation.

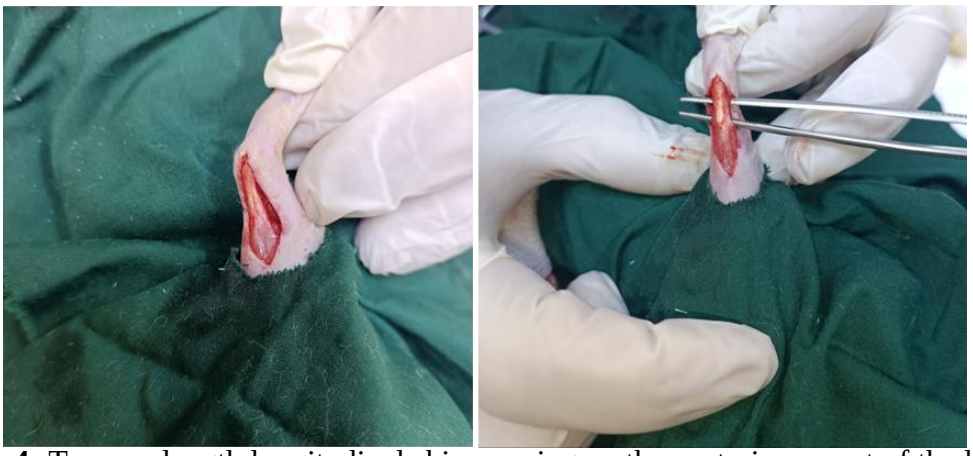

Figure 4: Two cm length longitudinal skin opening on the posterior aspect of the left hind limb.

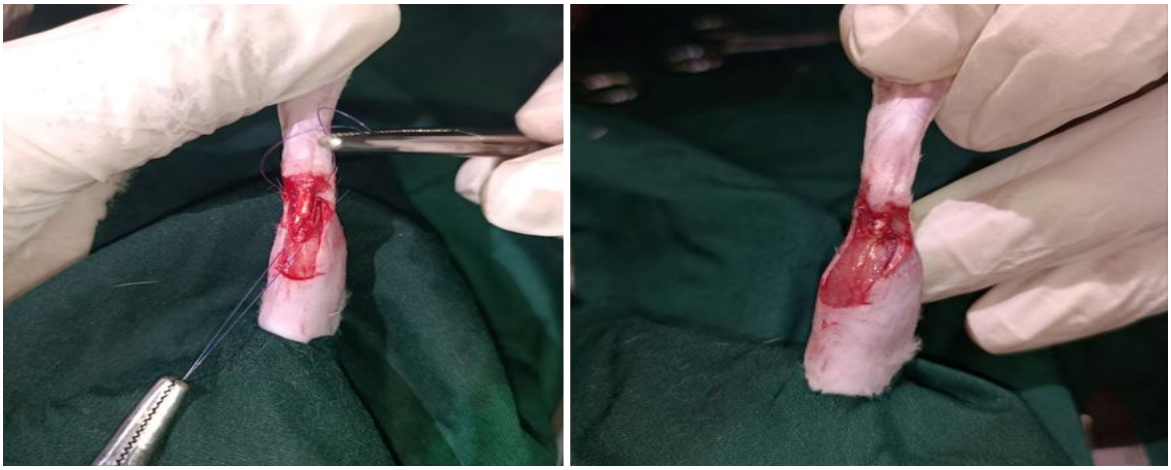

Figure 5: Cutting and suturing of Achilles tendon by use (Modified Kessler technique) using Polydioxanone (PDO) Absorbable Monofilament Surgical Suture size (5-0) USP.

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Figure 6: skin incision closure by simple continuous suturing using Polypropylene Non-absorbable Monofilament Surgical Suture size (4-0) USP.

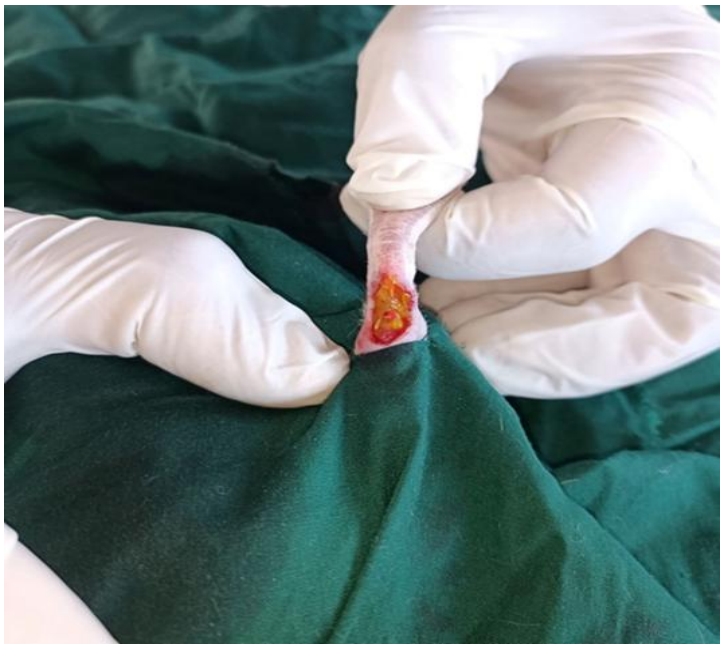

Figure 7: Turmeric extract nanoparticles gel application.

Statistical Analysis

To inspect the connection among the categorical variables (clinical features) an SPSS was achieved. The test measures whether there is a statistically significant related among variables by comparation. The level of significance was set at 0.05. (Abdul Rahman *et al*., 2025).

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Ethics approval:

The investigation was conducted in accordance with general strategies for the use and care of research laboratory animals. Each protocol was accepted by the University of Al Qadisiyah College of Veterinary Medicine Faculty's High Committee for Examining and Agreement of Research Proposals under the No. 5354 dated 9/12/2024.

Results:

Detection of prepared turmeric extract nanoparticles

1-Field Emission Scanning Electron Microscopy (FESEM)

Figure (8) presents a high-resolution Field Emission Scanning Electron Microscopy (FESEM) image illustrating the size and shape of the turmeric extract nanoparticles. The image is characterized by a significant degree of nanoparticle agglomeration. (FESEM) analysis demonstrated that the synthesized material displayed a spherical or quasi-spherical shape, with the agglomerates exhibiting a rough and uneven surface. This surface roughness is probably due to the dense packing and disordered arrangement of the constituent nanoparticles within the clusters.

A measurement of 100 randomly selected nanoparticles showed an average particle size of 43.56 ± 0.48 nm.

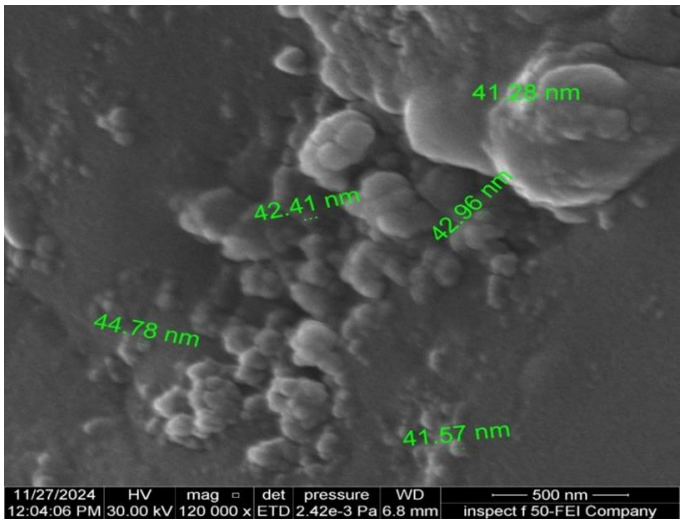

Figure 8: (FESEM) analysis image of turmeric extract nanoparticles.

2- X-Ray Diffraction (XRD)

As shown in the Figure (9) the typical X-ray diffraction (XRD) pattern in the 2Θ range of 30° to approximately 70° of the prepared curcumin nanoparticles, showing the presence of the various characteristic peaks. (XRD) is a powerful technique to identify the crystalline phases present in a

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material and can provide information about the size of the crystallites. The (XRD) pattern reveals several distinct peaks, indicating the crystalline nature of the turmeric extract nanoparticles. These main peaks, located at 17.6911, 18.3707, 20.9036, 24.3445, and 26.93 degrees (2θ) with their FWHM at 1.071, 1.077, 5.302, 2.783, 2.923 respectively suggest the presence of specific crystal planes within the curcumin structure. Based on the Scherrer equation, the estimated crystallite sizes of approximately 42-43 Å for the first two peaks and a smaller size of 11 Å for the most intense peak, indicating that the crystallites are in the nanometer range with potential size variations across crystal planes. The (XRD) analysis confirms that the curcumin nanoparticles are crystalline, characterized by small crystallite sizes and some micro-strain. The figure (14) provides essential data on peak positions, intensities, and other parameters for characterizing the nanoparticles' crystal structure.

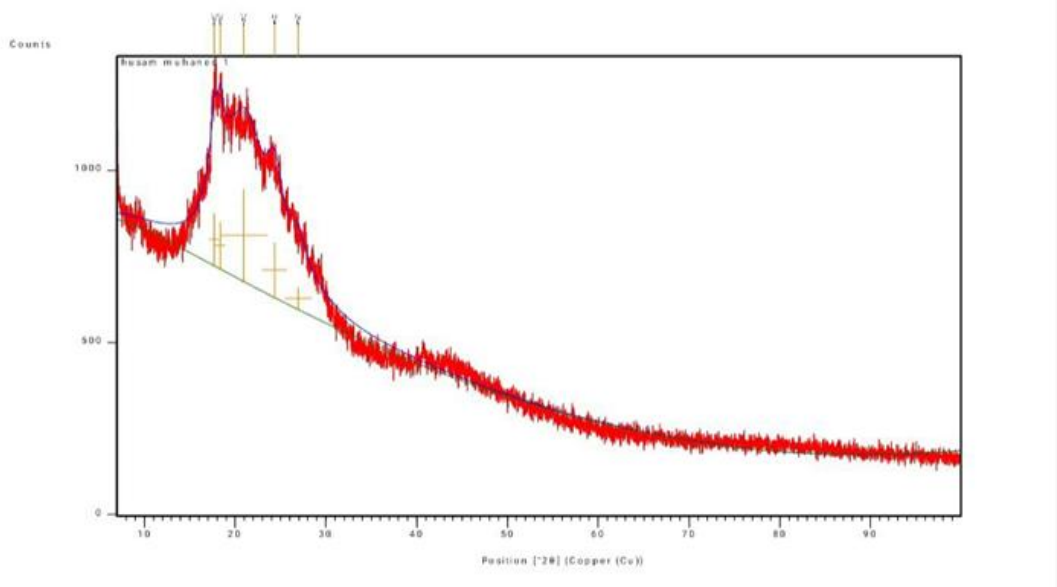

Figure 9: X- Ray diffractogram of turmeric extract.

3- UV-Visible Absorption Spectroscopy

As shown in the figure (10) the spectrum displays a characteristic absorption of prepared turmeric extract derived nanoparticles which recorded at characteristic absorbance at wavelength 420 nm (Abs. 0.545) and wavelength 240 nm (Abs. 0.233).

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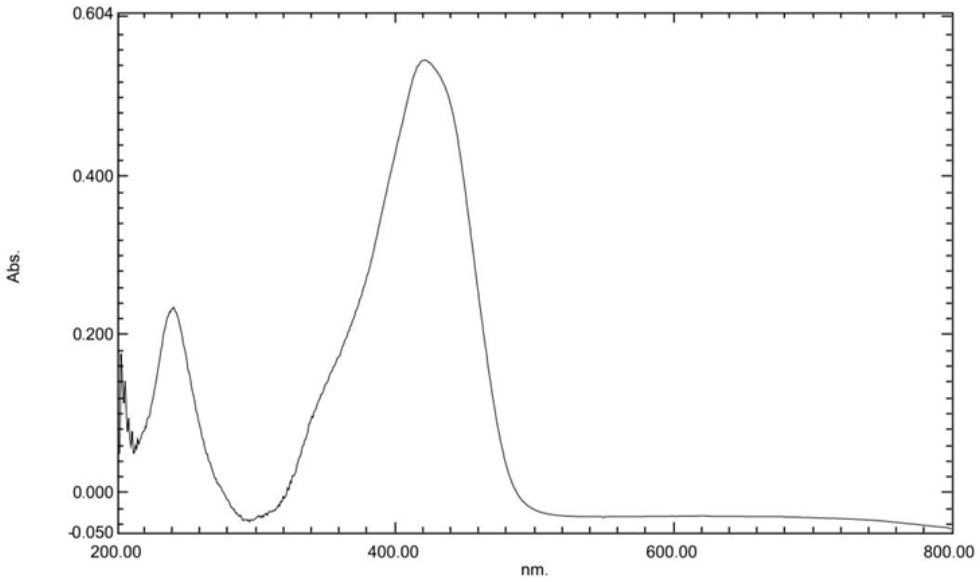

Figure 10: UV-visible Absorption spectroscopy of prepared turmeric extract nanoparticles.

4-Fourier Transform Infrared (FTIR) Spectroscopy

This FTIR (Fourier Transform Infrared) spectrum of turmeric extract nanoparticles provides detailed data around the efficient collections existing in the example based on the absorption of infrared light at different wavenumbers (cm^{-1}). The infrared spectrum of the curcumin nanoparticles was evaluated in the wavelength variety of 4000-500 cm^{-1} as shown in the figure (11). The FTIR spectra showed that the stretching of the hydroxyl groups (-OH) of the alcohols and phenol in the curcumin extract was represented by certain absorption bands at 3643.35 cm^{-1}. the presence of bands at 1593.52 and 3500.38 cm^{-1} qualified to strong broad OH stretch of alcoholic groups and medium NH stretching primary amine respectively. The band at 3255.23 cm^{-1} corresponds to the C=C-H:C-H stretching of alkynes. The absorption peaks at 2929.03 and 2870.17 cm^{-1} originate from asymmetric stretching vibrations of Csp2−H and Csp3−H bonds, respectively. The absorption peak at 1742.09 corresponds to the stretching vibration of the conjugated carbonyl (C = O). another peak recorded at 1680.49 represent strong C=O stretching bond. Medium C-H bending of alkane (methyl group recorded at 1453.3 cm^{-1}.

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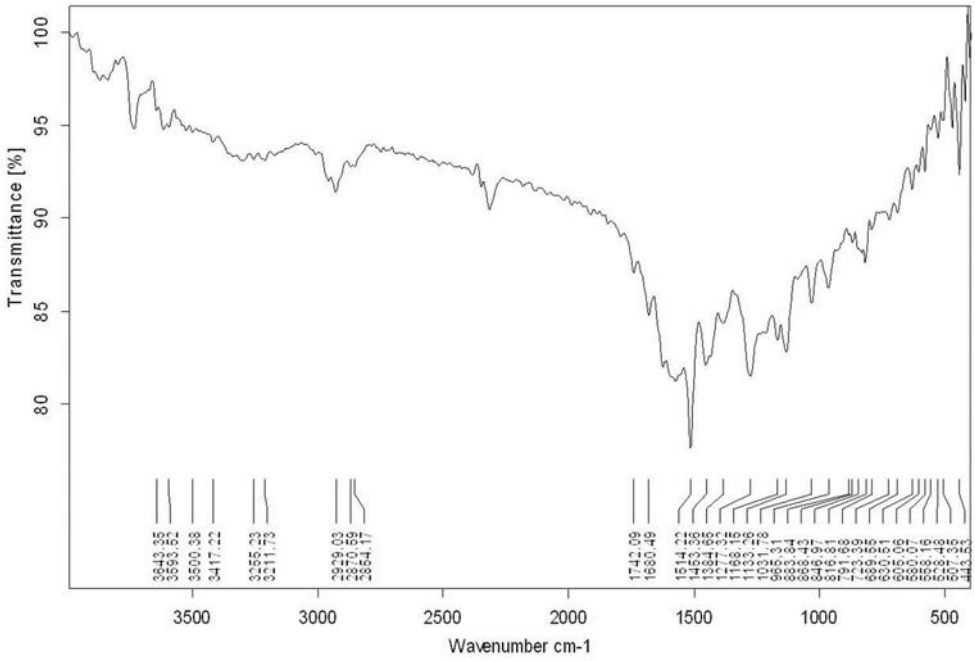

Figure 11: Fourier transform infrared spectroscopy (FTIR) analysis.

5-Gas Chromatography/Mass Spectrometry (GC/MS)

The GC-MS Spectrometry of the turmeric extract show twenty-five elements in the following results (Table 2) and figure (12). The main elements from high percentage to the low was: 1,4-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester 39.63 %, aR-Turmerone 15.33 %, Curlone 7.32 %, Glycerin 7.22 %, 9-Octadecenoic acid, 12-hydroxy-, methyl ester, [R-(Z)] 5.73%, Tumerone 4.6%, 2-Methoxy-4-vinylphenol 2.64%, 1,3-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester 2.43%, 13-Docosenamide, (Z) 2.28%, Squalene 1.8%, Eugenol 1.61%, (E)-Atlantone 1.3%, 4-Vinylphenol 1.12%, and other below 1 % trace elements were noticed.

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No. of peak	Retention time	Elements	Area%
1	37.229	1,4-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester	39.63
2	22.951	aR-Turmerone	15.33
3	23.692	Curlone	7.32
4	6.729	Glycerin	7.22
5	33.561	9-Octadecenoic acid, 12-hydroxy-, methyl ester, [R-(Z)]-	5.73
6	23.039	Turmerone	4.6
7	15.252	2-Methoxy-4-vinylphenol	2.64
8	39.866	1,3-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester	2.43
9	40.346	13-Docosenamide, (Z)-	2.28
10	41.129	Squalene	1.8
11	16.283	Eugenol	1.61
12	25.083	(E)-Atlantone	1.3
13	12.92	4-Vinylphenol	1.12
14	39.99	D(17a)-Homo-C,18-dinorcard-20(22)-enolide, 14-hydroxy-17a-methylene-3-oxo-, (5 beta.)-	0.85
15	24.992	Pentadecane, 2-methyl-2-phenyl-	0.78
16	35.105	Hexanedioic acid, bis(2-ethylhexyl) ester	0.69
17	35.53	Phenol, 2,2'-methylenebis[6-(1,1-dimethylethyl)-4-methyl-	0.61
18	25.811	2-Butanone, 4-(2,6,6-trimethyl-2-cyclohexen-1-ylidene)-	0.57
19	28.803	1-Eicosanol	0.57
20	28.901	2-Butenoic acid, 2-methyl-, 2-(acetyloxy)-1,1a,2,3,4,6,7,10,11,11a-decahydro-7,10-dihydroxy-1,1,3,6,9-pentamethyl-4a,7a-epoxy-	0.56
21	27.078	6-(5-Hydroxy-4-methylidenecyclohex-2-en-1-yl)-2-methylhept-2-en-4-one	0.54
22	33.17	1,2-Benzenedicarboxylic acid, butyl 2-ethylhexyl ester	0.54
23	27.977	Turmeronol A	0.5
24	24.555	(6R,7R)-Bisabolone	0.43
25	10.691	Triethyl phosphate	0.35

Table 2: Phytochemical elements recognized in turmeric extract nanoparticles by GC/MS.

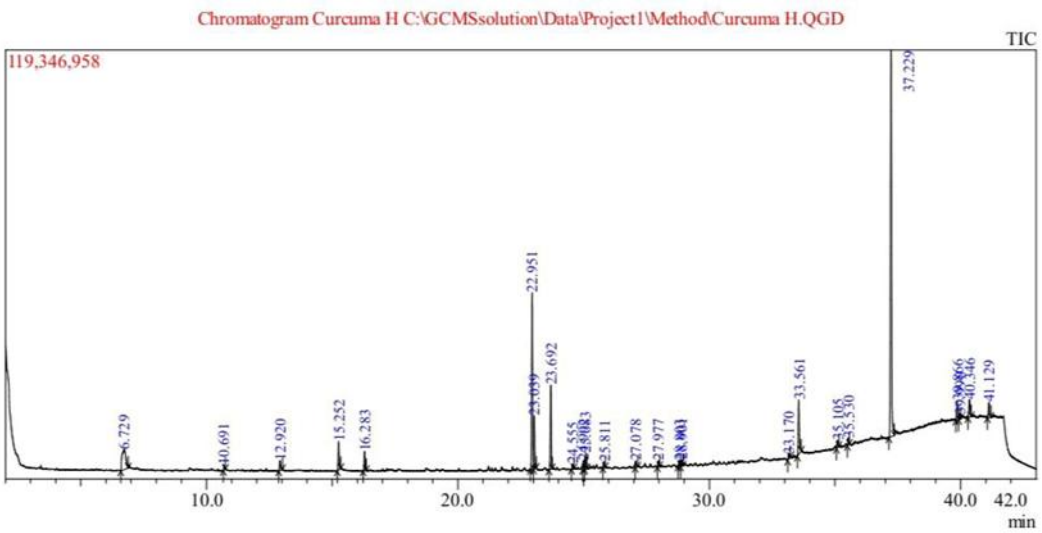

Figure 12: GC-MS analysis of turmeric extract nanoparticles sample.

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Clinical findings of the Tendon Healing

Rats used their hindlimbs very little during the first two days after surgery, according to postoperative clinical evaluations. After that, when stimulated, they began to walk, and by day six, they were able to move freely. All of the rats showed no symptoms of infection. By the fourth week, none of the animals had any complications and were free to move without bandages.

The quality of healing responses varied significantly ($p < 0.05$) across the treatment group when compared with control group according to macroscopic measurements at day 14 as indicated in Table (3).

The curcumin group registered 70%, 60%, 60%, 70%, 60%, 80%, 60% in an inflammation, Achilles tendon adhesion, connection to skin the color, tendon surface at injuries area, tendon shape and level of injury respectively where differed significantly ($p < 0.05$) when contrasted with control group in the clinical parameters, as well as the curcumin group differed significantly ($p < 0.05$) in all parameters.

The control group, on the other hand, showed much more variability where recorded 90%, 90%, 100%, 90%, 90%, 50% and 100% in an inflammation, Achilles tendon adhesion, connection to skin the color, tendon surface at injuries area, tendon shape and level of injury respectively, demonstrated with inflammation, which is attained by edema, swelling, and redness.

The Turmeric group total macroscopic assessment score showed noticeably better outcomes.

Table (3): Variations of macroscopic parameters in clinical evaluation of injured tendon at 14 days. of macroscopic parameters in evaluation of injured tendon.

Variables	Control	Curcumin
Inflammation	90 % B	70 % A
Achilles tendon adhesions	90 % B	60 % A
Connection to skin	100 % B	60 % A
The color	90 % B	70 % A
Tendon surface at injuries area	90 % B	60 % A
Tendon shape	50 % B	80 % A
Level of injury	100 % B	60 % A

*** Different letters in the rows mean significant difference (P<0.05).**

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The clinical evaluations at 28 days were displayed in Table (4). The curcumin group showed 30%, 10%, 10%, 30%, 30%, 80%, and 30% in an inflammation, Achilles tendon adhesion, connection to skin, color, tendon surface at the injury site, tendon shape, and degree of injury, respectively, which differed significantly ($p < 0.05$) when contrasted to the control group in the clinical parameters.

In contrast, the control group exhibited 70%, 50%, 40%, 60%, 50%, 50%, and 60% of inflammation, Achilles tendon adhesion, connection to skin, color, tendon surface at the site of injury, tendon shape, and degree of injury, respectively, as evidenced by oedema, swelling, and redness.

Table (4): Variations of macroscopic parameters in clinical evaluation of injured tendon at 14 days. of macroscopic parameters in evaluation of injured tendon.

Variables	Control	Curcumin
Inflammation	70 % B	30 % A
Achilles tendon adhesions	50 % B	10 % A
Connection to skin	40 % B	10 % A
The color	60 % B	30 % A
Tendon surface at injuries area	50 % B	30 % A
Tendon shape	50 % B	80 % A
Level of injury	60 % B	30 % A

***Different letters in the rows mean significant difference (P<0.05).**

Histopathological Assessment of the Tendon Healing

1-Control group (G1)

At 14 days post procedure:

A significant process of inflammatory reactions was seen categorized by occurrence of numerous inflammatory cells, scar tissue with presence of a narrow incision, accompanied by formation of small blood vessels and few collagen fibers in the dermis. There is a minor network of collagen fibers in the subcutaneous tissue. The collagen network is irregular and fine, particularly in areas where mild fibrosis is observed (Figures 13,14,15).

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At 28 days post procedure:

There has been minimal progress in the healing process, narrow incision with abundant granulation tissue is seen, characterized by significant infiltration of inflammatory cells particularly macrophages and lymphocytes with mild fibrosis and newly-formed blood vessels. There is marked fibrosis and an extensive presence of collagen fibers. The injury site exhibits engorged blood vessels, along with regular collagen fibers networks and proliferation of fibroblasts. Endothelial cells contribute to the formation of small new blood vessels. Profuse collagen fibers are seen stained blue with Masson's stain (Figures 16,17,18).

2- Turmeric extract nanoparticles gel group (G2)

At 14 days post operation:

The sections show a thin epidermis overlying wide scar tissue with abundant granulation tissue, marked fibrosis, and numerous newly formed dilated blood vessels, some engorged with RBCs. And there is proliferation of fibroblasts and infiltration of inflammatory cells, mainly macrophages, with focal areas of hemorrhage in the dermis (Figures 19,20,21,22).

At 28 days post operation:

The histopathological sections reveal thick and regularly arranged collagen fibers, with scattered inflammatory cell infiltration, formation of new blood vessels (some dilated and engorged with blood), limited areas of fibrosis, and the presence of macrophages (Figures 23,24,25).

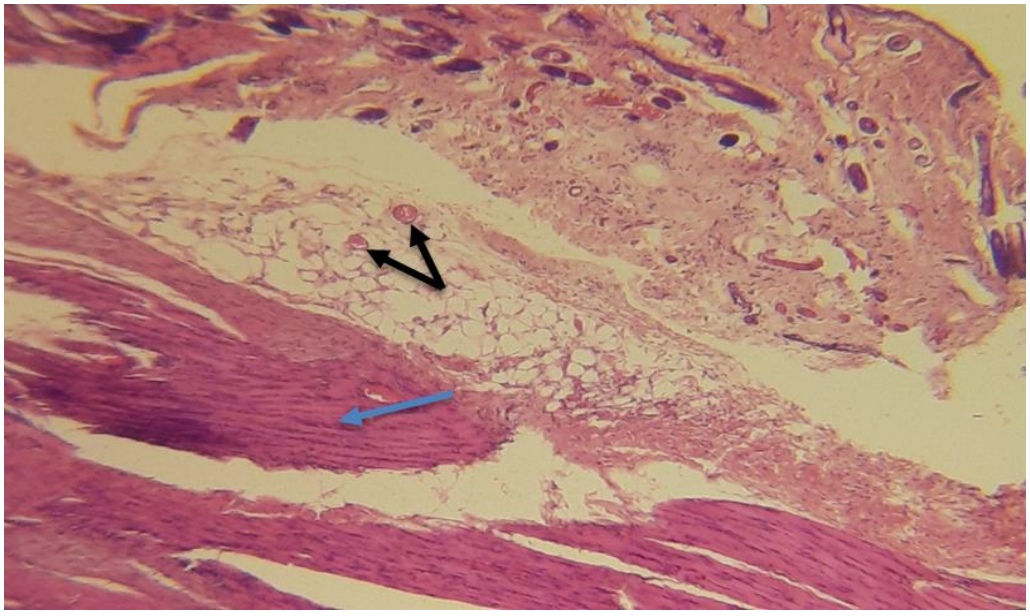

Figure (13): (G1 d14) Histopathological section show a wide scar tissue with presence of narrow incision with formation of small blood vessels (Black arrow) in the dermis. and small amounts of collagen network (Blue arrow) in the Subcutaneous tissue (4X H&E).

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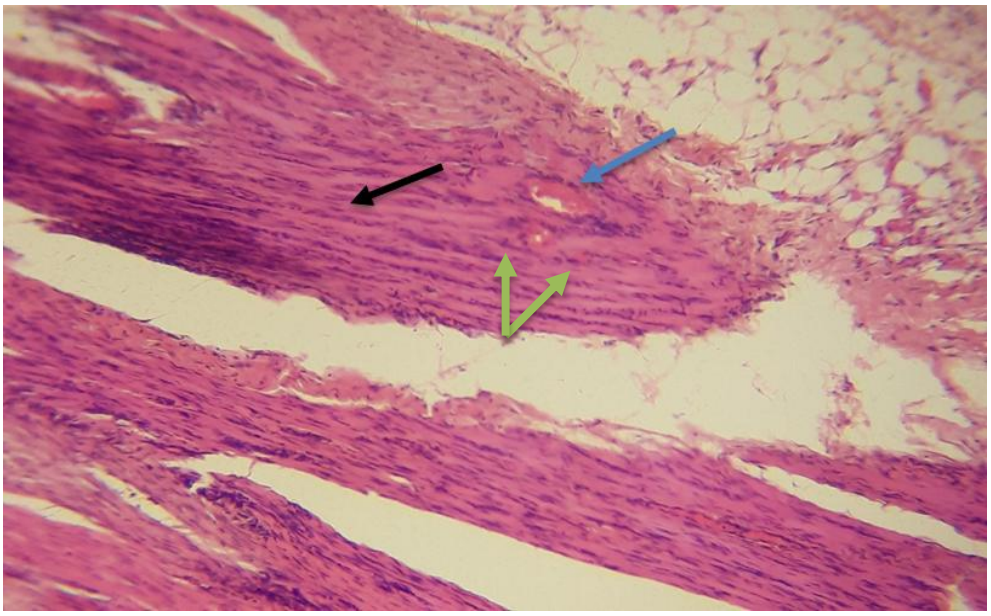

Figure (14): (G1 d14) Histopathological section show a small amount of irregular network of collagen fibers (Black arrow) with small newly-formed blood vessels (Blue arrow) with infiltration of inflammatory cells (Green arrow) (10x H&E).

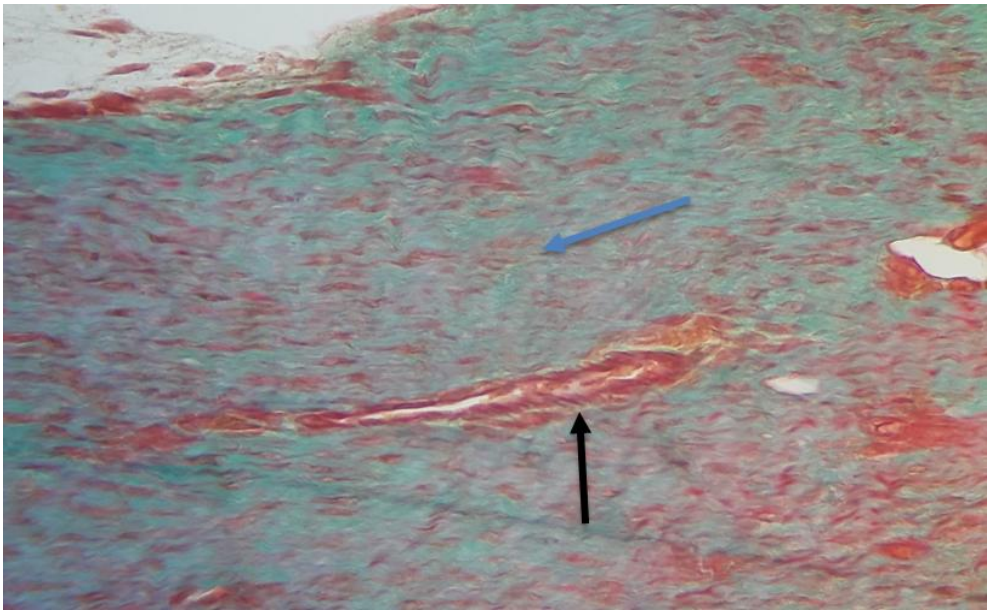

Figure (15): (G1 d14) Histopathological section show a few collagen networks (Blue arrow) stained blue in color with newly-formed blood vessels (Black arrow) (10X Masson trichrome).

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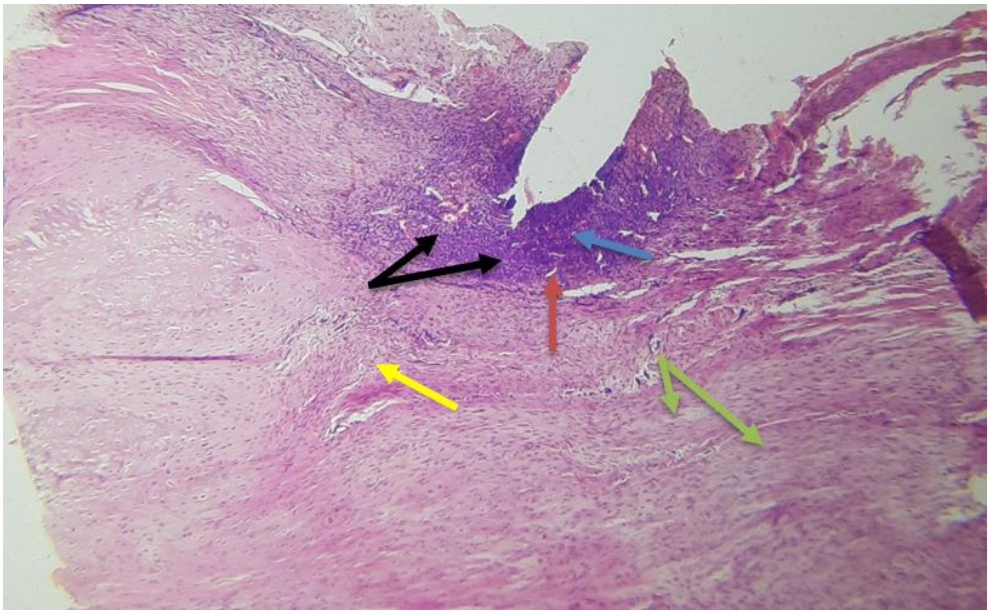

Figure (16): (G1 d28) Histopathological section show a very narrowed incision with profuse granulation tissue (Black arrow) in which there is high infiltration of inflammatory cells (Blue arrow) with new formation of blood vessels (Red arrow) with profuse fibrosis (Yellow arrow) and collagen fibers (Green arrow) (4X H&E).

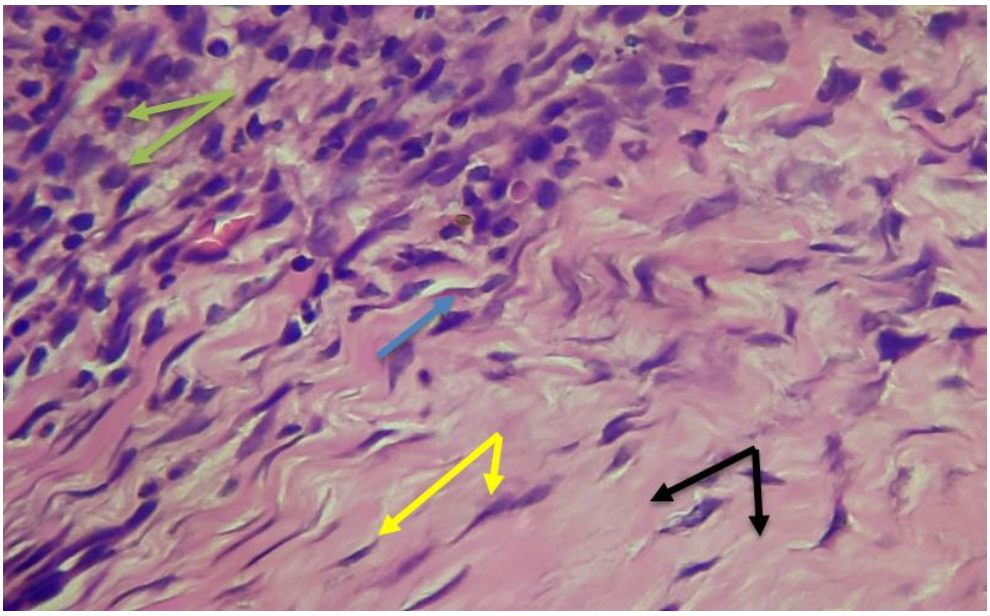

Figure (17): (G1 d28) Histopathological section show a regular collagen fiber (Black arrow) with proliferation of fibroblasts (Yellow arrow) with endothelial cells (Blue arrow) which formed new small blood vessels and infiltration of macrophages (Green arrow) (40XH&E).

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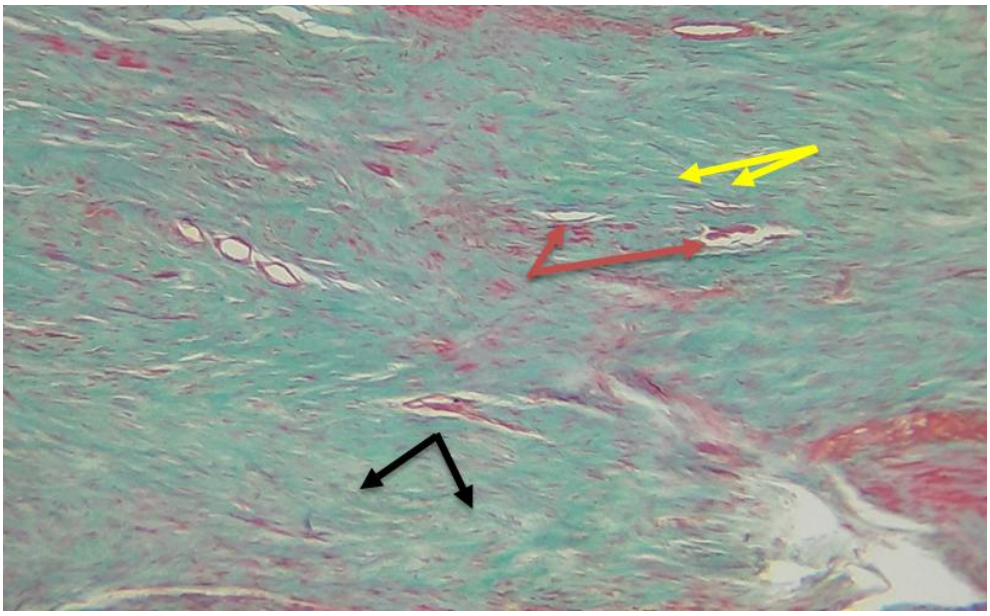

Figure (18): (G1 d28) Histopathological section show a Profuse collagen fiber (Black arrow) which stained blue color with Masson stain. few fibrosis (Yellow arrow) and new formation of small blood vessels (Red arrow) (10X Masson).

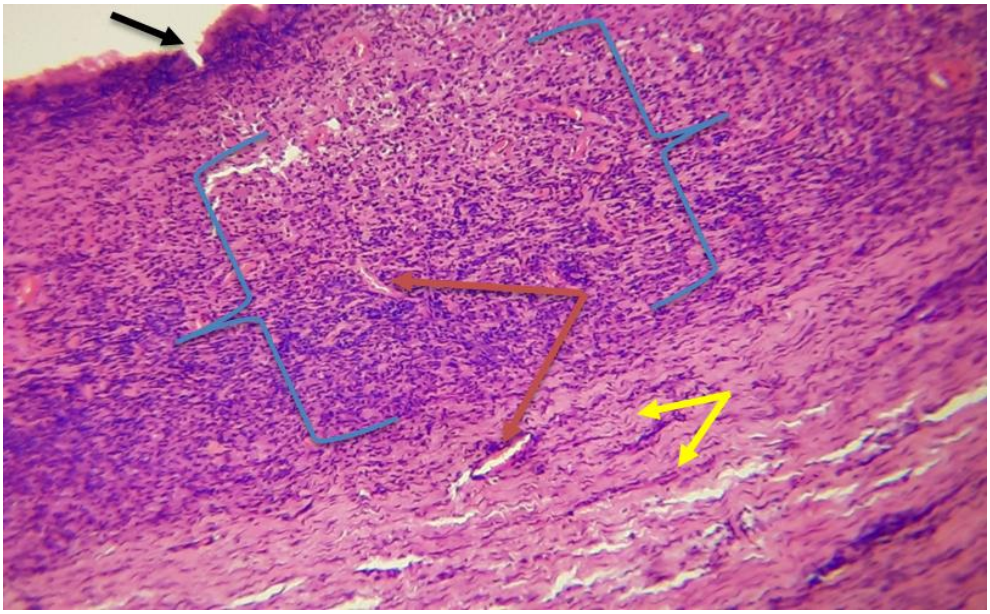

Figure (19): (G2 d14) Histopathological section show a thin epidermis with narrow incision (Black arrow) and wide scar tissue. There is profuse granulation tissue (Blue arches) in which there is new blood vessels formation (Red arrow) vertically on the site of incision and profuse fibrosis (Yellow arrow) horizontally on it (10X H&E).

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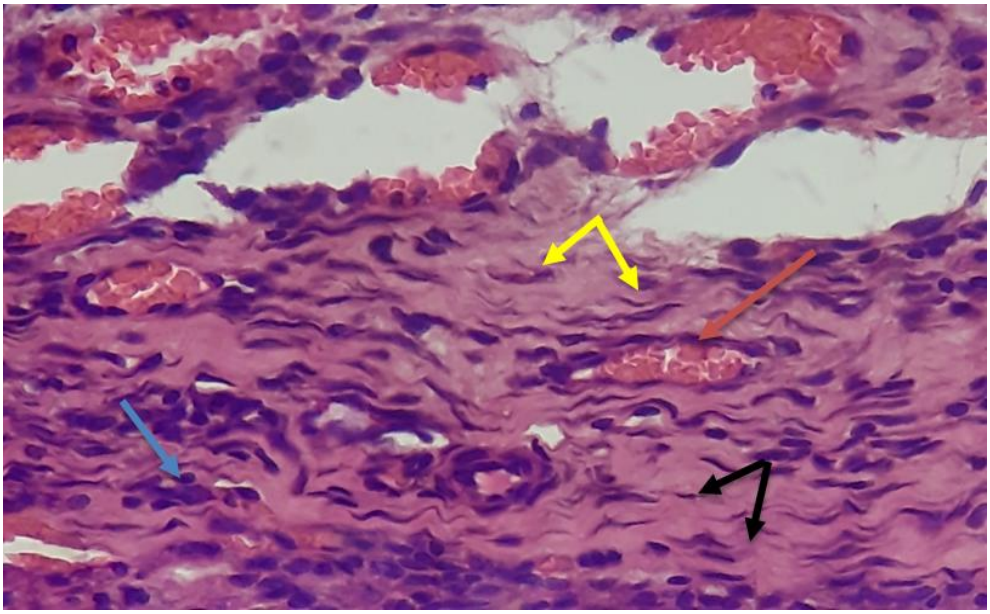

Figure (20): (G2 d14) Histopathological section show a dilated new blood vessels (Red arrow) formation with proliferation of fibroblasts (Yellow arrow). Few amounts of collagen fibers (Black arrow) with infiltration of macrophages (Blue arrow) (40X H&E).

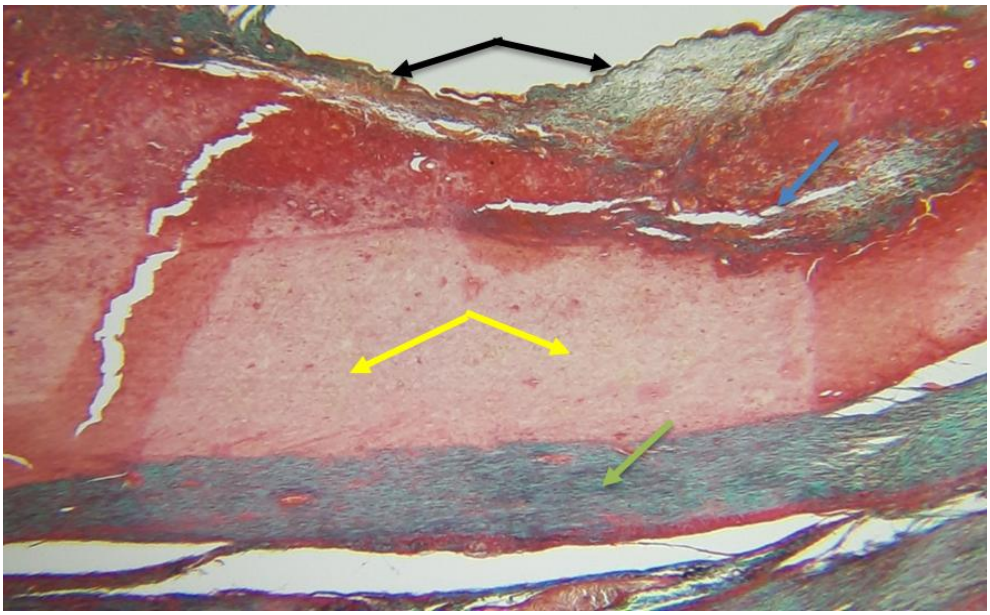

Figure (21): (G2 d14) Histopathological section show a thin epidermis. Wide Scar tissue (Black arrow). Also, there is profuse fibrosis (Yellow arrow) and newly formed blood vessels (Blue arrow), with presence of thin network of collagen fibers (Green arrow) (4X Masson trichrome).

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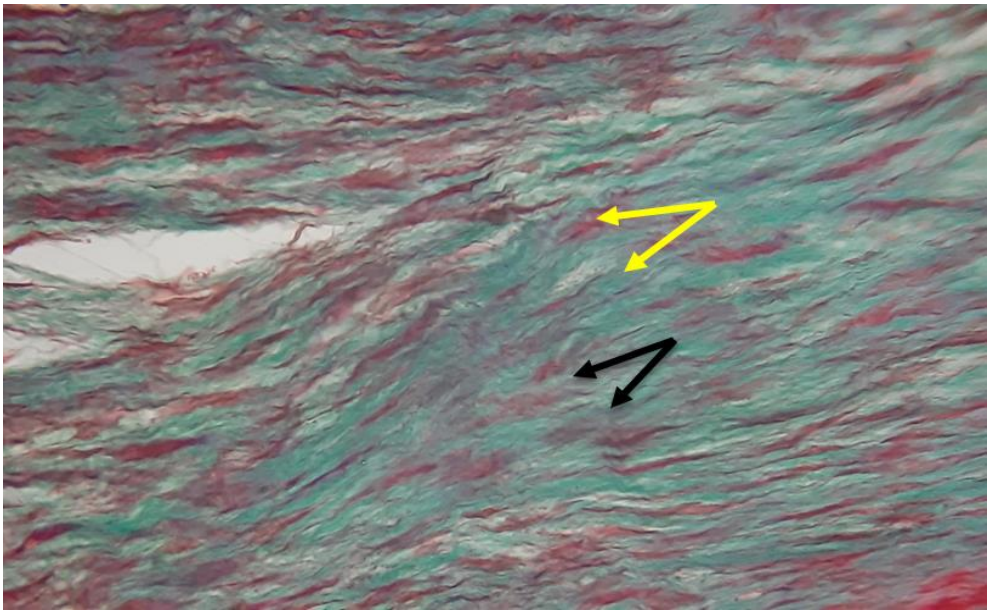

Figure (22): (G2 d14) Histopathological section show a thin and irregular network of collagen fibers (Black arrow) with proliferation of fibroblasts (Yellow arrow) (40x Masson trichrome).

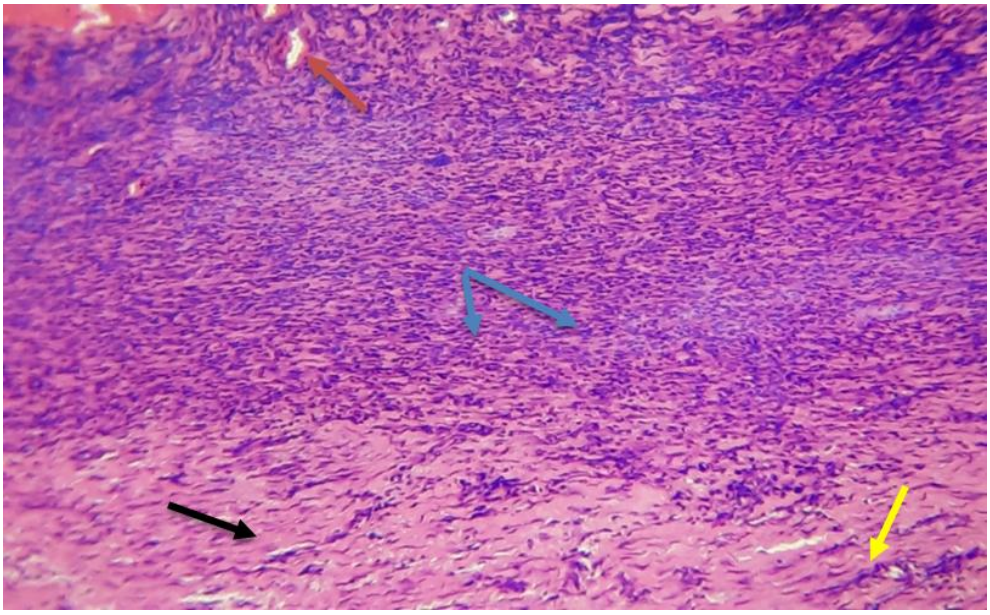

Figure (23): (G2 d28) Histopathological section show a thick regular network of Collagen fibers (Black arrow), infiltration of inflammatory cells (Blue arrow), Formation of new blood vessels (Red arrow) with few fibrosis (Yellow arrow) (10X H&E).

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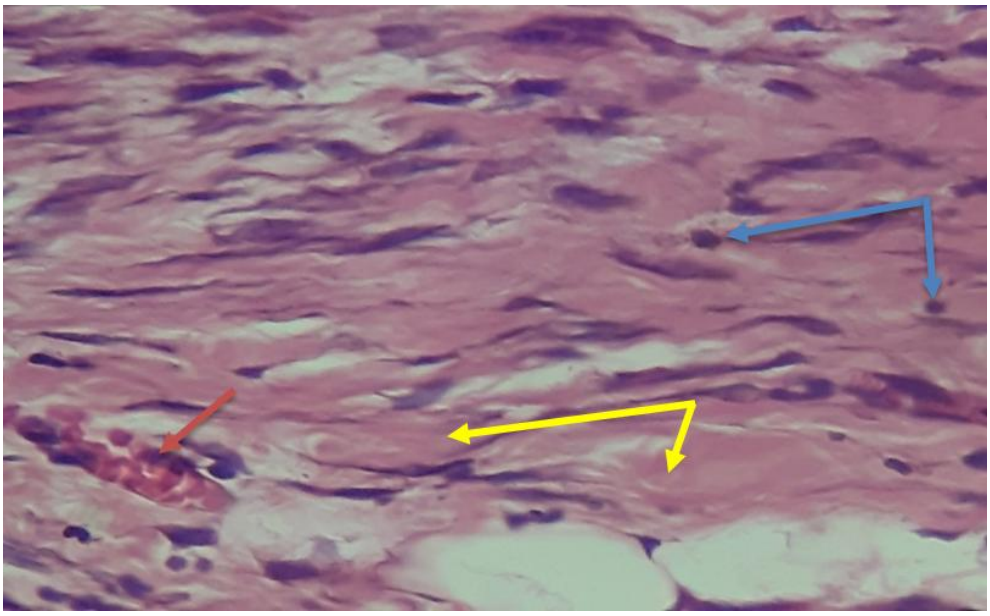

Figure (24): (G2 d28) Histopathological section show a thick collagen fiber (Yellow arrow) which arranged regularly with new dilated blood vessels (Red arrow) engorged with blood and Scattered macrophages (Blue arrow) also present (40X H&E).

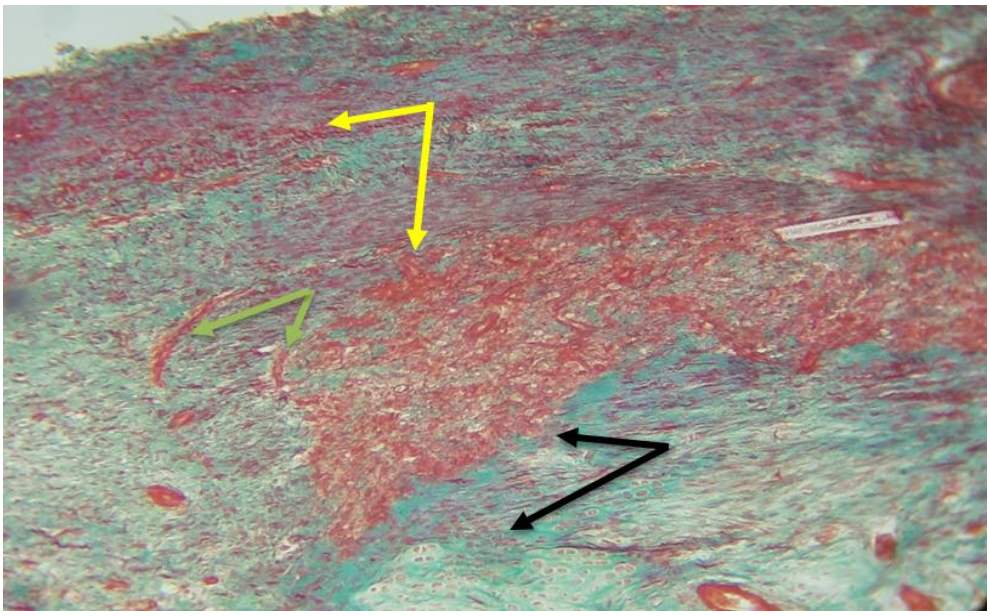

Figure (25): (G2 d28) Histopathological section show a Profuse and thick network of collagen fibers (Black arrow) in the dermis with few fibrosis (Yellow arrow) and newly formed blood vessels (Green arrow) (10X Masson trichrome).

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Discussion:

The present study aimed to evaluate the histopathological changes during the healing of the Achilles tendon injury in rats following treatment with turmeric extract nanoparticles gel, in comparison with the control group. The discussion below interprets the obtained results based on the known biological and histological mechanisms of tendon repair.

Wistar rats are one of the most popular lab animals since they are easy to breed, have a short reproductive cycle (gestation lasts around 21 to 23 days), and don't need much care. They are docile, easy to handle, and have been widely adopted in biomedical and pharmacological research for decades, making them suitable for studies requiring large sample sizes and reproducibility (Hickman *et al*., 2017).

The Achilles tendon is the most often injured tendon in an animal's body. It is a long, circular, rope-shaped extraarticular tendon that is coated by a paratenon rather than a synovial sheath (Nanka *et al*., 2024). Compared to intra-synovial tendons, these extra-synovial tendons are more prone to adhesions and scarring, and after an injury or laceration, additional surgery may be necessary to remove the scarred tissue (Liang *et al*., 2024).

Following an injury, tendons usually go through three stages: an inflammatory phase, a proliferative phase during which collagen is deposited, and a remodeling phase (Luttrell, 2024). The full recovery shown by the fourth week in the current study implies that the therapies given curcumin facilitated this natural healing process, hastening the resolution of inflammation and encouraging tissue repair.

Macroscopic Findings

Rats in this study showed restricted use of their hind limbs for the first 48 hours following surgery, but they gradually recovered their ability to move. By day 6, their ambulation was almost normal, and there were no obvious signs of infection during the follow-up period. This early postoperative recovery is in line with conventional tendon repair models in rodents, where initial inflammation and surgical stress temporarily limit function but do not prevent steady improvement as early repair processes start and the acute inflammatory phase fades.

Because inflammatory and fibrotic responses naturally occur following surgical injury, the elevated scores for inflammation, adhesion, and abnormal tendon morphology observed in the control group are expected in untreated tendon repair models. Unresolved acute inflammation and the development of scar tissue are reflected in persistent oedema, swelling, and redness, which compromise tendon gliding and structural integrity.

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Turmeric Extract and Tendon Repair

The anti-inflammatory, antioxidant, and pro-regenerative qualities of turmeric, a polyphenolic substance derived from Curcuma longa, have been thoroughly investigated in models of musculoskeletal injuries (Li, 2025).

Topical use of turmeric extract enhanced histological tissue organisation and biomechanical parameters like failure load, final stress, and strain in comparison to controls in a rat Achilles tendon injury model (Sufian *et al*., 2024). These results corroborate the fact that the curcumin group's macroscopic healing properties were noticeably superior to those of the controls.

The anti-inflammatory and antioxidant properties of turmeric lessen fibrosis and excessive inflammatory signaling. According to recent mechanistic research, turmeric reduces adhesion and inflammatory infiltration while increasing the expression of tendon-specific markers like β-catenin and EpCAM and promoting tenogenesis by activating signaling pathways like PI3K/Akt, these results accorded with Zhang *et al*., (2024) who detailed that in addition to its anti-inflammatory and antioxidant properties, curcumin may enhance tendon functional recovery by encouraging tenogenesis. Through the PI3K/Akt signaling pathway, curcumin caused tendon stem/progenitor cells to differentiate into tenocytes. This result supported the use of curcumin to stop adhesion during tendon restoration.

According to Li *et al*. (2025), turmeric has been demonstrated to inhibit proliferation while promoting the differentiation of tendon stem/progenitor cells into tenocytes, a step essential for appropriate tendon tissue regeneration and functional repair.

The benefits of turmeric are probably due to its capacity to regulate oxidative stress, NF-κB, and tenogenic differentiation pathways, which results in less fibrotic scar tissue and more ordered collagen deposition as concluded by Cozmin *et al*., (2024).

Microscopic findings

The results of the study showed powerful of the Turmeric extract group (G2) which used in rat Achilles tendon injury. When compared between (G2) and (G1) were founded (G2) have better results than (G1), Turmeric shows promising outcomes for tendon repair in rats, its help providing anti-inflammatory and antioxidant effects that promote collagen production and tenogenesis. That can lead to improved functional recovery and mechanical properties of the injured tendon that agree with (Güleç *et al*., 2018).

At 14th days post operation

Numerous inflammatory cells, as showed in (G1), scar tissue with presence of a narrow incision, accompanied by formation of small blood vessels and few collagen fibers in the dermis. There is a minor network of collagen fibers in the subcutaneous tissue. The collagen network is irregular and

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fine, particularly in areas where mild fibrosis, these were observed by (Nakazawa *et al*.,2024). Scar tissue is formed by fibroblasts and inflammatory cells during the inflammatory phase, within 1–2 weeks post-surgery. Most of the collagen produced during this process is type III as reported by (Nichols *et al*., 2019).

A scar tissue as clarified by (G2) with abundant granulation tissue, marked fibrosis, and numerous newly formed dilated blood vessels, some engorged with RBCs. There are proliferation of fibroblasts and infiltration of inflammatory cells, mainly macrophages, with focal areas of hemorrhage in the dermis accorded with Sufian *et al*., (2024). According to previous studies, Turmeric has the potential for elevation of synthesis of collogen, promoting angiogenesis, decreasing reactive oxygen radicals, and promoting healing process as gotten by (Ma *et a*l.,2013).

At 28th days post operation

Abundant granulation tissue, as displayed in (G1), characterized by significant infiltration of inflammatory cells particularly macrophages and lymphocytes with mild fibrosis and newly-formed blood vessels. There is marked fibrosis and an extensive presence of collagen fibers. The injury site exhibits a regular collagen fibers networks and proliferation of fibroblasts. Profuse collagen fibers are seen stained blue with Masson's stain. In the control group (G1), tendon repair followed the typical sequence of healing characterized by early granulation tissue formation, inflammatory cell infiltration, and progressive collagen deposition. However, the collagen fibers remained less organized with persistent inflammatory changes at 28 days, indicating a relatively slower and less efficient healing process. These findings are in line with previous reports describing the intrinsic limitations of tendon healing, where scar tissue predominates over true regenerative repair, often resulting in impaired biomechanical properties, that agree with (Voleti *et al*., 2012; Nourissat *et al*., 2015).

Thick and regular arranged collagen fibers, as represented in (G2) with scattered inflammatory cell infiltration, formation of new blood vessels (some dilated and engorged with blood), limited areas of fibrosis, and the presence of macrophages. In this group the healing response appeared more favorable compared to control group, collagen fibers became thick and well-organized, with limited fibrosis and reduced inflammatory cell infiltration. These findings suggest that turmeric nanoparticles accelerated the resolution of inflammation and promoted the maturation and alignment of collagen fibers. The observed effects may be attributed to the antioxidant, anti-inflammatory, and pro-collagen properties of Turmeric, which have been enhanced by its nanoparticle formulation improving bioavailability as mentioned by (Akbik *et al*., 2014; Sharifi-Rad *et al*., 2020). So, Turmeric could improve tendon functional recovery via promoting tenogenesis in addition to its antioxidant and anti-inflammatory activities, this approved by (Zhang *et al*., 2024).

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Conclusions:

Turmeric extract nanoparticles gel shows significantly higher anti-inflammatory and antioxidant effects better than control group and show powerful outcomes on tendon healing.

Recommendations:

1-Using Turmeric extract as a comparative study with another treatment techniques.

2- Studying the therapeutic effect of commercial Turmeric for its availability to improve healing of tendons injures.

3- Studying different types of tendons injuries, timing of treatment and doses to create a program of treatment.

Acknowledgment:

Special appreciation and thanks to the head and all staff members of the Surgery and Obstetrics Department, College of Veterinary Medicine, Al-Qadisiyah University for the advises and resources that provide for this study.

Conflict of Interest:

The authors confirm that there is no conflict of interest in this paper.

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Authors Contributions:

Both authors contributed equally to the study design, data collection and approval final version.

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