Comparative Clinicopathological Studies on Some Anti-Inflammatory Agents in Rats

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Abstract

The present work was performed to study the protective and curative anti-inflammatory role of natural moringa and licorice extract as anti-inflammatory agents on carrageenan-induced paw edema in rats. Seventy-two male albino rats (weighing 100-120 g) were divided into the following 6 groups; Control group (C): Kept as normal control that received normal saline 0.9% NaCl solution in a dose of 0.5 ml/kg body weight for 10 days, Moringa group (MO): Normal rats that received daily MO oil extract in a dose of 400 mg/kg body weight orally for 10 days , Licorice Group (L): Normal rats that received daily L extract in a dose of 250 mg/kg body weight orally for 10 days, Control arthritic group (CA): Normal rats received normal saline 0.9% NaCl solution in a dose of 0.5 ml/kg body weight for 10 days then a single injection of 0.1 ml of carrageenan-saline solution (1%, w/v) into the plantar surface of the hind paw of left leg at the 10th day of the experiment, Moringa arthritic group (MA): Normal rats that received daily MO oil extract in a dose of 400mg/kg body weight orally for 10 days then received a single injection of 0.1 ml of carrageenan-saline solution (1%, w/v) into the plantar surface of the hind paw of left leg at the 10^{th} day of the experiment, Licorice arthritic group (LA): Normal rats that received daily L extract in a dose of 250mg/kg body weight orally for 10 days with modification of route of administration then received a single injection of 0.1 ml of carrageenan-saline solution (1%, w/v) into the plantar surface of the hind paw of left leg at the 10^{th} day of the experiment. The results showed significant increase in paw edema

volume in CA group was more pronounced after five hours. On the other hand, MA and LA groups showed an improvement in the edema volume comparing with the CA group. MA and LA groups significantly ameliorated the levels of serum hepatic, antioxidant and cytokines that deteriorated by carrageenan. Histopathological examination of hind paws was in concurrence with the biochemical results. It is logical to consider that the anti-inflammatory, antioxidative properties of MO and L are mechanistically achieved. So, MO and L could be used as protective agents against inflammation.

Key words: Rats, carrageenan, Moringa oleifera, licorice, hematology, biochemistry, antioxidant, cytokines, histopathology.

Introduction:

Inflammation is the response of living tissues to damage. It causes a complex range of enzyme activation, mediator release, and extravasation of fluid, cell migration, tissue breakdown and healing ***(Perianayagam et al., 2006)***. It can be classified as either acute or chronic depending on the time of start ***(Ferrero-Miliani et al., 2007)***. Models of acute inflammation, which is induced by formalin, dextran, histamine, serotonin, bradykinin, prostaglandin and carrageenan, are used to examine the anti-inflammatory effects of treatments ***(Süleyman and Büyükokuroolu, 2001)***. Numerous herbal preparations are being prescribed usually for the treatment of inflammatory conditions **(Bagul et al., 2005)**. Moringa oleifera is an important tropical yield that is used in human nutrition, medicine and in oil production ***(Ramnath et al., 2002; Chakraborty et al., 2007)***. *Glycyrrhiza glabra* (licorice) which has a wide variety of pharmacological activities including expectorant, antitussive, emollient, anti-inflammatory, antipyretic, antiviral, antibacterial, antiprotozoal, hepatoprotective, antitumor, immunomodulatory, endocrinological, antidepressant, memory enhancing, sedative, muscle relaxant and antifungal effects ***(Asl and Hosseinzadeh, 2008)***.

The current study aimed to investigate the protective role of *Moringa oleifera* (MO) and *Glycyrrhiza glabra* (L) as anti-inflammatory agents on carrageenan-induced paw edema in rats through the following: - Determination of their effect on some hematological factors. Analysis of some serum biochemical parameters as total protein (TP), albumin, globulin, A/G ratio, alanine aminotransferase (ALT), creatinine

and urea. Estimation of lipid peroxidation as malondialdehyde (MDA) and antioxidant as superoxide dismutase (SOD) and nitric oxide (NO). Estimation of some pro-inflammatory cytokines as tumor necrosis factor (TNF-α) and interleukin 6 (IL-6).

Materials and Methods:

λ-carrageenan: obtained from Sigma chemical (St. Louis, MO, USA) and used for initiation of inflammation (paw edema). Moringa oil extract: MO oil extract was purchased from Grenera Nutrients Private Limited, India. Licorice extract: L extract was purchased from Dongtai Hanfangyuan Biological Technology Co, Ltd, China.

Sampling:

Blood sampling: were taken 2 times, the first time at the 9th day and the second time at the 10th day (5 hrs after carrageenan injection). Five ml of blood was collected from retro-orbital venous plexus divided into two portions. The blood was drawn into 2 tubes; the first tube (0.5 ml blood) contained dipotassium salt of EDTA as anticoagulant. This tube was used for hematological evaluation (RBCs count, Hb concentration, PCV value, blood indices and WBCs count). The second tube (4.5 ml blood) was plain tube for serum separation. The separated sera were used for biochemical, lipid peroxidation and antioxidants, pro-inflammatory cytokines and arthritic markers analysis. Tissue sampling: after scarification, hind paw samples were obtained and fixed in 10% formalin for histopathological examination.

Hematological parameters were performed by standard techniques according to ***Jain (1986)***. Serum alanine aminotransferase (ALT) was measured according to ***Reitman and Frankel (1957).*** Serum total proteins (TP) and serum creatinine (Creat.) was determined according to ***Henry (1974).*** Serum albumin (Alb.) and globulin was determined by the method of ***Doumas et al. (1981).*** Albumin /globulin ratio was calculated by dividing albumin over globulin according to ***Noverraz (1953).*** Serum urea was determined by colorimetric test according to the method of ***Fawcett and Scott (1960).*** Serum Malondialdehyde (MDA) was measured according to ***Armstrong and Browne (1994).*** Serum Superoxide Dismutase (SOD) was measured according to ***Koivunen and Krogsrud (2006).*** Serum Nitric Oxide (NO) was measured according to ***Armando et al. (1992).*** Serum Tumor necrotic factor-α (TNF-α) was measured according to ***Wajant et al. (2003).*** Serum Interleukin estimation (IL-6) was measured according to ***Koivunen and Krogsrud (2006).***

Serum Rheumatoid factor (RF) was measured according to ***Mahajan et al. (2008).*** Serum C-reactive protein (CRP) level: was measured according to ***Tatiya and Saluja (2011).***

Statistical Analysis:

Data of the present study were expressed as mean ± standard error (SE) and analyzed using One-Way Analysis of Variance (ANOVA) for all tested groups according to ***Snedecor and Cochran (1989)***. Means separations were done by Duncan's Multiple Range test according to ***Duncan (1955)***. The present data were analyzed using (SPSS, 20) for windows. Results are considered significant at probability level of 0.05 ($P \leq 0.05$).

Results:

1.Clinical signs

Regarding the edema volume, a significant increase in paw edema volume in CA group was more pronounced after five hours. On the other hand, MA and LA groups showed an improvement in the edema volume comparing with the CA group.

2 .Hematological result:

At the 9th and the 10th day of the experimental period, MO, L, CA, MA and LA groups showed non-significant changes in all erythrogram parameters when compared with C group.

At the 10th day of the experimental period, CA group showed significant leukocytosis at ($P\leq 0.05$) when compared with C group. While, MA and LA groups showed significant decline toward normal in WBCs when compared with CA group.

3.Biochemical results

At the 10th day of the experimental period, CA group showed non-significant in all tested biochemical parameters except in liver enzyme (ALT) activity there was significant increased activity at ($P\leq 0.05$) when compared with C group. While, MA and LA groups showed non-significant changes in all tested biochemical parameters except in liver enzyme (ALT) activity there was significant decreased toward normal when compared with CA group.

4. Lipid peroxidation and antioxidants results

At the 10th day of the experimental period, CA group showed significant increase at ($P\leq 0.05$) in MDA and NO values when compared with C group. While, there was significant decrease at ($P\leq 0.05$) in SOD activity when compared with C group. However, MA and LA groups showed significant decrease toward normal in MDA and NO values but there was significant increase toward normal in SOD activity when compared with CA group.

5. Pro-inflammatory cytokines results

At the 10th day of the experimental period, CA group showed significant

increase at (P≤ 0.05) in TNF-α and IL-6 values when compared with C group. While, MA and LA groups showed significant decrease toward normal in TNF-α and IL-6 levels when compared with CA group.

6. Arthritis markers results

At the 10th day of the experimental period, CA group showed significant increase at (P≤ 0.05) in RF and CRP levels when compared with C group. While, MA and LA groups showed significant decrease toward normal in RF and CRP levels when compared with CA group.

7. Histopathological results

At the 10th day of the experimental period, the joints obtained from CA group showed disrupted articular surface, erosion of articular cartilage, severe hyperplasia of the synovial membrane. While, MA group showed smooth articular surface with thickened synovium with moderated obliteration of joint space. The sections prepared from LA group exhibited smooth articular surface with a mild hyperplasia of synovial membrane.

Table (1): *The effects of MO and L oil extracts on edema volume of rats at the 9th and the 10th day of the experimental period.*

Parameters / **Groups**	**Edema Volume (mm)**	
	At 9th day	At 10th day
C	29.33±0.42[a]	29.33±0.33[d]
MO	28.50±0.43[a]	29.27±0.31[d]
L	29.17±0.48[a]	29.17±0.40[d]
CA	29.17±0.31[a]	37.67±0.33[a]
MA	29.33±0.33[a]	33.50±0.22[b]
LA	29.00±0.37[a]	32.17±0.31[c]

Values are presented as means ±SE; a-d different superscripts mean in the same column considered significant at (P≤ 0.05).

Table (2): *The effects of MO and L oil extracts on hematological parameters of rats at the 9th and the 10th day of the experimental period.*

Values are presented as means ±SE; a-d different superscripts mean in the same column

Parameters / Groups	RBCs(10^6/µl)	Hb(g/dl)	PCV (%)	MCV (fl)	MCH (pg)	MCHC (%)	WBCs(10^3/µl)
At 9th day							
C	8.74±0.19^a	14.90±0.22^a	49.33±0.42^a	56.55±1.10^a	17.05±0.30^a	29.60±0.63^a	10.22±0.36^a
MO	8.46±0.12^a	14.95±0.16^a	49.30±0.79^a	57.30±0.92^a	17.42±0.37^a	30.35±0.38^a	10.63±0.42^a
L	8.45±0.08^a	14.62±0.25^a	48.93±0.43^a	58.53±1.06^a	17.30±0.39^a	29.58±0.64^a	10.65±0.29^a
CA	8.69±0.18^a	14.73±0.26^a	49.78±0.47^a	57.37±1.05^a	16.98±0.38^a	29.25±0.79^a	10.00±0.28^a
MA	8.48±0.12^a	14.90±0.21^a	49.15±0.71^a	57.97±0.49^a	17.55±0.21^a	30.33±0.58^a	9.94±0.29^a
LA	8.49±0.08^a	14.90±0.26^a	49.03±0.47^a	57.77±0.56^a	17.57±0.39^a	30.38±0.53^a	10.48±0.23^a
At 10th day							
C	8.66±0.12^a	14.77±0.17^a	49.17±1.75^a	56.82±2.10^a	17.07±0.38^a	30.18±0.89^{ab}	10.52±0.28^d
MO	8.45±0.08^a	14.80±0.25^a	48.67±1.66^a	57.60±1.84^a	17.50±0.34^a	30.57±0.97^a	10.38±0.23^d
L	8.54±0.08^a	14.53±0.18^a	49.82±0.75^a	58.35±1.01^a	17.00±0.09^a	29.22±0.53^{ab}	10.38±0.23^d
CA	8.65±0.16^a	14.60±0.18^a	50.22±0.50^a	58.12±1.14^a	16.92±0.36^a	29.07±0.23^{ab}	16.11±0.24^a
MA	8.41±0.15^a	14.13±0.28^a	50.10±0.85^a	59.62±0.79^a	16.83±0.23^a	28.23±0.69^b	14.61±0.14^b
LA	8.80±0.14^a	14.75±0.29^a	49.62±0.94^a	56.45±1.39^a	16.77±0.37^a	29.77±0.73^{ab}	12.71±0.29^c

considered significant at ($P \leq 0.05$).

Table (3): *The effects of MO and L oil extracts on biochemical values of rats at the 9th and the 10th day of the experimental period.*

Params / Grous	Tp. (g/dl)	Alb. (g/dl)	Glob. (g/dl)	A/G ratio	ALT (U/L)	Creat. (mg/dl)	Urea (mg/dl)
9th day							
C	7.43±0.^{a}	4.02±0.1^a	3.41±0.1^a	1.17±0.1^a	46.83±1.6^a	0.46±0.0^a	34.00±2.3^a
MO	7.13±0.^{a}	4.22±0.1^a	2.91±0.0^a	1.45±0.0^a	47.00±2.0^a	0.45±0.0^a	28.33±2.2^a
L	7.40±0.^{a}	4.33±0.0^a	3.07±0.1^a	1.4±0.10^a	47.17±1.7^a	0.44±0.0^a	33.50±3.5^a
CA	7.40±0.^{a}	4.00±0.1^a	3.40±0.1^a	1.17±0.0^a	46.67±1.7^a	0.46±0.0^a	34.33±2.4^a
MA	7.11±0.^{a}	4.23±0.1^a	2.88±0.0^a	1.47±0.0^a	46.17±1.5^a	0.45±0.0^a	28.67±1.8^a
LA	7.38±0.^{a}	4.32±0.0^a	3.06±0.1^a	1.41±0.0^a	45.83±1.8^a	0.45±0.0^a	33.17±3.8^a
10th day							
C	7.05±0.^{a}	4.17±0.0^a	2.88±0.1^a	1.44±0.0^a	47.00±1.5^c	0.43±0.0^a	39.66±1.3^a
MO	6.90±0.^{a}	3.87±0.1^a	3.03±0.1^a	1.27±0.1^a	48.50±2.3^c	0.39±0.0^a	37.16±2.7^a
L	7.07±0.^{a}	3.92±0.1^a	3.15±0.1^a	1.24±0.0^a	48.00±2.1^c	0.44±0.0^a	38.66±1.9^a
CA	7.05±0.^{a}	4.02±0.0^a	3.03±0.0^a	1.32±0.0^a	68.67±1.7^a	0.41±0.0^a	39.33±0.8^a
MA	7.00±0.^{a}	4.02±0.0^a	2.98±0.1^a	1.34±0.0^a	57.00±1.1^b	0.42±0.0^a	37.66±3.2^a
LA	6.95±0.^{a}	4.13±0.07^a	2.82±0.06^a	1.46±0.04^a	45.17±1.66^c	0.42±0.02^a	34.83±3.35^a

Values are presented as means ±SE; a-c different superscripts mean in the same column considered significant at ($P \leq 0.05$).

Table (4): *The effects of MO and L oil extracts on lipid peroxidation and antioxidants values of rats at the 9th and the 10th day of the experimental period.*

Parameters / Groups	MDA (nm)	SOD (μm)	NO (nm)
At 9th day			
C	1.67±0.04[a]	3.88±0.02[a]	54.74±0.37[a]
MO	1.68±0.03[a]	3.88±0.03[a]	54.20±0.16[a]
L	1.67±0.02[a]	3.87±0.05[a]	54.64±0.27[a]
CA	1.67±0.05[a]	3.88±0.02[a]	54.76±0.38[a]
MA	1.68±0.04[a]	3.88±0.04[a]	54.27±0.17[a]
LA	1.67±0.02[a]	3.87±0.05[a]	54.64±0.27[a]
At 10th day			
C	1.68±0.02[d]	3.87±0.01[a]	54.95±0.20[d]
MO	1.67±0.01[d]	3.88±0.02[a]	54.98±0.16[d]
L	1.67±0.01[d]	3.88±0.01[a]	54.49±0.19[d]
CA	5.03±0.02[a]	2.02±0.02[d]	95.04±0.37[a]
MA	2.42±0.03[b]	3.35±0.03[c]	65.85±0.17[b]
LA	2.06±0.02[c]	3.57±0.02[b]	62.04±0.34[c]

Values are presented as means ±SE; a-d different superscripts mean in the same column considered significant at (P≤ 0.05).

Table (5): *The effects of MO and L oil extracts on pro-inflammatory cytokines values of rats at the 9th and the 10th day of the experimental period.*

Parameters / Groups	TNF-α (pg/ml)	IL-6 (pg/ml)
At 9th day		
C	5.24±0.02[a]	8.53±0.02[a]
MO	5.21±0.02 [a]	8.53±0.01[a]
L	5.22±0.01 [a]	8.47±0.02[a]
CA	5.23±0.03 [a]	8.53±0.02[a]
MA	5.21±0.02 [a]	8.53±0.01[a]
LA	5.22±0.01 [a]	8.48±0.02[a]
At 10th day		
C	5.28±0.01[d]	8.51±0.02[d]
MO	5.14±0.02[d]	8.52±0.01[d]
L	5.29±0.14[d]	8.42±0.02[d]
CA	15.91±0.05[a]	20.02±0.11[a]
MA	8.47±0.09[b]	11.27±0.06[b]
LA	7.38±0.05[c]	10.06±0.08[c]

Values are presented as means ±SE; a-d different superscripts mean in the same column considered significant at (P≤ 0.05).

Table (6): *The effects of MO and L oil extracts on arthritic values of rats at the 9th and the 10th day of the experimental period.*

Parameters / Groups	RF (IU/ml)	CRP (ng/ml)
At 9th day		
C	3.78±0.24[a]	0.33±0.04[a]
MO	3.96±0.19[a]	0.25±0.02[a]
L	4.43±0.16[a]	0.37±0.07[a]
CA	3.78±0.25[a]	0.35±0.07[a]
MA	3.90±0.20[a]	0.28±0.06[a]
LA	4.45±0.15[a]	0.40±0.05[a]
At 10th day		
C	4.03±0.16[d]	0.31±0.04[c]
MO	4.08±0.07[d]	0.30±0.05[c]
L	4.26±0.14[d]	0.26±0.03[c]
CA	7.10±0.09[a]	1.08±0.09[a]
MA	5.86±0.12[b]	0.77±0.06[b]
LA	4.20±0.16[c]	0.33±0.05[c]

Values are presented as means ±SE; a-d different superscripts mean in the same column considered significant at (P≤ 0.05).

Photo 1: Histopathological picture of hind paw of C group showed normal articular cartilage structures with smooth articular surface (AC), normal synovium and normal joint space (asterisk). H&E. X 100.

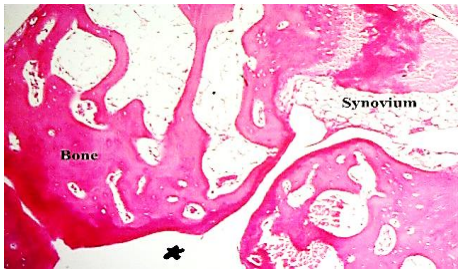

Photo 2: Histopathological picture of hind paw of MO group showed normal articular cartilage structures with smooth articular surface (AC), normal synovial membrane and normal joint space (asterisk). H&E. X 100.

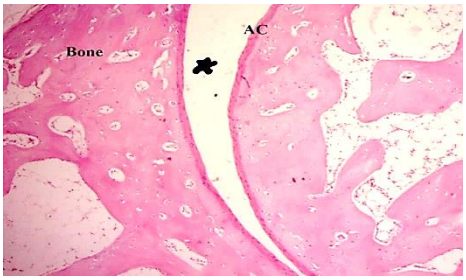

Photo 3: Histopathological picture of hind paw of L group showed normal articular cartilage structures with smooth articular surface (AC), normal synovial membrane and normal joint space (asterisk). H&E. X 100.

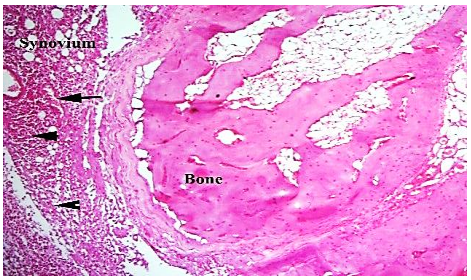

Photo 4: Histopathological picture of hind paw of CA group showed hyperplasia of synovial membrane with severe leucocytic infiltrations (arrow heads) and congestion of blood vessels (arrows) and obliterated joint space. H&E. X 100.

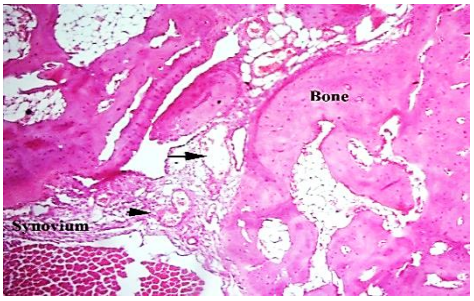

Photo 5: Histopathological picture of hind paw of MA group showed moderate hyperplasia of synovial membrane congestion of blood vessels (arrows), edema, mild leucocytic infiltrations (arrow heads) and narrowing of joint space. H&E. X 100.

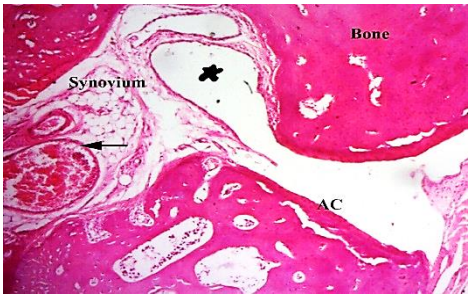

Photo 6: Histopathological picture of hind paw of LA group showed normal articular cartilage articular surface (AC), mild hyperplasia of synovial membrane with severe congestion of blood vessels (arrows), edema and maintained joint space. H&E. X 100.

Discussion:

Current results revealed that arthritic rats significantly increased total WBCs than normal control but no significant changes in erythrogram compared with control group. These results agreed with ***Weiner et al., (2006)*** who decided that male and female rats-controlled kappa carrageenan in

their diet at concentrations of 25,000 or 50,000 ppm for 90 consecutive days showed no treatment-related hematological disorders**.** The administration of MO and L extracts to inflamed rats significantly reduced toward normal the elevated TLC in arthritic rats. These results coincided with results of ***Auwal et al. (2013)*** for MO oil extract. ***Jayanthi et al. (2016)*** and ***Maksoud et al. (2019)*** got similar results for L extract with arthritic rats.

Current results revealed that MA and LA groups showed no significant changes in TP, albumin, globulin, A/G ratio, creatinine and urea but there was improvement in ALT compared to arthritic group. These results agreed with ***Haldar et al. (2010), Dolai et al. (2012) and Islam et al. (2014).*** The improvement in the cellular membrane integrity of the hepatic cell which is a clear manifestation of antihepatotoxic effect of MO administration ***(El-bakry et al., 2016).*** The constituents of L also exhibit hepatoprotective activity by lowering serum liver enzyme levels and improving tissue pathology in hepatitis patients **(Kim et al., 2012).**

Current results revealed that arthritic rats significantly increased in MDA and NO but there was significant decrease in SOD compared with control group. These results agreed with ***Dolai et al. (2012)***, ***Samudrala et al. (2015)*** *and* ***Ceylan et al. (2018).*** Current results revealed that MA and LA groups showed decline in MDA and NO with significant increase in SOD compared to arthritic groups. ***Masood (2010)*** got the similar results in case of MO oil extract. Results of ***Hemieda et al. (2016)*** were coincided with our results concerning L extract.

Current results revealed that arthritic rats significantly increased in TNF- α and IL-6 compared to control group. ***Mohammad et al. (2015)*** confirmed our results as they demonstrated TNF- α and IL-6 levels were significantly increased following injection of 0.10 ml of 1% carrageenan-induced paw edema in rats. Current results revealed that MA and LA groups showed improvement in TNF-α and IL-6 compared to arthritic group. The administration of MO and L extracts significantly reduced the levels of (TNF-α) in arthritic rats near to normal control rats. ***Karthivashan et al. (2016)*** and ***Yousef et al. (2018)*** got the similar results in case of MO oil extract.

Current results revealed that MA and LA groups showed improvement in RF and CRP compared to arthritic group. These

results agreed with ***Mansour et al., (2021)*** who said that treatment with *Moringa oleifera* extract showed highly significant improvement of RF and CRP compared to diseased treated groups. These results agreed with ***Jichun et al., (2014)*** that compared with I/R group, CRP level decreased significantly in the group treated with 1 μg/mL licochalcone B (which belongs to the retrochalcone family, is isolated from the roots of Chinese licorice).

Current results revealed that the joints of arthritic rats showed disrupted articular surface, erosion of articular cartilage, severe hyperplasia of the synovial membrane with marked congestion and diffuse leucocytic infiltrations. These results agreed with ***Pauline Hansra et al. (2000)*** that reported the histological sections severe, chronic and erosive inflammatory arthritis was present in all arthritic animals. Our results of the joints of MA group showed smooth articular surface with thickened synovium with moderated obliteration of joint space. The joints of LA group exhibited smooth articular surface with a mild hyperplasia of synovial membrane. These results agreed with ***Ammara et al. (2020)*** that showed percentage inhibition in paw volume exhibited by methanolic extract at 600 mg/kg and aqueous extracts of *Moringa rivae* at 600 mg/kg was significantly higher than piroxicam-treated rats at 28th day. ***Ki et al. (2009)*** indicated that oral administration of LE and rLE to CIA-immunized mice reduced the progression of arthritis by inhibiting the increase in arthritis score and paw swelling, as compared to the vehicle-treated immunized mice.

Conclusion:

It could be concluded that:

Licorice is a potent, fast acting anti-inflammatory agent and gives better result than moringa. It significantly decreases pro-inflammatory cytokines and oxidative stress markers.

It is recommended to:

Use moringa and licorice as prophylaxis in order to get optimum anti-inflammatory effect.

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دراسات باثولوجية إكلينيكية مقارنة علي بعض المواد المضادة للإلتهاب في الفئران

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الكلمات الدالة

فئران , كاراجينان , مورينجا أوليفيرا, عرقسوس , علم الدم , كيمياء حيوية, مضادات أكسدة ,سيتوكين , دراسات باثولوجية.

الملخص العربي

أجريت هذه الدراسة لمعرفة الدور الوقائى والعلاجي لمستخلصي المورينجا أوليفرا والعرقسوس كمضادات للإلتهاب فى الفئران المستحدث بها إلتهاب فى القدم باستخدام مادة الكاراجينان وذلك بقياس بعض دلالات الإلتهاب بالإضافة إلى دراسة الآثار الجانبية المترتبة عن إستخدام كلاً منهما على حدة.

أجريت هذه الدراسة على عدد إثنان وسبعون من الجرذان البيضاء والتى قسمت إلى ست مجموعات وتمت الدراسة على النحو الآتي:-

المجموعة الأولى:مجموعة ضابطة للتجربة والتى أعطيت (0.5 ملي/كجم) محلول ملحي عن طريق الفم لمدة عشرة أيام. المجموعة الثانية: أعطيت مستخلص المورينجا أوليفيرا عن طريق الفم بجرعة (400 مجم لكل كيلو جرام من وزن الجسم) لمدة عشرة أيام.المجموعة الثالثة: أعطيت مستخلص العرقسوس عن طريق الفم بجرعة (250 مجم لكل كيلو جرام من وزن الجسم) لمدة عشرة أيام . المجموعة الرابعة: مجموعة تم حقنها في القدم اليسري الخلفية بمادة الكاراجينان في اليوم العاشر. المجموعة الخامسة : أعطيت مستخلص المورينجا أوليفيرا عن طريق الفم بجرعة (400 مجم لكل كيلو جرام من وزن الجسم) لمدة عشرة أيام ثم حقنها في القدم اليسري الخلفية بمادة الكاراجينان في اليوم العاشر.المجموعة السادسة: أعطيت مستخلص العرقسوس عن طريق الفم بجرعة (250 مجم لكل كيلو جرام من وزن الجسم) لمدة عشرة أيام ثم حقنها في القدم اليسري الخلفية بمادة الكاراجينان في اليوم العاشر.

بعد ساعة من حقن الكاراجينان فى القدم تم ملاحظة أعراض الإلتهاب من إحمرار وإنتفاخ حتي خمس ساعات. ثم ذبحت الفئران في اليوم التاسع والعاشر لتجميع عينات الدم لعمل صورة الدم وفصل السيروم لإجراء القياسات البيوكيميائية وعوامل الأكسدة ودلالات الإلتهاب كما تم تجميع القدم لقياس التورم وعمل الفحص النسيجي. أثبتنا أنه عند إستخدام مستخلص العرقسوس قد أعطى نتائج أفضل من مستخلص المورينجا أوليفيرا.فقد قلل من الآثار الجانبية للإلتهاب وزاد من كفاءته كمضاد للإلتهاب. نوصى بتناول مستخلص المورينجا أوليفيرا والعرقسوس كعلاج مضاد للإلتهاب أكثر أمانا من المواد الكيميائية.