AIM OF THE WORK

The aim of the present study was to assess the efficacy and safety of 4,5-diacetyloxy-9,10-dioxo-anthracene-2-carboxylic acid on osteoarthritic knee pain; and the effect of this agent on IL-1β levels.

SUBJECTS

The present prospective study was conducted on:

(1) 60 patients with knee pain due to primary knee OA and aged above 50 years.
(2) 6 subjects of matching age and sex with asymptomatic mild knee effusion who did not have knee OA or any inflammatory arthritis (control group).

Patients and controls were recruited from the outpatient clinic of the department of Physical Medicine, Rheumatology and Rehabilitation, Alexandria University.

Patients with the following criteria[123] were included:

1. Knee pain.
2. Radiologic osteophytes.
3. One of the following features:

   - Age > 50 years.
   - Morning stiffness < 30 minutes, and/or.
   - Joint crepitus.

The exclusion criteria were:

1. Patients with knee instability.
2. Patients with inflammatory arthritis.
3. Patients with bleeding tendency.
4. Overweight patients (>80 kg).
5. Patients with associated knee bursitis.
6. Patients with contraindications to NSAIDs, e.g. gastropathy, peptic ulcer, renal insufficiency, liver disease, asthma, hypertension, etc.
7. Patients with severe functional impairment (severe cardiac or chest problem, amputation, ischemic heart disease, etc).
8. Patients or subjects with haemoarthrosis.

METHODS

Detailed history, complete clinical and radiological examination were conducted to ensure the diagnosis of OA. Clinical examination included a routine general examination of the patients to confirm the diagnosis and to exclude other causes of knee pain.

Clinical examination of the affected knee was conducted according to the systematic methods of knee examination. The range of motion was determined with a goniometer. The presence of any joint effusion was documented.

The duration of study was 3 years from July 2011 to July 2014. It was reviewed and approved by the Local Ethics Committee of Alexandria University.

Radiological study: Weight-bearing postroanterior and supine lateral radiographs of the affected knees were obtained.

Patient's groups:

The patients were divided randomly into three groups, each of at least 10 patients:

Group I: received diacerein (osteocerein) (50 mg capsules twice daily) for 2 months.

Group II: received diclofenac sodium (75 mg capsules once per day) for 2 months.

Group III: received both diclofenac and diacerein in the previous doses for 2 months.

Patient's first assessment:

Patients were evaluated for pain severity using both the visual analogue scale and the verbal rating scale at the start of the study (This was not done in the control group).

Twenty meter fast walking time was measured (by a stopwatch) for all patients at the start of the study (This was not done in the control group).

Determination of the level of IL-1β in the serum of all patients and in the control subjects was performed just before the enrollment in the study.

Determination of the level of IL-1β in the synovial fluid was performed in 10 patients of Group I & 10 patients of Group III as well as control subjects.

Patient's reassessment:

- Reassessment of pain severity and the walking time was done in all patients 1 month and 2 months after the start of treatment, in the same way as before treatment.

Also, patients with knee OA were evaluated after medical treatment with laboratory screening of blood samples and aspirated synovial fluid from the knee to determine IL-1β level in the serum and synovial fluid 1 month and 2 months following the start of the study (in at least 10 patients after 1 month) in the same way as before treatment and in the same patients.

Assessment of any side effects:

Patients were asked about any side effects during treatment (e.g. heartburn, nausea, vomiting, diarrhea, etc.).

- An informed consent was taken from all subjects.

Measurement of Interleukin (IL) 1β in Synovial fluid and Serum in patients with knee osteoarthritis:

Principle:

IL-1β was measured in patient synovial fluid and serum, using the WKEA Human IL-1β ELISA (Enzyme-Linked Immunosorbent Assay) kit, according to manufacturer's instructions. It is an in vitro enzyme-linked immunosorbent assay for quantitative measurement of human IL-1β in serum, plasma, and other biological fluids.

Materials supplied:

Table (1): Materials supplied for measurement of IL-1β

Reagents	Specification
Microtiter plate	96 wells
Standard: 27ng/L	0.5mlx1bottle
Standard diluent	1.5mlx1bottle
Enzyme Conjugate	6mlx1bottle
Sample diluent	6mlx1bottle
Substrate A	6mlx1bottle
Substrate B	6mlx1bottle
Stop Solution	6mlx1bottle
Wash Solution	(20mlx30 fold)x1bottle

Assay procedure:

1. Sample was diluted and added to Standard: 10 Standard wells were set on the microtiter plate coated, Standard 100µl was added to the first and the second well, then Standard dilution 50µl was added to the first and the second well, and was mixed; 100µl was taken out from the first and the second well then added to the third and the forth well separately, then Standard dilution 50µl was added to the third and the fourth well, and was mixed; then 50µl was taken out from the third and the fourth well and was discarded, 50µl was added to the fifth and the sixth well, then Standard dilution 50µl was added to the fifth and the sixth well, and was mixed; 50µl was taken out from the fifth and the sixth well and was added to the seventh and the eighth well, then Standard dilution 50µl was added to the seventh and eighth well, and was mixed; 50µlwas taken out from the seventh and the eighth well and was added to the ninth and the tenth well, Standard dilution 50µl was added to the ninth and the tenth well, and was mixed, 50µl was taken out from the ninth and the tenth well and was discarded, sample 50µl was added to each well after diluting, (density: 18ng/L, 12ng/L, 6ng/L, 3ng/L, 1.5ng/L).

2. Sample was added: wells set back separately (blank comparison wells sample and Enzyme Conjugate were not added, other each step operation is same). Sample dilution 40µl was added to sample well, then sample 10µl was added (sample final dilution is 5-fold), sample was added to wells, the well wall was not touched as far as possible, and was gently mixed.
3. Incubation: After closing plate with closure plate membrane, incubation was done for 30 minutes at 37°C.
4. Solution preparation: 30-fold (or 20-fold) wash solution was diluted 30-fold (or 20-fold) with distilled water and reserved.
5. Manual washing: Incubation mixture was removed by aspirating contents of the plate into a sink or proper waste container. Using a squirt bottle, each well was completely filled with wash solution, then contents of the plate were aspirated into a sink or proper waste container. This procedure was repeated four more times for a total of FIVE washes. After final wash, plate was inverted and blot dried by hitting plate onto absorbent paper or paper towels until no moisture appears. Note: The slides of the plate frame were held firmly when washing the plate to assure that all strips remain securely in frame.
6. Enzyme addition: 50µl Enzyme Conjugate reagent was added to each well, except blank well.
7. Incubation: Operation with 3.
8. Washing: Operation with 5.
9. Color: 50µl Substrate A and Substrate B were added to each well, covered and incubated for 15 minutes at 37°C.
10. The reaction stopping: 50µl Stop Solution was added to each well and mixed well.
11. Assay: The optical density of each well was determined within 15 minutes by a microplate reader.

Calculation of results:

The standard density was taken as the horizontal, the OD value for the vertical, the standard curve is drawn on graph paper. The corresponding density was found out according to the sample OD value by the Sample curve, multiplied by the dilution multiple, or the straight line regression equation of the standard curve was calculated with the standard density and the OD value, with the sample OD value in the equation, the sample density was calculated, multiplied by the dilution factor, the result is the sample actual density.

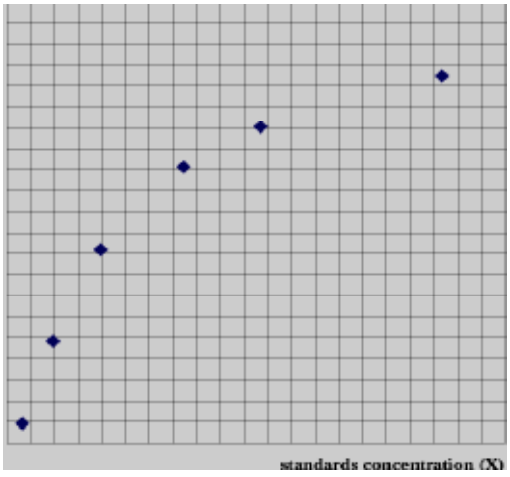

Fig. (1): Standard curve of IL-1β.

Statistical analysis:

Statistical analysis was carried out using SPSS statistics software version 20. Categorical variables were described using frequencies and percentages. Fisher's exact test and Monte Carlo test were used for testing associations between categorical variables. Quantitative data were given as median (minimum-maximum). Non-parametric statistical tests of significance were applied; Kruskal-Wallis test was used to compare more than two independent groups and Friedman test was used to compare more than two dependent groups. Any significant Kruskal-Wallis or Friedman comparison was followed by adjusted post-hoc pair-wise comparisons. Statistical significance was accepted as $p < .05$. All applied statistical tests of significance were two-tailed.

Case-study sheet adopted in the present work:

Date: / /20							
PERSONAL HISTORY:							
ID #							
Name							
Age (year)							
Sex							
Occupation							
Weight(kg)							
Height(meter)							
BMI							
HISTORY OF PRESENT ILLNESS:							
Onset							
Course							
Disease duration							
Involved knee(Rt/Lt/Bil)							
EXAMINATION:							
Alignment							
Tenderness over joint line							
Effusion							
Joint crepitus							
Wasting							
Morning stiffness							
PAIN SEVERITY ASSESSMENT:							
		At the start of treatment(0)		**1 month later**		**2months later**	
Visual analogue scale							
Verbal rating scale							
FUNCTIONAL ASSESSMENT:							
Muscle power		**Rt.**	**Lt.**	**Rt.**	**Lt.**	**Rt.**	**Lt.**
	QFM.						
	Hamstrings.						
Knee ROM							
	Flexion.						
	Extension.						
20 meters walking time							
LAB.STUDIES:							
Blood IL-1(µg)							
Synovial fluid IL-1(µg)							

RESULTS

The current work was done on 60 patients. 44 patients were bilaterally involved, 7 patients with right knee OA, while 9 patients were with left knee OA.

As 10 patients were dropped out from Group II during follow up, so their follow-up data are missed.

Table (2): Distribution of cases enrolled in each of the study groups.

	Group I	Group II	Group III	Total
Number of cases at enrollment	20	20	20	60
Number of cases who completed the whole course of the study	20	10	20	50

The patients' demograghic data are displayed in table 3, and the patients' clinical characteristics are displayed in table 4.

As shown in table 3, there was no statistically significant difference between the 3 groups of patients enrolled in the study regarding their age (P = 0.771), weight (P = 0.089), or body mass index (BMI) (P = 0.427). There was statistically significant difference between the 3 groups regarding their height (P = 0.03)

In group I and III patients 15% were males and 85% were females, while all patients of group II were females with no significant difference in sex between the 3 groups (P = 0.623).

In group I, 75% of patients were housewives, 5% were office workers, and 20% were manual workers. In group II, all patients were housewives, and in group III 50% of patients were housewives, 30% were office workers, 15% were manual workers and 5% were on bench with statistically significant difference between the 3 groups (P = 0.038).

Table (3): Demographic data of the studied patients.

		Group I	Group II	Group III	Test statistic	p
		n= 20	n=10	n= 20		
Age (years)	**Median (Min- Max)**	55 (51- 73)	55 (51- 64)	56.5 (51- 70)	*H= 0.519	.771
Weight (Kg)	**Median (Min- Max)**	70 (47–79)	70 (52 – 79)	72 (66 – 79)	**H**= 4.831	0.089
Height (m)	**Median (Min- Max)**	1.6 (1.4 – 1.9)	1.6 (1.4 – 1.8)	1.6 (1.5 – 1.8)	**H**= 6.992	0.030**
BMI	**Median (Min-Max)**	28.8 (21.1–29.9)	29.1 (26.3 – 29.9)	28.5 (23.6 – 29.7)	**H**= 1.701	.427
Gender	**Male n (%)**	3 (15)	0 (0)	3 (15)	†**MCp** =.623	
	Female n (%)	17 (85)	10 (100)	17 (85)		
Occupation	**Housewife n (%)**	15(75)	10(100)	10(50)	**MCp**=.038**	
	Office worker n (%)	1(5)	0(0)	6(30)		
	Manual worker n (%)	4(20)	0(0)	3(15)		
	On bench n (%)	0(0)	0(0)	1(5)		

*H: Kruskal Wallis test
† MCp: Monte Carlo test
** $p < 0.05$ (significant)

There was no statistically significant difference between the 3 groups regarding disease onset (P = 0.341). Also, there was no statistically significant difference between the 3 groups as regard to disease duration, side affected, course of the disease, quadriceps femoris muscle (QFM) wasting, tenderness, crepitus or joint alignment (table 4).

50% of patients in group I and group III had effusion, while no patient in group II had effusion. This was statistically significant between the 3 groups (P = 0.016).

The QFM and hamstring muscle power for the different groups are displayed in tables 5 and 6, respectively. In group I, patients with mild weakness of the right QFM were 30% before treatment and decreased to 15% after 1 month of treatment and decreased again to 10% after 2 months of treatment and those with moderate weakness of the right QFM were 5% before treatment and decreased to 0% after 1 month of treatment, while in group II, patients with moderate weakness of the right QFM were 10% before treatment and decreased to 0% after 1 month of treatment.

In group I, patients with mild weakness of the left QFM were 30% before treatment and decreased to 15% after 1 month of treatment and decreased again to 5% after 2 months of treatment.

In group I, patients with mild weakness of the right hamstring muscles were 50% before treatment and decreased to 20% after 1 month of treatment and decreased again to 10% after 2 months of treatment, while in group II, patients with moderate weakness of the right hamstring muscles were 10% before treatment and decreased to 0% after 1 month of treatment.

In group I, patients with mild weakness of the left hamstring muscles were 40% before treatment and decreased to 15% after 1 month of treatment.

The ROM of the affected knees are displayed in tables 7a and 7b.

In group I, patients with mild right knee flexion were 10% before treatment and decreased to 0% after two months of treatment. Also, in group III, patients with mild right knee flexion were 5% before treatment and decreased to 0% after two months of treatment.

In group I, patients with mild left knee flexion were 10% before treatment and decreased to 0% after two months of treatment. Also, in group III, patients with mild left flexion were 10% before treatment and decreased to 5% after two months of treatment.

In group II, patients with restricted right knee extension were 20% before treatment and decreased to 5% after two months of treatment with no statistically significant difference between the 3 groups (P> 0.05).

Table (4): Clinical characteristics of knee osteoarthritis in the studied groups.

		Group I	Group II	Group III	Test statistic (*p* value)
		n= 20	n=10	n= 20	
Onset n (%)	**Gradual**	14(70)	10(100)	13(65)	**MCp=** .341
	Sudden	2(10)	0(0)	2(10)	
	Acute	4(20)	0(0)	5(25)	
Duration of disease (Months)	**Median (Min- Max)**	12 (1- 180)	12 (1- 60)	15 (1- 240)	**H**= 0.532 (*p*= .767)
Course n (%)	**Progressive**	18(90)	10(100)	20(100)	¶**FEp=** .350
	Intermittent	2(10)	0(0)	0(0)	
Side n (%)	**Bilateral**	15(75)	8(80)	15(75)	**MCp=** .967
	Right	2(10)	1(10)	1(5)	
	Left	3(15)	1(10)	4(20)	
Quadriceps Wasting n (%)	**Quadriceps Bilateral**	1(5)	0(0)	1(5)	**MCp=** .820
	Left Quadriceps	2(10)	1(10)	4(20)	
	Right Quadriceps	1(5)	0(0)	1(5)	
	Left Vastus medialis	5(25)	3(30)	3(15)	
	Right and Left Vastus medialis	4(20)	1(10)	1(5)	
	Right Vastus medialis	2(10)	1(10)	0(0)	
	No wasting	5(25)	4(40)	10(50)	
Effusion n (%)	**Yes**	10(50)	0(0)	10(50)	**FEp=** .016*
	No	10(50)	10(100)	10(50)	
Tenderness n (%)	**Yes**	17(85)	10(100)	18(90)	**FEp=** .611
	No	3(15)	0(0)	2(10)	
Crepitus n (%)	**Palpable**	13(65)	3(30)	13(65)	**MCp=** .241
	Audible	6(30)	6(60)	4(20)	
	Right audible / left palpable	1(5)	0(0)	2(10)	
	Right palpable/ left audible	0(0)	1(10)	1(5)	
Alignment n (%)	**Normal**	13(65)	7(70)	7(35)	**MCp=** .238
	Genu varum	6(30)	2(20)	9(45)	
	Genu valgum	1(5)	1(10)	4(20)	

¶FEp: Fisher Exact test
*p < 0.05 (significant)

Table (5): Quadriceps muscle power in the 3 studied groups during initial and the two follow up assessment.

Muscle power	Studied group	n	Grade	Before	After 1 month	After 2 month
				n (%)	n (%)	n (%)
Right quadriceps	Group I	20	Normal	13 (65%)	17 (85%)	18 (90%)
			Mild weakness	6 (30%)	3 (15%)	2 (10%)
			Moderate weakness	1 (5%)	0 (0%)	0 (0%)
	Group II	10	Normal	9 (90%)	9 (90%)	8 (80%)
			Mild weakness	0 (0%)	1 (10%)	2 (20%)
			Moderate weakness	1 (10%)	0 (0%)	0 (0%)
	Group III	20	Normal	19 (95%)	20 (100%)	19 (95%)
			Mild weakness	1 (5%)	0 (0%)	1 (5%)
			Moderate weakness	0 (0%)	0 (0%)	0 (0%)
Left quadriceps	Group I	20	Normal	14 (70%)	17 (85%)	19 (95%)
			Mild weakness	6 (30%)	3 (15%)	1 (5%)
			Moderate weakness	0 (0%)	0 (0%)	0 (0%)
	Group II	10	Normal	9 (90%)	9 (90%)	8 (80%)
			Mild weakness	0 (0%)	0 (0%)	1(10%)
			Moderate weakness	1 (10%)	1 (10%)	1(10%)
	Group III	20	Normal	19 (95%)	20 (100%)	19 (95%)
			Mild weakness	1 (5%)	0 (0%)	1 (5%)
			Moderate weakness	0 (0%)	0(0%)	0 (0%)

Table (6): Hamstring muscle power in the 3 studied groups during initial and the two follow up assessment.

Muscle power	Studied group	n	Grade	Before	After 1 month	After 2 month
				n (%)	n (%)	n (%)
Right hamstring	Group I	20	Normal	10 (50%)	16 (80%)	18(90%)
			Mild weakness	10 (50%)	4(20%)	2 (10%)
			Moderate weakness	0 (0%)	0 (0%)	0 (0%)
	Group II	10	Normal	9 (90%)	10 (100%)	9 (90%)
			Mild weakness	0 (0%)	0 (0%)	1 (10%)
			Moderate weakness	1 (10%)	0(0%)	0 (0%)
	Group III	20	Normal	20 (100%)	20 (100)	19 (95%)
			Mild weakness	0 (0%)	0 (0%)	1 (5%)
			Moderate weakness	0 (0%)	0 (0%)	0 (0%)
Left hamstring	Group I	20	Normal	12 (60%)	17 (85%)	17 (85%)
			Mild weakness	8 (40%)	3 (15%)	3 (15%)
			Moderate weakness	0 (0%)	0 (0%)	0 (0%)
	Group II	10	Normal	9 (90%)	10 (100%)	9 (90%)
			Mild weakness	1 (10%)	0 (0%)	1 (10%)
			Moderate weakness	0 (0%)	0 (0%)	0 (0%)
	Group III	20	Normal	20 (100%)	19 (95%)	19 (95%)
			Mild weakness	0 (0%)	1 (5%)	1 (5%)
			Moderate weakness	0 (0%)	0 (0%)	0 (0%)

Table (7a): Range of motion (ROM) of affected knees of the patients in the 3 studied groups during initial and the two follow up assessment.

	Studied group	n	Grade	Before	After 1 month	After 2 month
				n (%)	n (%)	n (%)
Right Flexion	**Group I**	20	Mild	1 (10)	1 (10)	0 (0)
			Moderate	0 (0)	0 (0)	1 (10)
			Full	19 (90)	19 (90)	19 (90)
	Group II	10	Mild	0 (0)	0 (0)	0 (0)
			Moderate	0 (0)	0 (0)	1 (10)
			Full	10 (100)	10 (100)	9 (90)
	Group III	20	Mild	1 (5)	0 (0)	0 (0)
			Moderate	2 (10)	1 (10)	1 (10)
			Full	17(85)	19 (90)	19 (90)
Left Flexion	**Group I**	20	Mild	1 (10)	1 (10)	0 (0)
			Moderate	0 (0)	0 (0)	1 (10)
			Full	19 (90)	19 (90)	19 (90)
	Group II	10	Mild	0 (0)	0 (0)	0 (0)
			Moderate	0 (0)	0 (0)	1 (10)
			Full	10 (100)	10 (100)	9 (90)
	Group III	20	Mild	1 (10)	1 (10)	1 (5)
			Moderate	0 (0)	0 (0)	3 (15)
			Full	19 (90)	19 (90)	16 (80)

Table (7b): Range of motion (ROM) of affected knees of the patients in the 3 studied groups during initial and the two follow up assessment.

	Studied group	n	Grade	Before	After 1 month	After 2 month	Cochran Q	p
				n (%)	n (%)	n (%)		
Right Extension	**Group I**	20	Full	20(100)	19(95)	19 (95)	1.000	.607
			Restricted	0 (0)	1 (5)	1 (5)		
	Group II	10	Full	8 (80)	8 (80)	19 (95)	.667	. 717
			Restricted	2 (20)	2 (20)	1 (5)		
	Group III	20	Full	15 (70)	15(70)	15 (70)	.667	. 717
			Restricted	5 (25)	5 (25)	5 (25)		
Left Extension	**Group I**	20	Full	20(100)	20(100)	19(95)	2.000	.368
			Restricted	0 (0)	0 (0)	1(5)		
	Group II	10	Full	9 (90)	9 (90)	9 (90)	.000	1.000
			Restricted	1 (10)	1 (10)	1 (10)		
	Group III	20	Full	15 (70)	15 (70)	15 (70)	.000	1.000
			Restricted	5 (25)	5 (25)	5 (25)		

The median of pain visual analogue scale (VAS) in the 3 studied groups during initial and two follow up assessment is demonstrated in table 8. In group I there was statistically significant difference between before, 1 month and 2 months after treatment(P = 0.031).Post hoc paired comparisons revealed median VAS was significantly higher before receiving treatment than 2 months after treatment. In group III there was statistically significant difference between before, 1month and 2 months after treatment (P = 0.045), but no statistical significant pair wise comparisons.

The median of verbal rating scale (VRS) in the 3 studied groups during initial and two follow up assessment is demonstrated in table 8. In patients enrolled in group I there was statistically significant difference between before, 1month and 2 months after treatment (P = 0.033). Post hoc paired comparisons revealed median VRS was significantly higher before receiving treatment than 2 months after treatment.

In group III there was statistically significant difference between before, 1month and 2 months after treatment (P = 0.048), but no statistical significant pair wise comparisons.

The median of 20 meters fast walking time in the 3 studied groups during initial and two follow up assessment is demonstrated in table 8. In group I there was statistically significant difference between before, 1month and 2 months after treatment (P = 0.001). Post hoc paired comparisons revealed median 20 meters fast walking time was significantly higher before receiving treatment than 2 months after treatment.

Table (8): Pain visual analogue, pain verbal rating scales and 20 meters fast walking time in the 3 studied groups before and after treatment (Intra-group changes).

Patient assessment	Studied group	n	Before	After 1 month	After 2 month	Friedman $\chi 2$	p
Pain VAS	Group I	20	70_a (30-100)	$50_{a,b}$ (0-100)	50_b (10-90)	11.742	.031*
	Group II	10	90 (50-100)	85 (20-100)	70 (10-100)	5.586	.06
	Group III	20	70_a (50-100)	55_a (0-90)	60_a (20-100)	6.083	.045*
Pain VRS	Group I	20	70_a (30- 100)	$50_{a,b}$ (0-100)	50_b (10-90)	11.742	.033*
	Group II	10	90 (50-100)	85 (20-100)	70 (10-100)	5.586	.061
	Group III	20	70_a (50-100)	55_a (0-90)	60_a (20-100)	6.083	.048*
20 meters fast walking time	Group I	20	20_a (13-34)	$18_{a,b}$ (11-33)	17_b (12-23)	14.147	.001*
	Group II	10	20.5 (17-36)	20.5 (16-43)	21 (16-30)	1. 967	.393
	Group III	20	19.5 (10-59)	20 (10-56)	26 (10-63)	3.647	.161

Note: Minimum and maximum appear in parentheses below medians. Medians with differing subscripts within rows are significantly different at the adjusted $p < .05$ based on post hoc paired comparisons.
* $p < 0.05$ (significant)

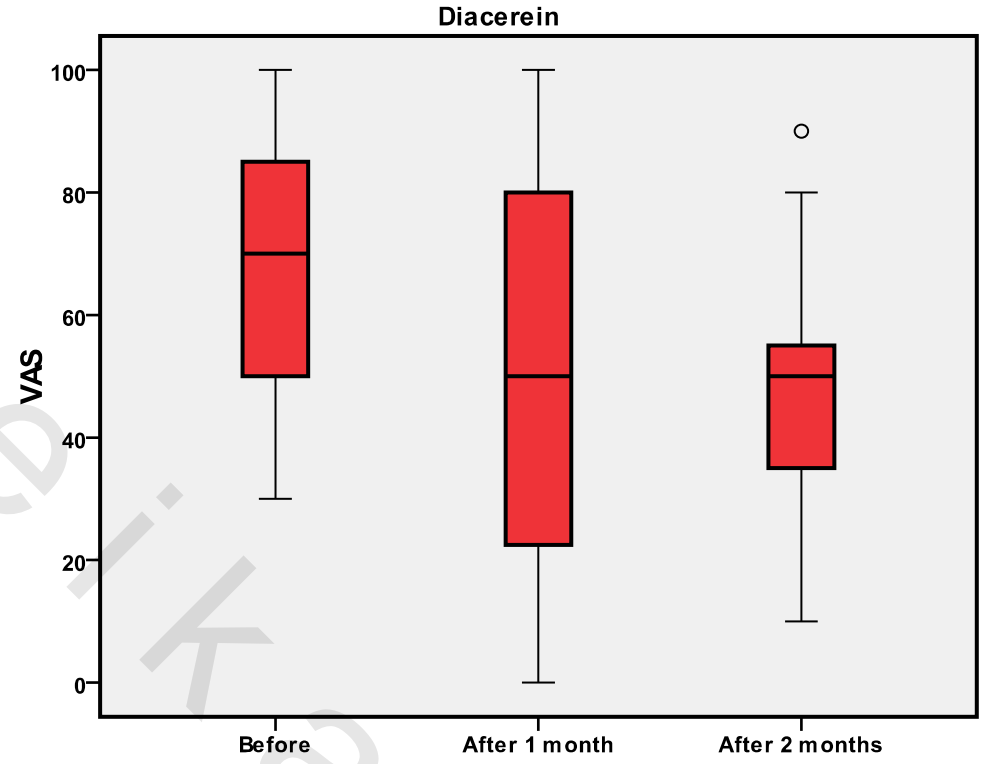

Fig. (2) Box plot for median VAS and its range in Group I between before, 1month and 2 months after treatment.

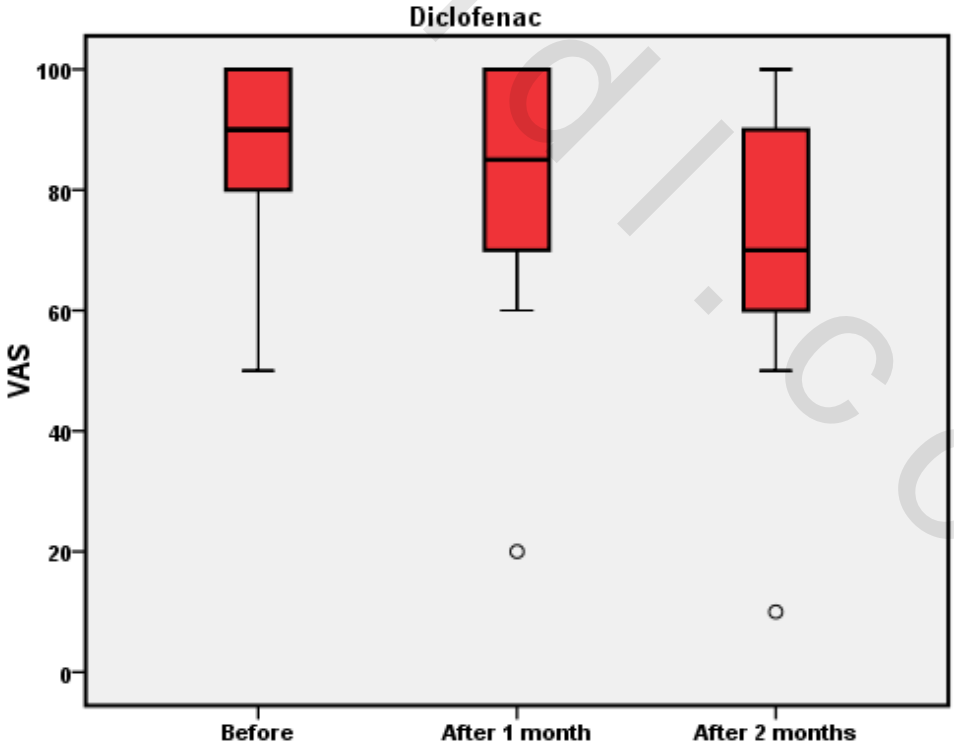

Fig. (3): Box plot for median VAS and its range in Group II between before, 1month and 2 months after treatment

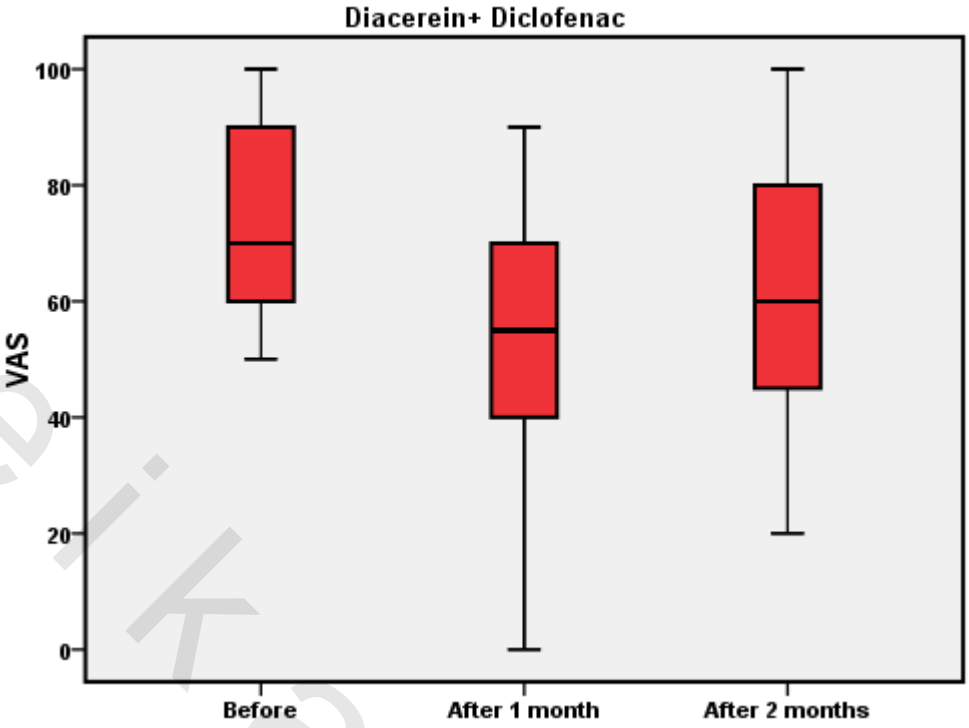

Fig. (4): Box plot for median VAS and its range in Group III between before, 1month and 2 months after treatment.

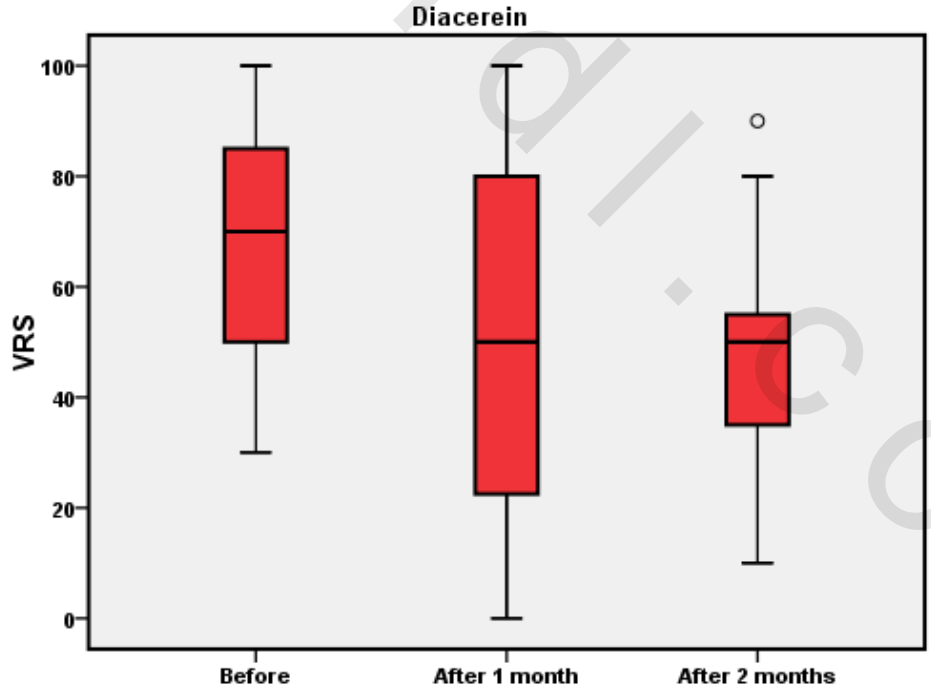

Fig. (5): Box plot for median VRS and its range in Group I between before, 1month and 2 months after treatment.

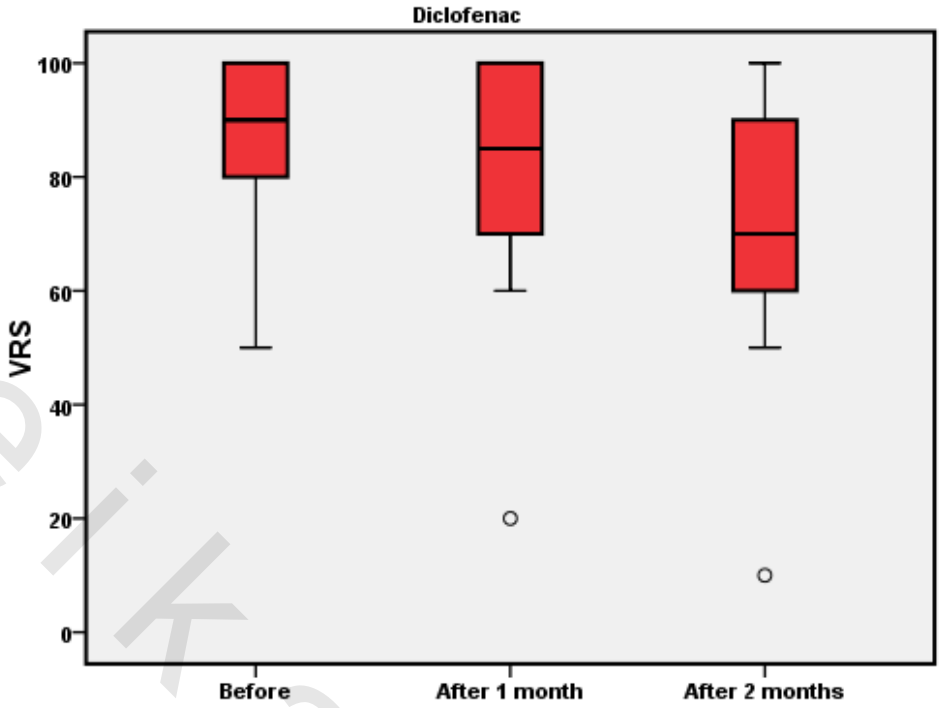

Fig. (6): Box plot for median VRS and its range in Group II between before, 1month and 2 months after treatment.

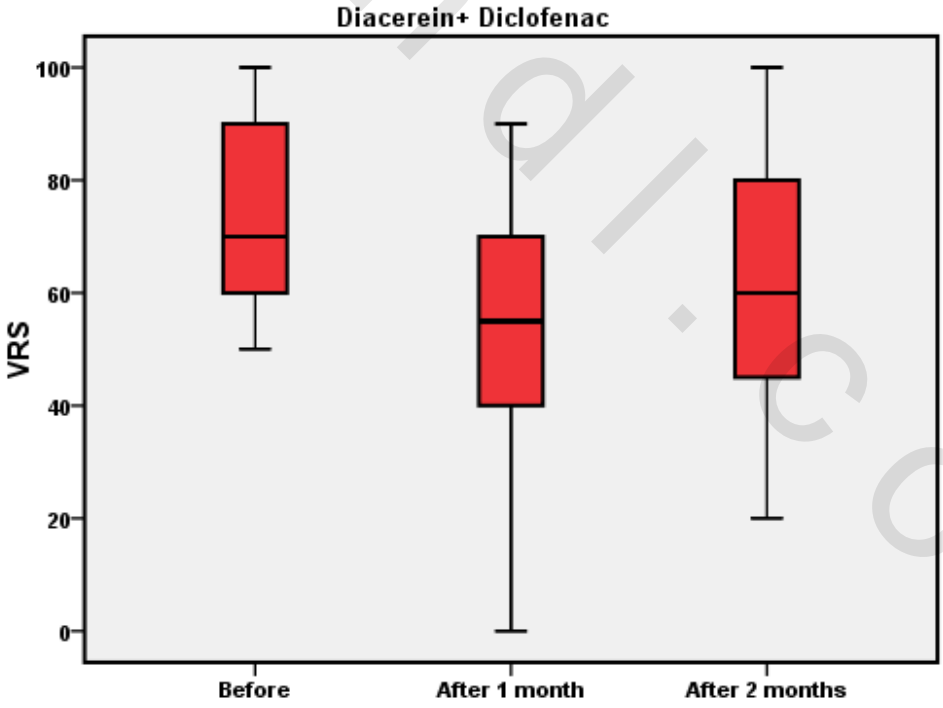

Fig. (7): Box plot for median VRS and its range in Group III between before, 1month and 2 months after treatment.

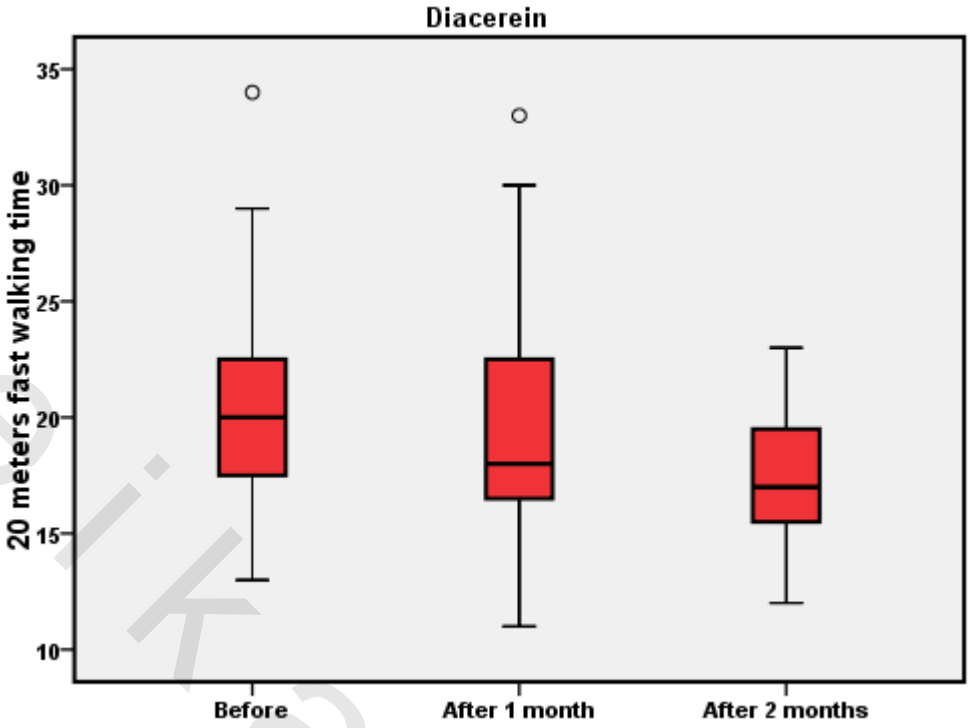

Fig. (8): Box plot for median 20 meters fast walking time and its range in Group I between before, 1month and 2 months after treatment.

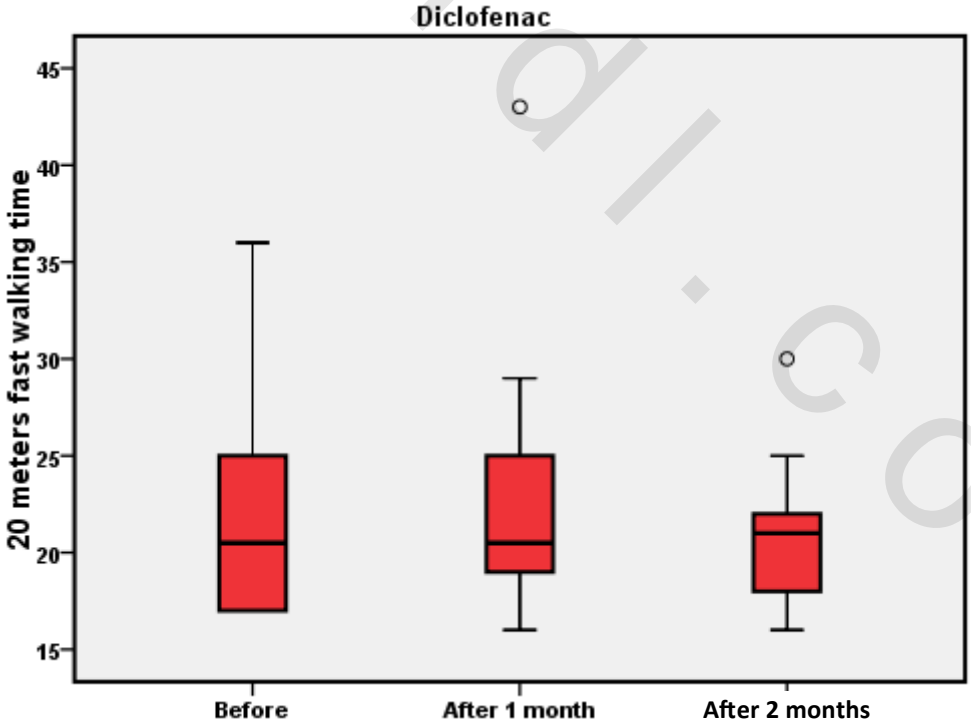

Fig. (9): Box plot for median 20 meters fast walking time and its range in Group II between before, 1month and 2 months after treatment.

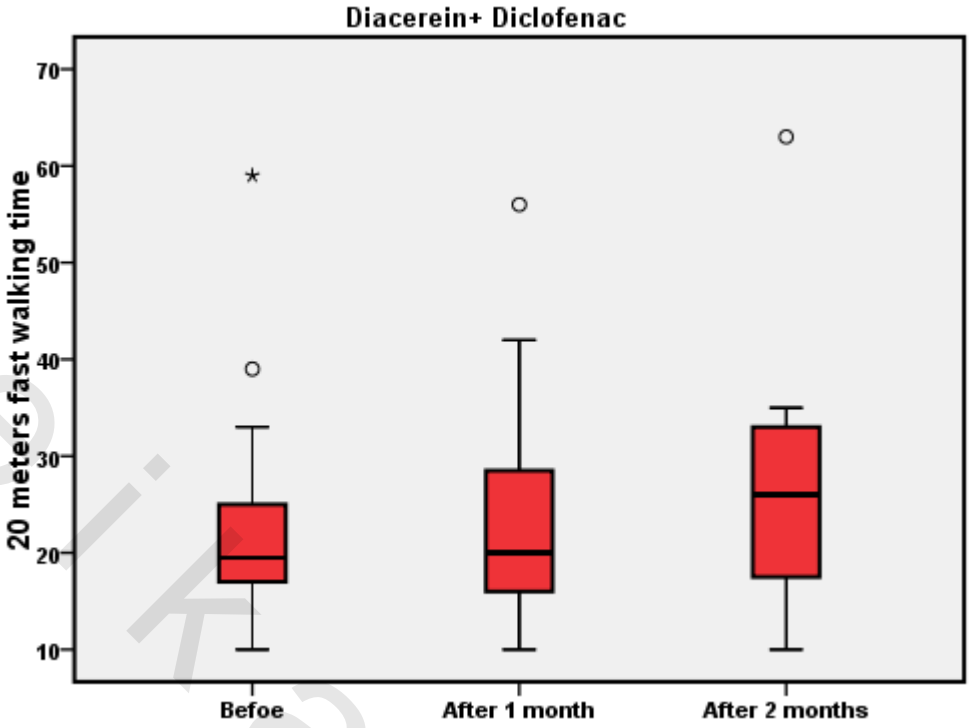

Fig. (10): Box plot for median 20 meters fast walking time and its range in Group III between before, 1month and 2 months after treatment.

As shown in table 9, the median difference in VAS, VRS and 20 meters fast walking in the 3 studied groups was tabulated.

The median difference in 20 meters fast walking time between 1month and 2 months after treatment was statistically significant between the 3 groups (P = 0.006).Also, the median difference in 20 meters fast walking time between before treatment and 2 months after treatment was statistically significant between the 3 groups (P = 0.008). Post hoc pair wise comparisons revealed that median difference of 20 meters fast walking time between before treatment and 2 months after treatment was significantly higher in group I patients than those of group III.

Table (9): Comparison between the 3 studied groups regarding pain VAS, pain VRS and 20 meters fast walking time (inter-group comparisons).

Patient assessment		Studied group			H	p	Adjusted p*
		Group I	Group II	Group III			
		n= 20	n=10	n= 20			
Pain VAS	Before treatment- After 1 month	10 (-40- 90)	0 (-20- 40)	20 (-20- 90)	1.419	.492	.159
	After1month treatment- After 2 month	0 (-40- 50)	5 (-10- 50)	0 (-60- 40)	1.650	.438	.924
	Before treatment- After 2 month	30 (-40- 70)	10 (0- 40)	25 (-20- 40)	2.247	.325	.263
Pain VRS	Before treatment- After 1 month	10 (-40- 90)	0 (-20- 40)	20 (-20- 90)	1.419	.492	.159
	After1month treatment- After 2 month	0 (-40- 50)	5 (-10- 50)	0 (-60- 40)	1.650	.438	.924
	Before treatment- After 2 month	30 (-40- 70)	10 (0- 40)	25 (-20- 40)	2.247	.325	.263
20 meters fast walking time	Before treatment- After 1 month	1.5 (-13- 6)	0 (-7- 4)	1 (-20- 7)	3.497	.174	.290
	After1month treatment- After 2 month	1_a (-2- 17)	5_a (-3- 13)	-1_a (-21- 8)	7.207	. 027	.006**
	Before treatment- After 2 month	2.5_a (-2- 16)	$5_{a,b}$ (-3- 6)	0_b (-21- 13)	8.454	. 015	.008**

Note: Minimum and maximum appear in parentheses below medians. Medians with differing subscripts within rows are significantly different at the adjusted $p < .05$ based on post hoc paired comparisons.
*p value after adjustment for height, occupation and effusion by linear regression model.
** $p< 0.05$ (significant)

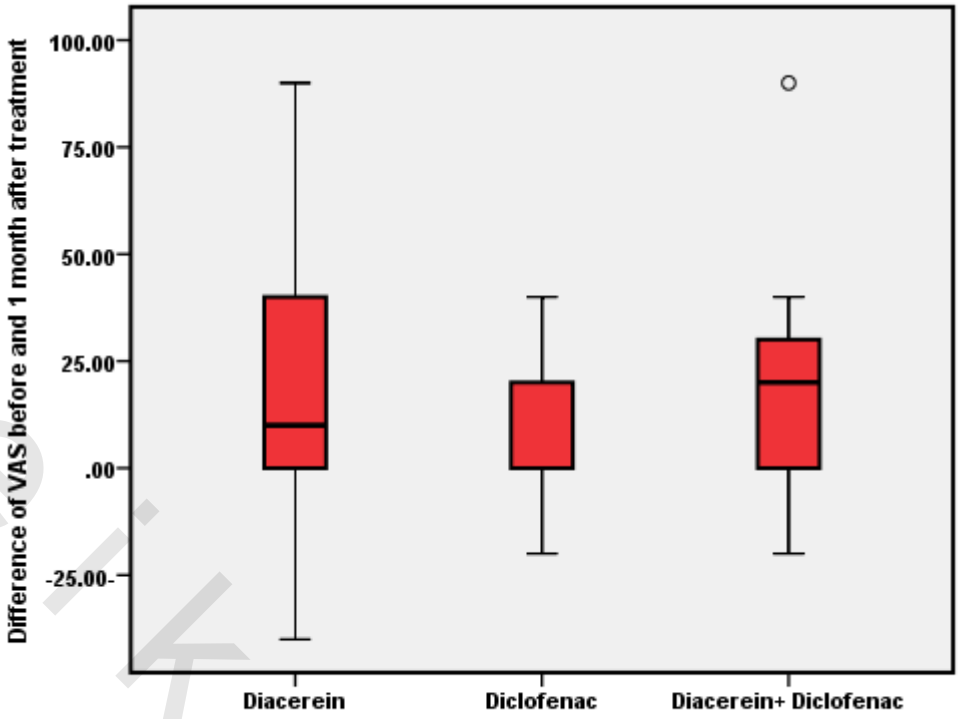

Fig. (11): Box plot for median difference of VAS between before and 1 month after treatment and its range in 3 different groups of treatment.

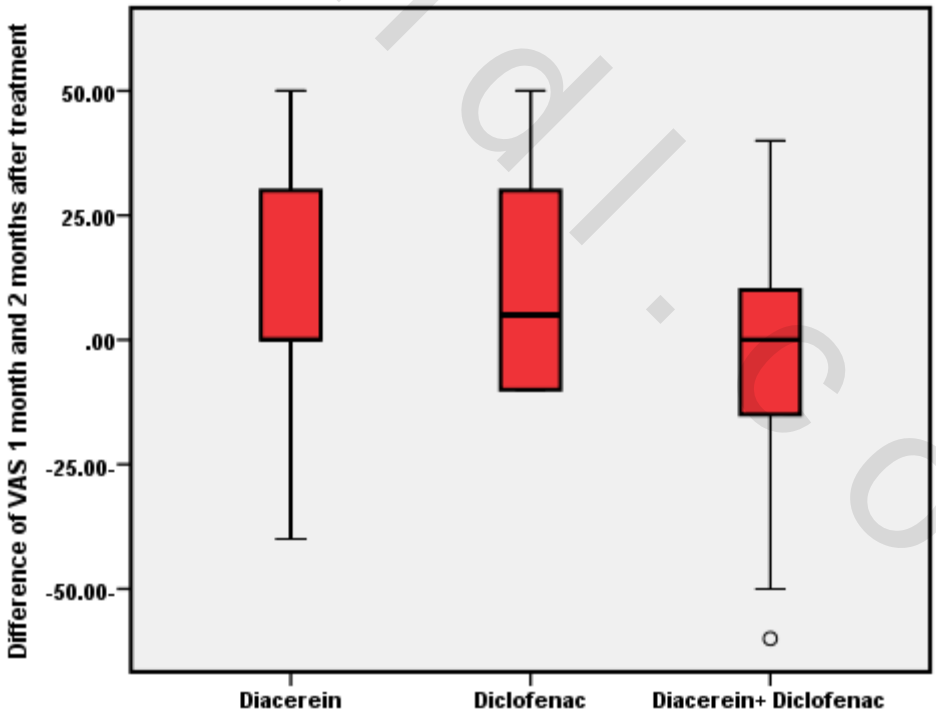

Fig. (12): Box plot for median difference of VAS between 1 month and 2 months after treatment and its range in 3 different groups of treatment.

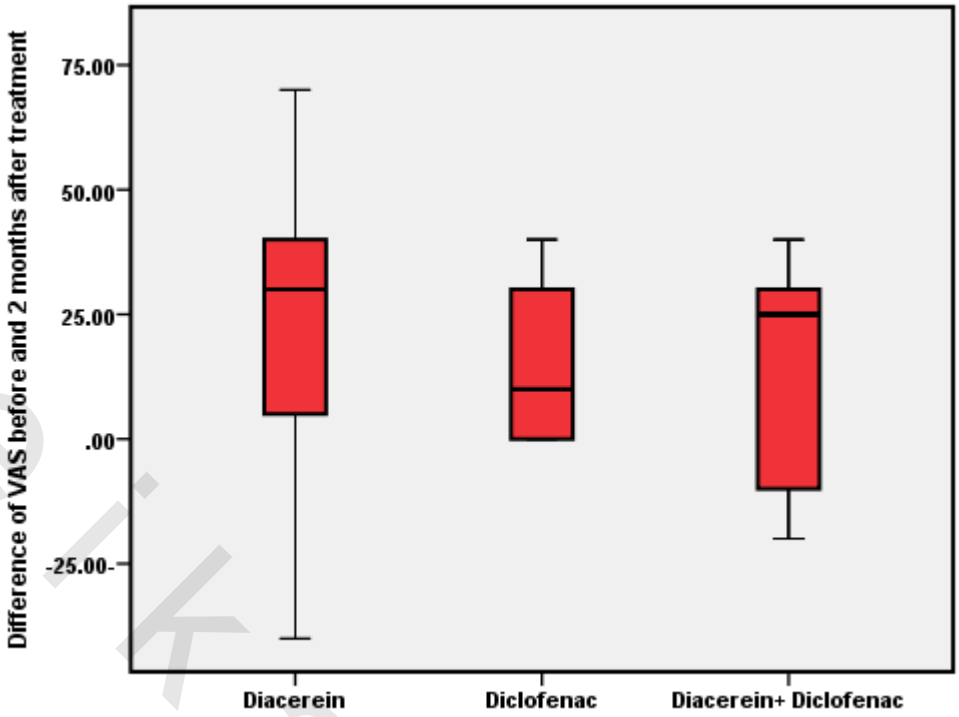

Fig. (13): Box plot for median difference of VAS between before and 2 months after treatment and its range in 3 different groups of treatment.

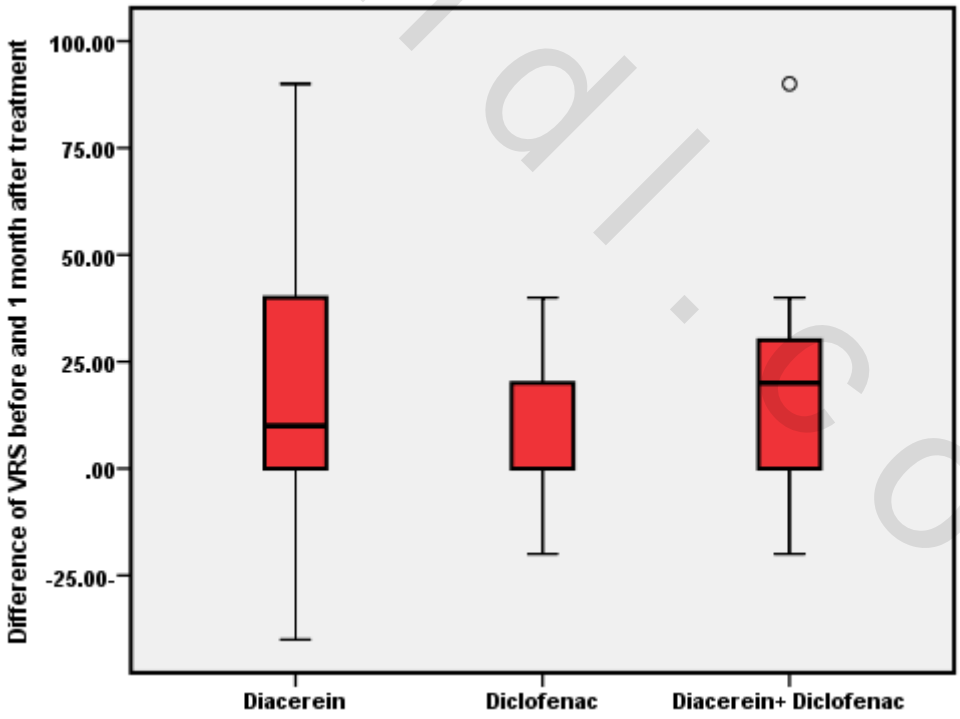

Fig. (14): Box plot for median difference of VRS between before and 1 month after treatment and its range in 3 different groups of treatment.

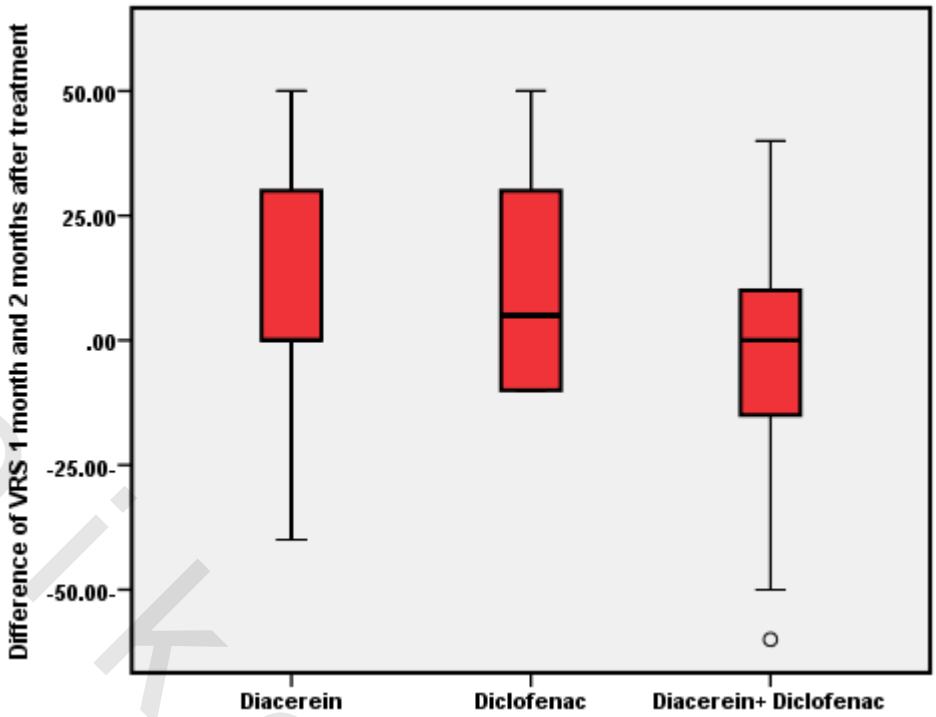

Fig. (15): Box plot for median difference of VRS between 1 month and 2 months after treatment and its range in 3 different groups of treatment.

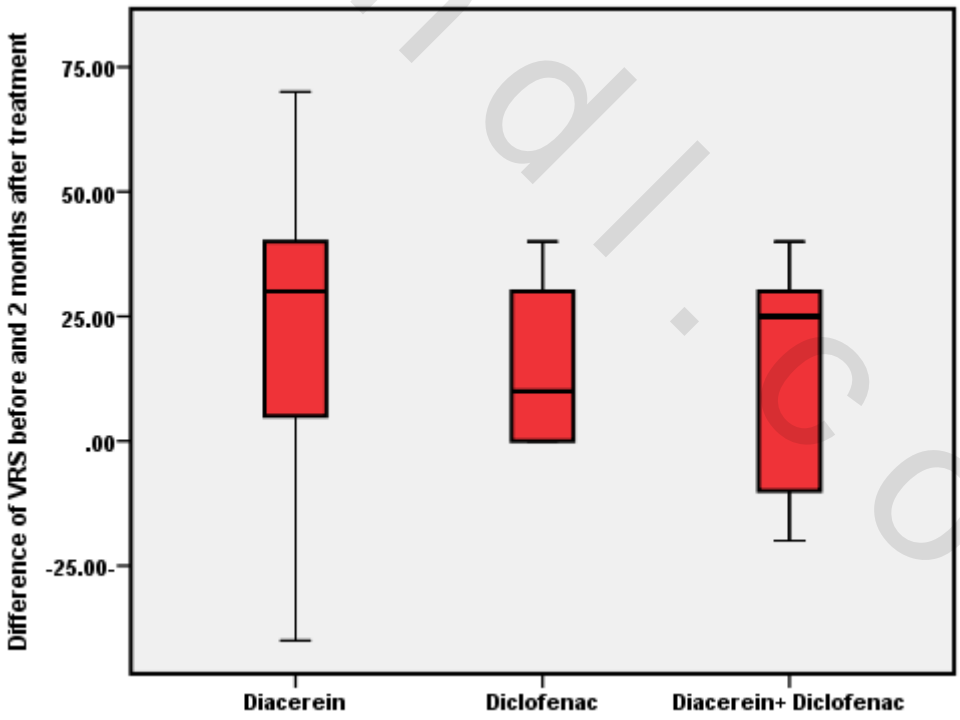

Fig. (16): Box plot for median difference of VRS between before and 2 months after treatment and its range in 3 different groups of treatment.

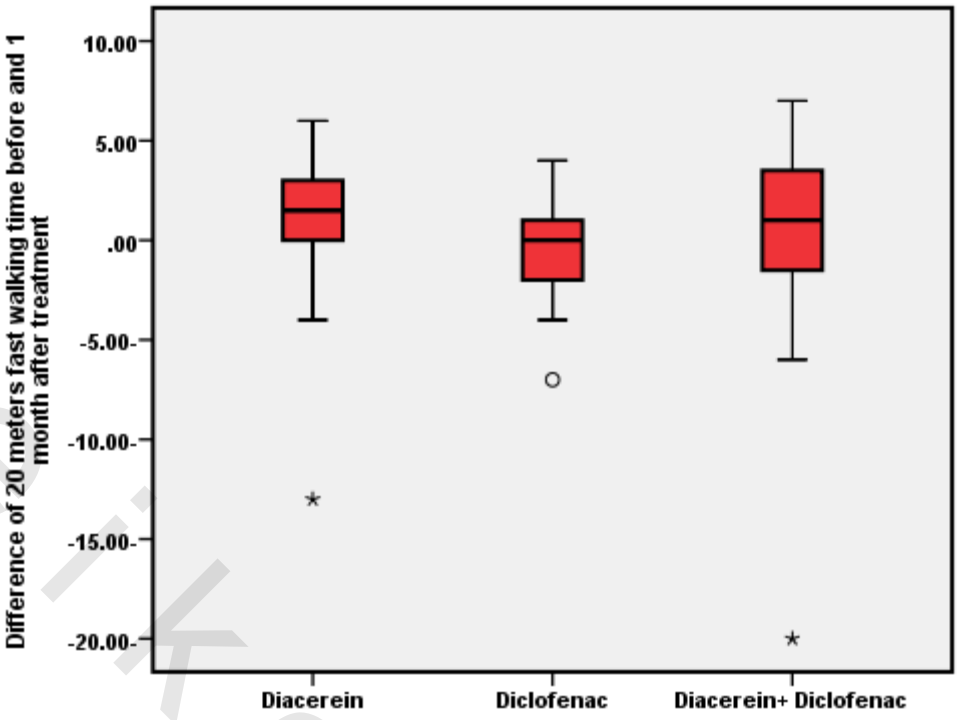

Fig. (17): Box plot for median difference of 20 meters fast walking time between before and 1 month after treatment and its range in 3 different groups of treatment.

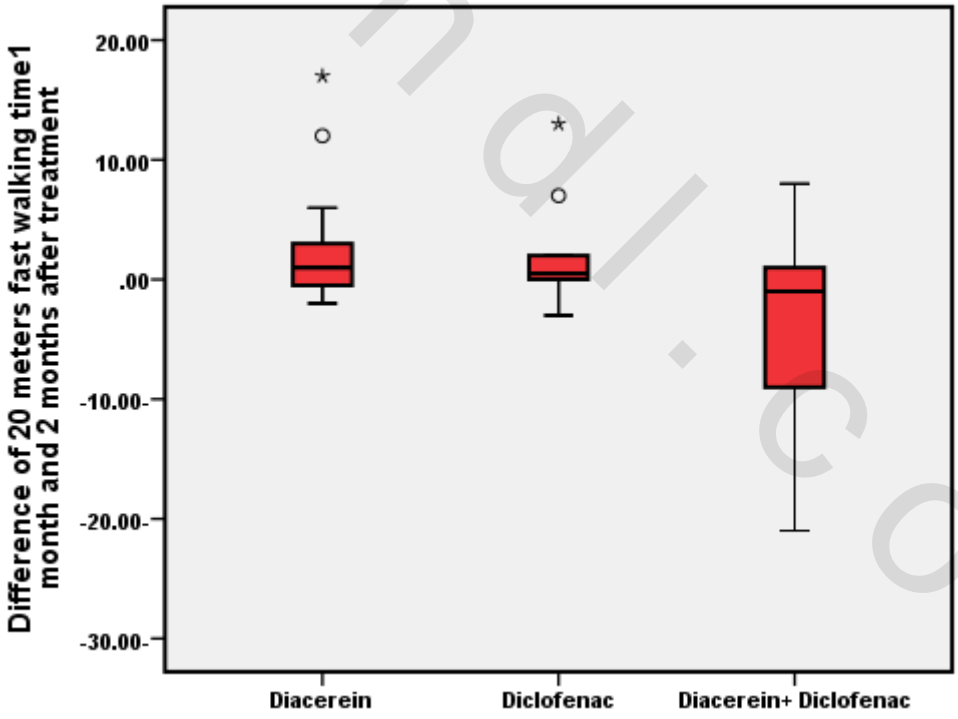

Fig. (18): Box plot for median difference of 20 meters fast walking time between 1 month and 2 months after treatment and its range in 3 different groups of treatment.

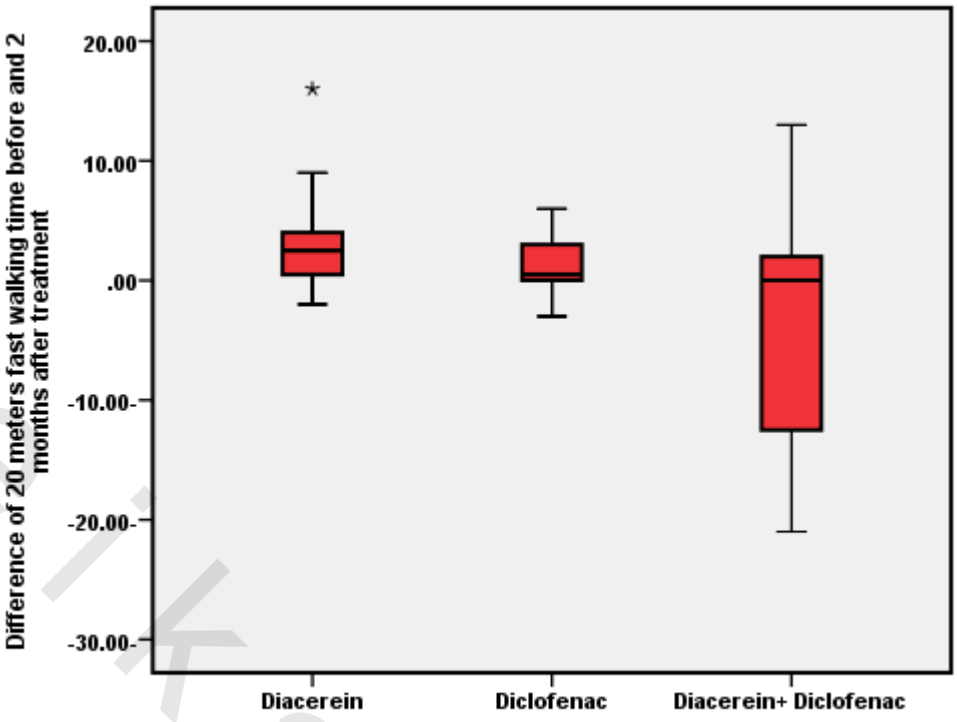

Fig. (19): Box plot for median difference of 20 meters fast walking time between before and 2 months after treatment and its range in 3 different groups of treatment.

In tables 10 and11, QFM and hamstring muscle power in the 3 studied groups before treatment and at 1 month and 2 months follow up assessment are displayed.

Regarding the muscle power at in case of right quadriceps, the proportion of patients with one grade improvement between before treatment and 2 months after treatment were 20% in group I patients, 10% in group II patients and 5% in group III patients while the proportion of patients with two grade improvement between before treatment and 2 months after treatment were 5% in group I patients, 0% in group II and III patients with no statistically significant difference between the 3 groups(P = 0.110).

Regarding the muscle power in case of left quadriceps, the proportion of patients with one grade improvement between before treatment and 2 months after treatment were 20% in group I patients, 10% in group II patients and 5% in group III patients. Also, the proportion of patients with two grade improvement between before treatment and 2 months after treatment were 5% in group I patients, 0% in group II and III patients with statistically significant difference between the 3 groups (P = 0.037), but no statistical significant pair wise comparisons.

Regarding the muscle power in case of right hamstring, the proportion of patients with one grade improvement between before treatment and 1 month after treatment were 30% in group I patients, 0% in group II and III patients with statistically significant difference between the 3 groups (P = 0.029). Post hoc paired comparisons showed that the difference in muscle power of the right hamstring between before treatment and 1 month after treatment was significantly different between group I patients and group III patients. Also, the proportion of patients with one grade improvement between 1 and 2 months after treatment was 10% in group 1 patients, 0% in group II and III patients with no statistically significant difference between the 3 groups (P = 0.117), while the proportion of patients with one grade improvement between before treatment and 2 months after treatment were 40% in group 1 patients, 0% in group II and III patients with statistically significant difference between the 3 groups (P = 0.005). Post hoc paired comparisons showed that the difference in muscle power of the right hamstring between before treatment and 2 months after treatment was significantly different between group I patients and group III patients.

Regarding the muscle power in case of left hamstring, the proportion of patients with one grade improvement between before treatment and 1 month after treatment were 25% in group I patients, 10% in group II and 0% in group III patients with statistically significant difference between the 3 groups (P = 0.034).Post hoc paired comparisons showed that the difference in muscle power of the left hamstring between before treatment and 1 month after treatment was significantly different between group I patients and group III patients. Also, the proportion of patients with one grade improvement between 1 and 2 months after treatment were 5% in group 1 patients, 0% in group II and 5% in group III patients with no statistically significant difference between the 3 groups (P = 0.676), while the proportion of patients with one grade improvement between before treatment and 2 months after treatment were 25% in group 1 patients, 10% in group II and 0% in group III patients with statistically significant difference between the 3 groups (P = 0.042). Post hoc paired comparisons showed that the difference in muscle power of the left hamstring between before treatment and 2 months after treatment was significantly different between group I patients and group III patients.

Table (10): Comparison between the 3 studied groups regarding quadriceps muscle power at before treatment and 1 and 2 months follow up assessment.

Muscle power			Studied group			H	p
			Group I	Group II	Group III		
			n= 20	n=10	n= 20		
			n (%)	n (%)	n (%)		
Right quadriceps	Before treatment- After 1 month	Deteriorated	0(0)	9 (90)	0 (0)	2.224	.329
		No improvement	16 (80)	0(0)	19 (95)		
		One grade improvement	3 (15)	1(10)	1 (5)		
		Two grades improvement	1 (5)	0 (0)	0 (0)		
	After 1month - After 2 months	Deteriorated	0 (0)	1 (10)	1 (5)	1.744	.418
		No improvement	1 (5)	9 (90)	19 (95)		
		One grade improvement	17 (85)	0 (0)	0 (0)		
		Two grades improvement	2 (10)	0 (0)	0 (0)		
	Before treatment- After 2 months	Deteriorated	0 (0)	1 (10)	1 (5)	4.423	.110
		No improvement	15 (75)	8 (80)	18 (90)		
		One grade improvement	4 (20)	1 (10)	1 (5)		
		Two grades improvement	1 (5)	0 (0)	0 (0)		
Left quadriceps	Before treatment- After 1 month	Deteriorated	0 (0)	0 (0)	0 (0)	2.397	.302
		No improvement	17 (85)	10 (100)	19 (95)		
		One grade improvement	3 (15)	0 (0)	0 (0)		
		Two grades improvement	0 (0)	0 (0)	1 (5)		
	After1month - After 2 months	Deteriorated	1 (5)	1 (10)	1 (5)	2.858	.240
		No improvement	16 (20)	9 (90)	19 (95)		
		One grade improvement	3 (15)	0 (0)	0 (0)		
		Two grades improvement	0 (0)	0 (0)	0 (0)		
	Before treatment- After 2 months	Deteriorated	0 (0)	1 (10)	1 (5)	6.596	.037*
		No improvement	15 (75)	8 (80)	18 (90)		
		One grade improvement	4 (20)	1 (10)	1 (5)		
		Two grades improvement	1 (5)	0 (0)	0 (0)		

* $p < 0.05$ (significant)

Table (11): Comparison between the 3 studied groups regarding hamstring muscle power before and after treatment.

Muscle power			Studied group			H	P
			Group I	Group II	Group III		
			n= 20	n=10	n= 20		
			n (%)	n (%)	n (%)		
Right hamstring	Before treatment- After 1 month	Deteriorated	0 (0)	0 (0)	0 (0)	7.095	.029*
		No improvement	14 (70)	9 (90)	20 (100)		
		One grade improvement	6 (30)	0 (0)	0 (0)		
		Two grades improvement	0 (0)	1 (10)	0 (0)		
	After1month - After 2 months	Deteriorated	0 (0)	1 (10)	1 (5)	4.288	. 117
		No improvement	18 (85)	9 (90)	19 (95)		
		One grade improvement	2 (10)	0 (0)	0 (0)		
		Two grades improvement	0 (0)	0 (0)	0 (0)		
	Before treatment- After 2 months	Deteriorated	0 (0)	1 (10)	1 (5)	10.660	.005*
		No improvement	12 (60)	8 (80)	19 (95)		
		One grade improvement	8 (40)	0 (0)	0 (0)		
		Two grades improvement	0 (0)	1 (10)	0 (0)		
Left hamstring	Before treatment- After 1 month	Deteriorated	0 (0)	0 (0)	1 (5)	6.780	.034*
		No improvement	15 (75)	9 (90)	19 (95)		
		One grade improvement	5 (25)	1 (10)	0 (0)		
		Two grades improvement	0 (0)	0 (0)	0 (0)		
	After1month - After 2 months	Deteriorated	1 (5)	1 (10)	1 (5)	0.784	.676
		No improvement	18 (90)	9 (90)	18 (90)		
		One grade improvement	1 (5)	0 (0)	1 (5)		
		Two grades improvement	0 (0)	0 (0)	0 (0)		
	Before treatment- After 2 months	Deteriorated	0 (0)	1 (10)	1 (5)	6.318	.042*
		No improvement	15 (75)	8 (80)	19 (95)		
		One grade improvement	5 (25)	1 (10)	0 (0)		
		Two grades improvement	0 (0)	0 (0)	0 (0)		

* $p < 0.05$ (significant)

Illustrative Radiographs

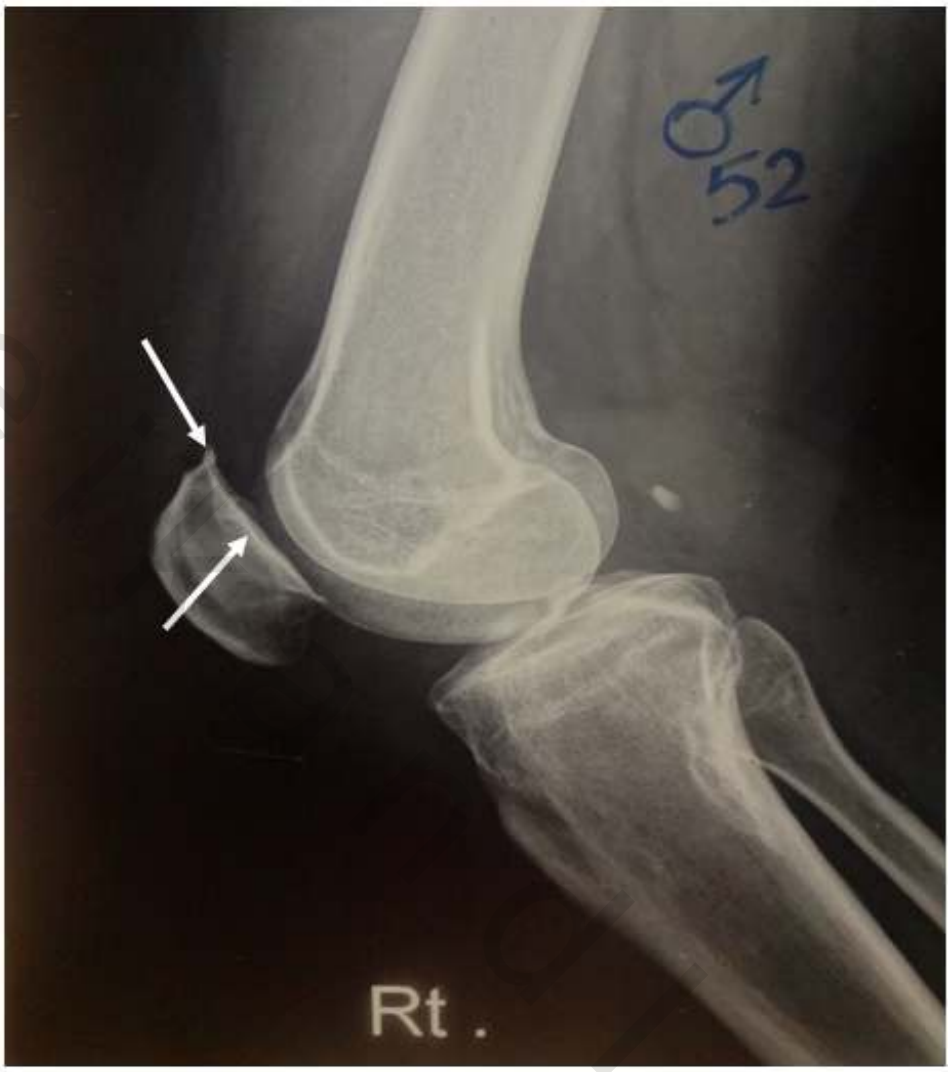

Fig (20): Lateral radiograph of the knee joint showing the patellofemoral articulation and demonstrating the following features of OA:

a) Sharpening of the articular ends of the patella
b) Subchondral sclerosis of the patella
c) Spiking of the articular ends of the patella

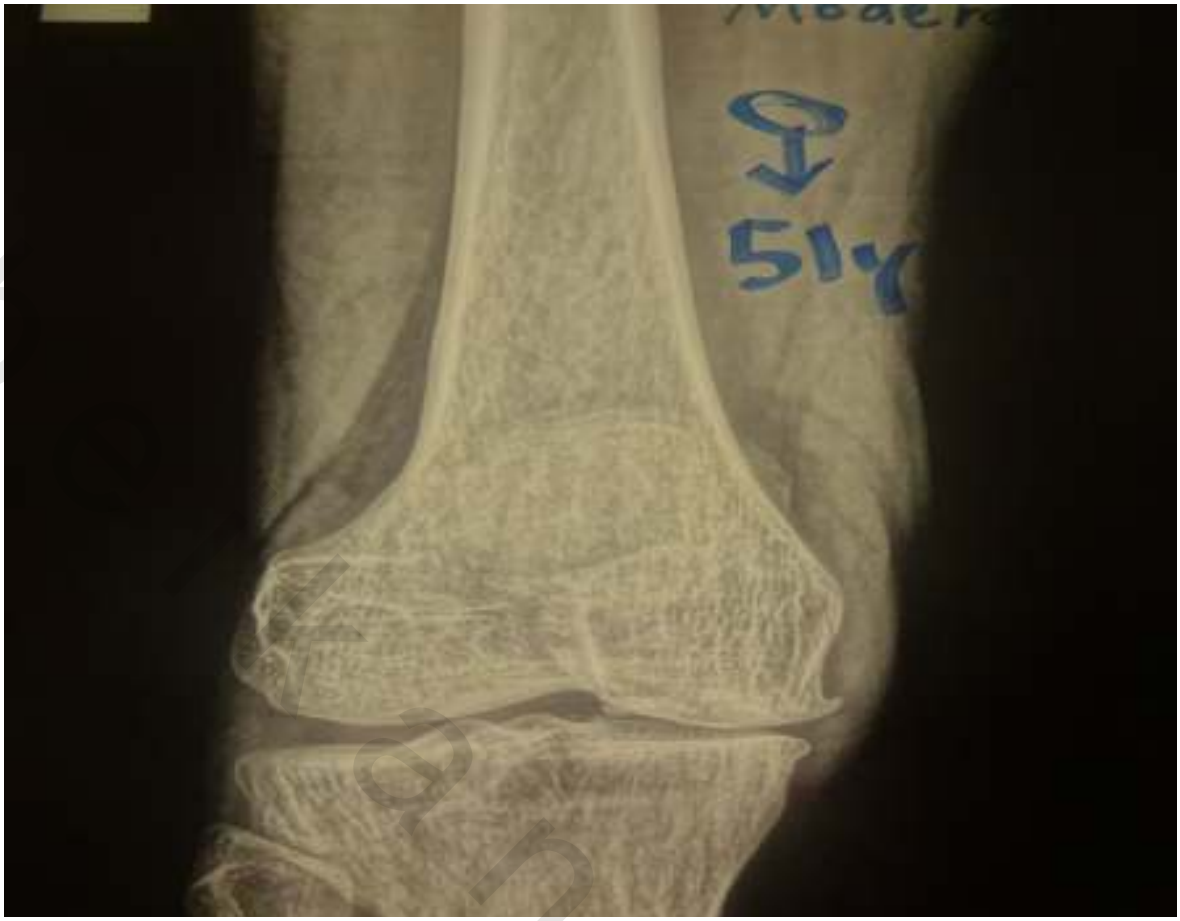

Fig.(21): Radiograph showing the anteroposterior view of the knee joint and demonstrating the following features of OA:

a) Presence of marginal osteophytes
b) Reduced joint space
c) Subchondral sclerosis

As shown in table 12, no statistically significant difference in synovial IL-1β between before treatment compared to 1 and 2 months after treatment in group I or group III (P = 0.565 and 0.581 respectively).

Also, no statistical significant difference in blood IL-1β between before treatment compared to 1 and 2 months after treatment in group I, II and III (P = 0.590, 0.293 and 0.154 respectively).

Table (12): Synovial and serum IL-1β level in the studied groups at initial and follow up assessment.

Patient assessment	Studied group	n	Before	After 1 month	After 2 month	Friedman χ^2	p
Synovial IL-1β (μg)	Group I	10	6 (5-9)	5 (1.5-12.5)	5 (1.3-7)	1.143	.565
	Group III	10	1.35 (1.2-27)	1.4 (1.1-3.3)	1.8 (0.8-3)	1.086	.581
Blood IL-1β (μg)	Group I	20	7 (5-30)	7 (5-59)	7.5 (4.5-57.5)	1.054	.590
	Group II	10	6.25 (5-8)	5.75 (4.5-8)	6 (5-7)	2.457	.293
	Group III	20	6.5 (5-77.5)	6.5 (5-65)	7 (6-40)	3.735	.154

Note: Minimum and maximum appear in parentheses below medians.

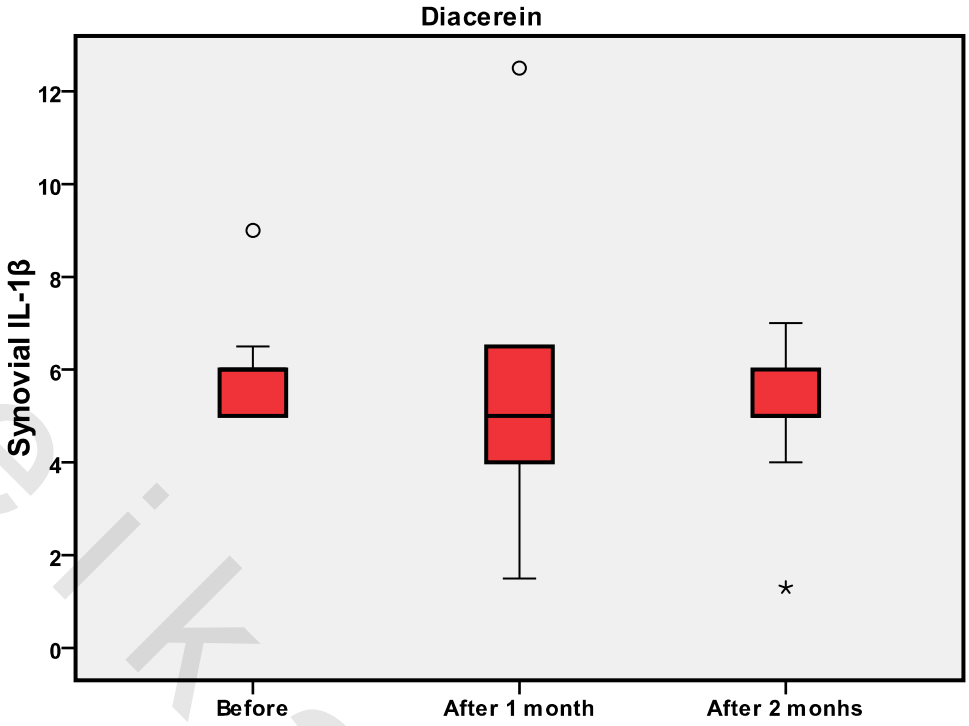

Fig. (22): Box plot for median synovial IL-1β and its range in group I between before, 1 and 2 month after treatment.

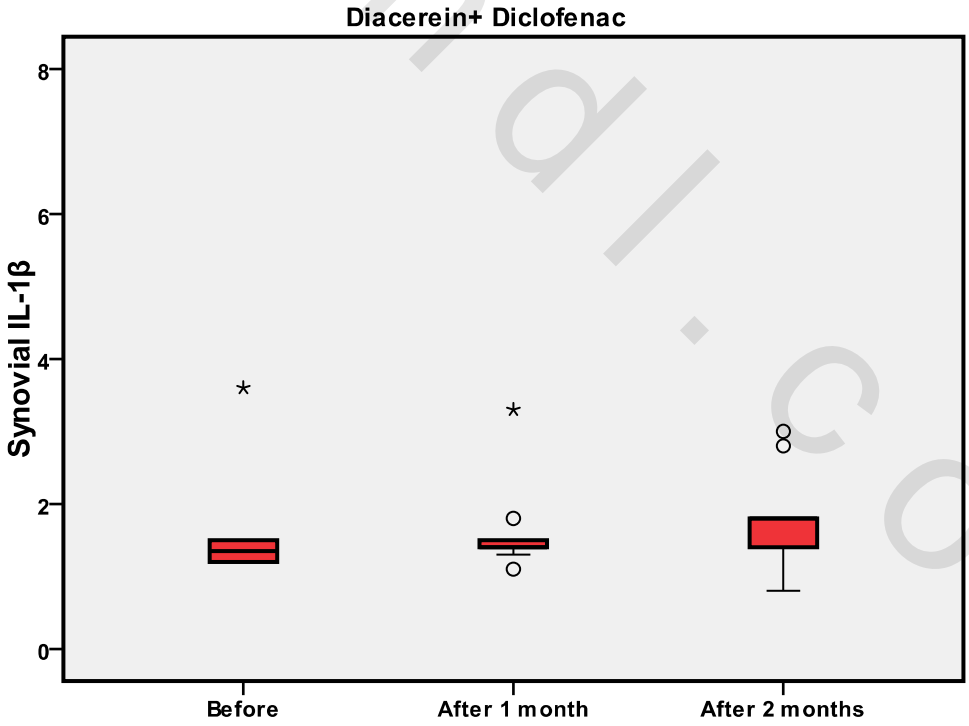

Fig. (23): Box plot for median synovial IL-1β and its range in group III between before, 1 and 2 month after treatment.

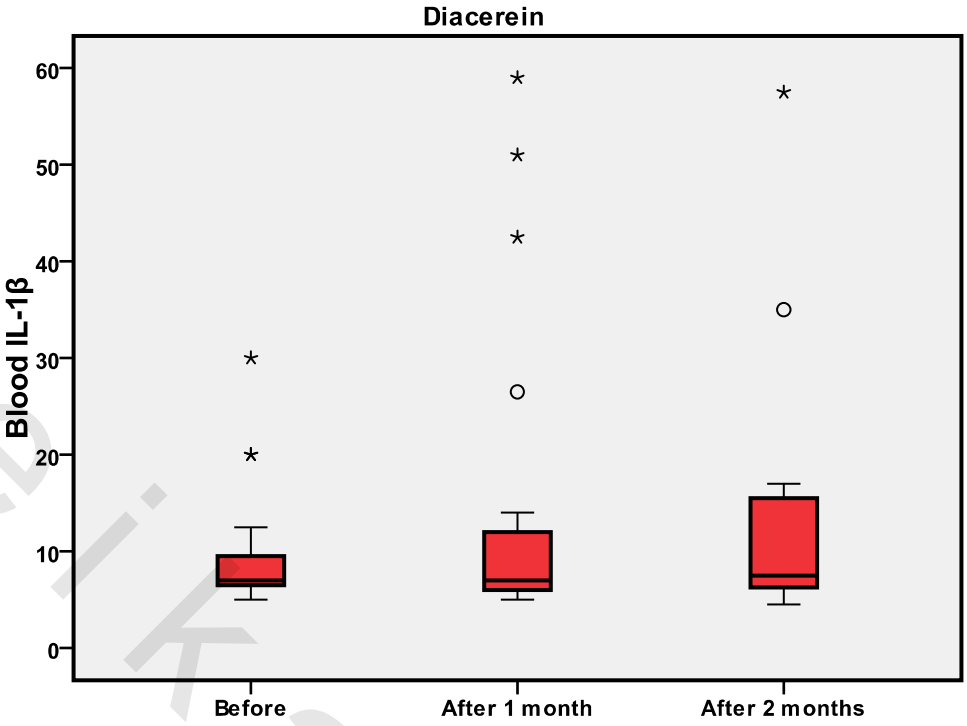

Fig. (24): Box plot for median blood IL-1β and its range in group I between before, 1 and 2 month after treatment.

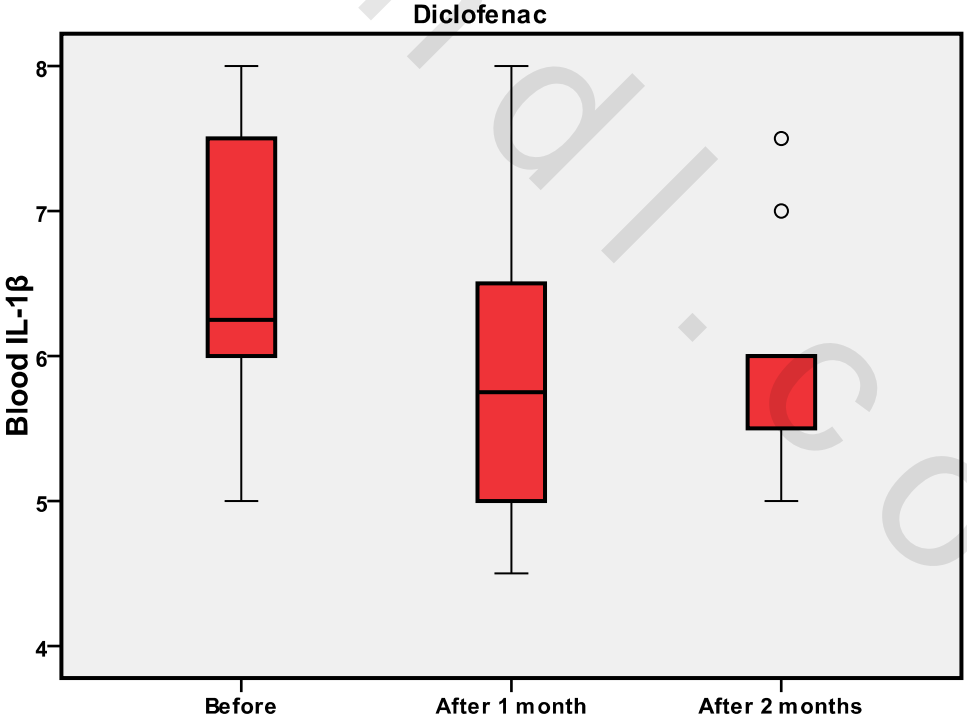

Fig. (25): Box plot for median blood IL-1β and its range in group II between before, 1 and 2 month after treatment.

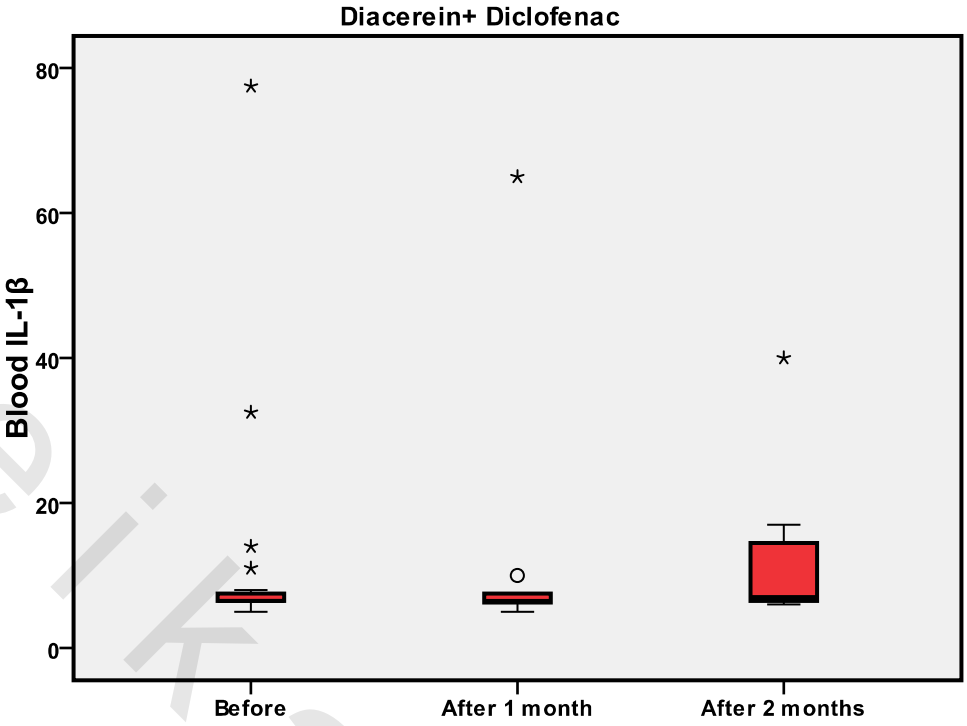

Fig. (26): Box plot for median blood IL-1β and its range in group III between before, 1 and 2 month after treatment

As shown in table 13, the median of synovial IL-1β level before treatment shows statistically significant difference between group I, group III and controls (P= 0.001). Post hoc paired comparisons showed that synovial IL-1β was statistically higher in patients of group I than controls and that synovial IL-1β was statistically higher in patients of group I than patients of group III before treatment. Also, the median of synovial IL-1β level after 2 months of treatment shows statistically significant difference between group I, group III and controls (P= 0.002). Post hoc paired comparisons showed that median synovial IL-1β was statistically higher in patients of group I after 2 months of treatment than controls and that median synovial IL-1β was statistically higher in patients of group I than patients of group III after 2 months of treatment.

The median of blood IL-1β level after 2 months of treatment shows statistically significant difference between 4 groups (P=0.007). Post hoc paired comparisons showed that median blood IL-1β was statistically higher in patients of group I than those of group II. Also, median blood IL-1β was significantly higher in patients of group III than those of group II.

Table (13): Synovial and serum IL-1β level in the studied patients and control group before treatment and 2 months after treatment.

Patient assessment			Studied group				H	p
			Group I	Group II	Group III	Control*		
Synovial IL-1β (μg)	n		10	-	10	6		
	Before treatment	Median (Min-Max)	6_a (5- 9)	-	$1.35_{b,c}$ (1.20- 27)	1.45_c (1.30- 2.20)	13.908	.001**
	After 2 month	Median (Min-Max)	5_a (1.30- 7)	-	$1.8_{b,c}$ (0.80- 3)	1.45_c (1.30- 2.20)	12.387	.002**
Blood IL-1β (μg)	n		20	10	20	6		
	Before treatment	Median (Min-Max)	7 (5- 30)	6.25 (5- 8)	6.5 (5- 77.5)	6.5 (5- 7)	3.262	.353
	After 2 month	Median (Min-Max)	$7.5_{a,c}$ (4- 57.50)	6_b (5- 7)	7_c (6- 40)	$6.5_{a,b,c}$ (5- 7)	12.175	.007**

Note: Minimum and maximum appear in parentheses below medians. Medians with differing subscripts within rows are significantly different at the adjusted $p < .05$ based on post hoc paired comparisons.
*IL-1β grade is assumed to be the same over time in controls (i.e.: same grades before and after 2 month treatment in either blood or synovial fluid)
** $p < 0.05$ (significant)

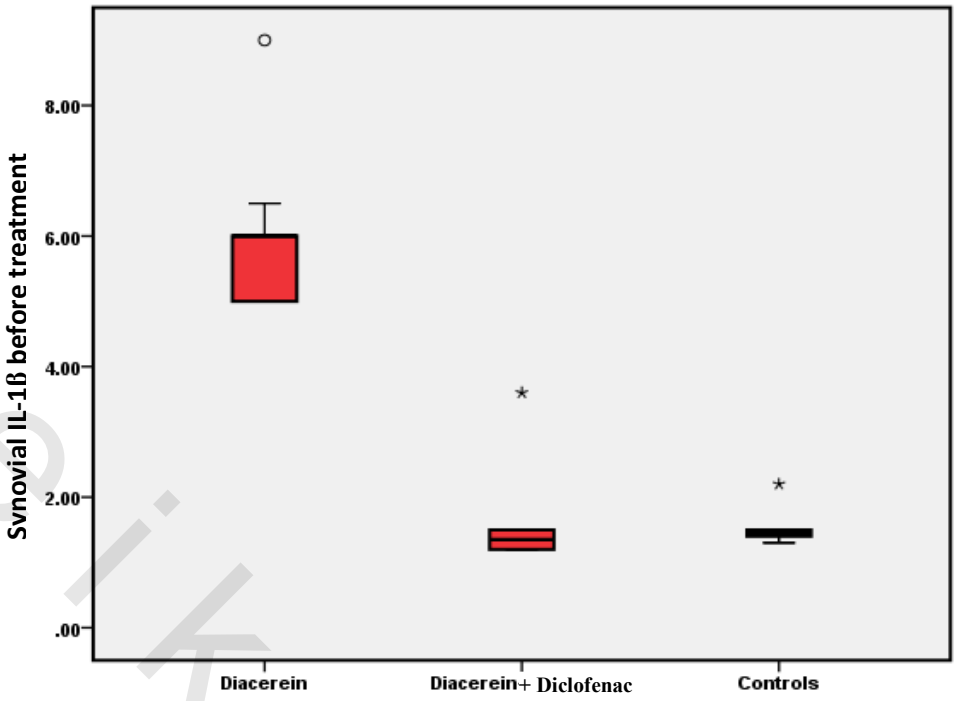

Fig. (27): Box plot for median synovial IL-1β before treatment and its range between the 3 groups of treatment and controls.

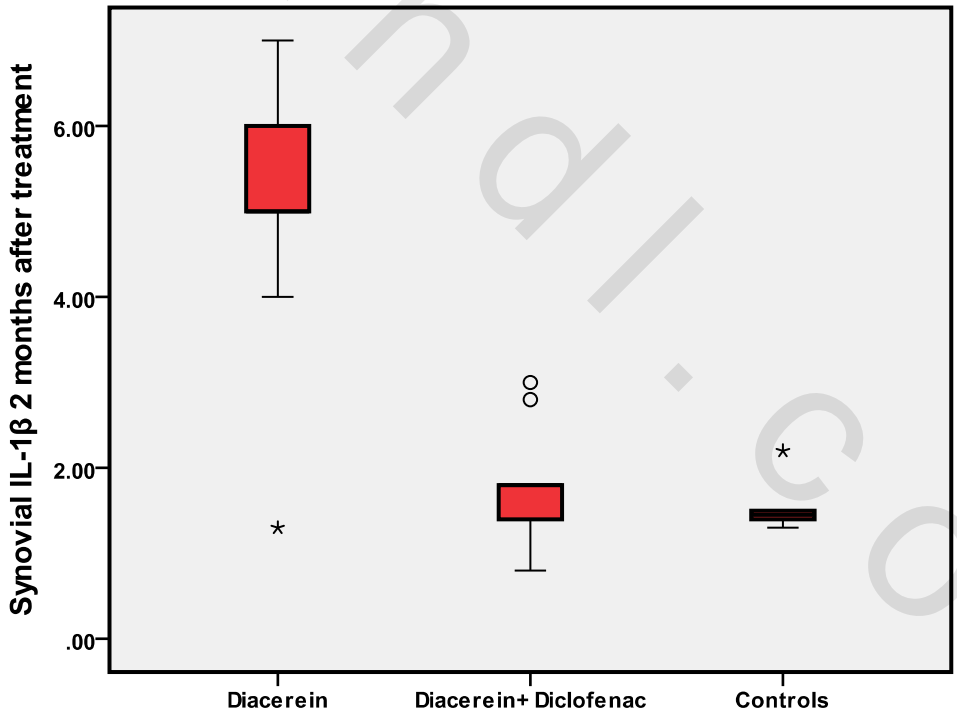

Fig. (28): Box plot for median synovial IL-1β 2 months after treatment and its range between the 3 groups of treatment and controls.

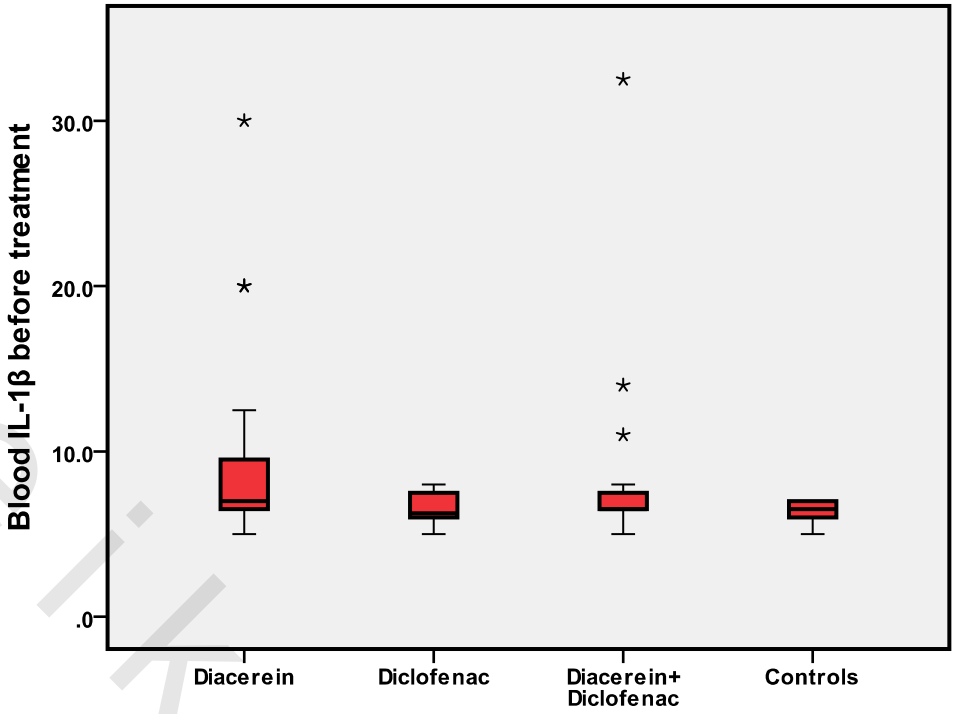

Fig. (29): Box plot for median blood IL-1β before treatment and its range between the 3 groups of treatment and controls.

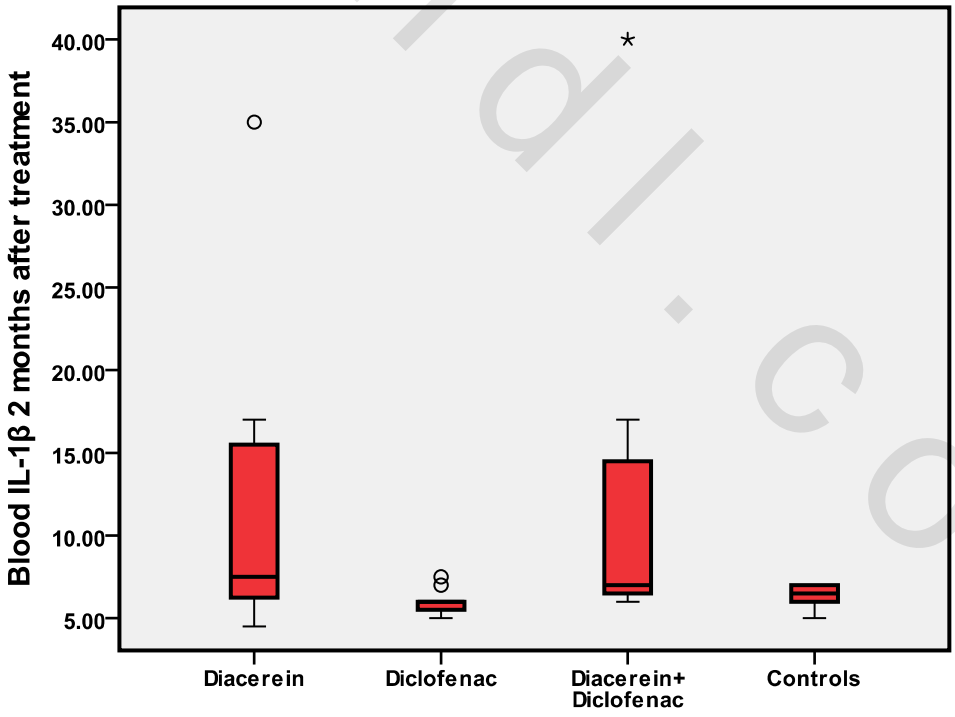

Fig. (30): Box plot for median blood IL-1β 2 months after treatment and its range between the 3 groups of treatment and controls.

The frequency of side effects of diclofenac sodium and diacerein is displayed in table 14. Heartburn was found in 20% of group II and 25% of group III patients. Nausea was reported in 20% of patients of groups II and III. Diarrhea was found in 40% and urine discoloration in 100% of patients of groups I and III.

Table (14): Frequency of side effects of diclofenac sodium and diacerein.

Side effect	Patient's group		
	Group I (n=20)	**Group II (n=10)**	**Group III (n=20)**
	n (%)	**n (%)**	**n (%)**
Heartburn	-	2(20%)	5(25%)
Nausea	-	2(20%)	4(20%)
Diarrhea	8(40%)	-	8(40%)
Urine discoloration	20(100%)	-	20(100%)