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Research Article

Ulcer Index and Anti-Inflammatory Testing of Some Benzimidazole Derivatives

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Abstract

In this work, we carry out the testing of some Benzimidazole derivatives as anti-inflammatory using Indomethacin 10mg/Kg, diclofenac sodium 7mg/Kg, celecoxib 100mg/Kg and tested compounds of 200mg/Kg. The tested compounds showed anti-inflammatory activity in comparison with the standard reference drugs. In additionally, carrying out the testing of ulcer index for some testing compounds 600mg/Kg comparing with indomethacin 100mg/Kg, the testing revealed indomethacin causes ulcer in stomach of the testing animals, while the testing compounds no ulcerated the stomach of the testing animal.

Keywords: Benzimidazole; Anti-inflammatory; Ulcer index

Introduction

The Inflammation is a hard problem when somebody suffers from it. So the inflammation in any part of the body must be treated until no aggravate causing serious problems, one of these problems is the Tumor. So the inflammation when treated must take care in the choice of drug which used in the treatment.

Indomethacin used in treatment of many inflammations due to its activity as a powerful anti-inflammatory agent, but along time term of using it causes ulcerated stomach of the patient. Hance this work testing the some of benzimidazole derivatives 200mg/Kg as anti-inflammatory agent comparing with Indomethacin 10mg/Kg, diclofenac sodium 7mg/Kg, celecoxib 100mg/Kg as a reference drugs, and testing for some of benzimidazole 600mg/Kg comparing with Indomethacin 100mg/Kg

The chemical structure of heterocyclic compounds specially the structures which have benzo diazine and benzo diazole (phthalazine, Quinoxaline, Quinazoline and benzimidazole) have many of biological activities antibacterial, anticonvulsant, oral hypoglycemics, anticancer, anthelmintics, antiprotozoal, anti-inflammatory, analgesics, antioxidants, growth regulators, and other biological uses. so, we choose the benzimidazoles which is promising nucleus. it has analgesic activity like diclofenac and also has anticancer activity as doxirubsin i.e., six membered ring fused with five membered ring containing tow nitrogen like some compounds of anticancer, so we synthesized the following structure [1-25].

Chemistry

All of these compounds are reported [1] most of selected compounds (intermediate of final one) are tested as anti-inflammatory and carried out the ulcer index. Not all because of high cost of the biological studies.

The compounds prepared by the following Scheme 1.

Biological Studies

Experimental Animal for anti-inflammatory activity

Adult healthy Male Wister albino rats weighting between 140-160 gm were used for the study. The animals were housed in standard conditions (temperature 25±2°C with 55±5% relative humidity and a 12-hour light dark cycle). All animals had free access to water and normal diet. The study was approved by Institutional Animal Ethical Committee (IAEC) and was in accordance with the guideline of the Committee for the Purpose of Control and Supervision of Experimental Animal (CPCSEA).

In vivo anti-inflammatory activity

The initial paw volume of each rat was noted by the usage of Digital Vernier Caliper. One hundred fourty four animals were used in this study and divided into 24 groups (six animals per each).

Group-1 was served for saline injection in the right hind paw.

Group-2 was served for carrageenan injection in the right hind paw.

Group-3 was served for voltarin injection at a dose of 7mg/kg body weight.

Group-4 was received indomethacin at a dose of 10mg/kg body weight.

Group-5 was received celecoxib at a dose of 100mg/kg body weight.

Whereas groups 6-24 received the test samples at a dose of 200mg/kg body weight.

One hour post oral administration of test compounds at a dose of 200mg/kg, isotonic saline (0.1ml/paw), standards: diclofenac sodium (7mg/kg I.P), indomethacin (10mg/kg orally) and celecoxib (100mg/kg orally) 1% w/v from carrageenan solution (0.1ml/paw) was injected subcutaneously into the plantar surface of the rat right leg hind paw.

The paw volume of the left legs; negative control for each animal was measured with the help of official Digital Vernier caliper during the time intervals of 1, 2, 3, 6, 12 and 24 h after carrageenan injection.

Percentage protection (or inhibition) was calculated by using the formula,

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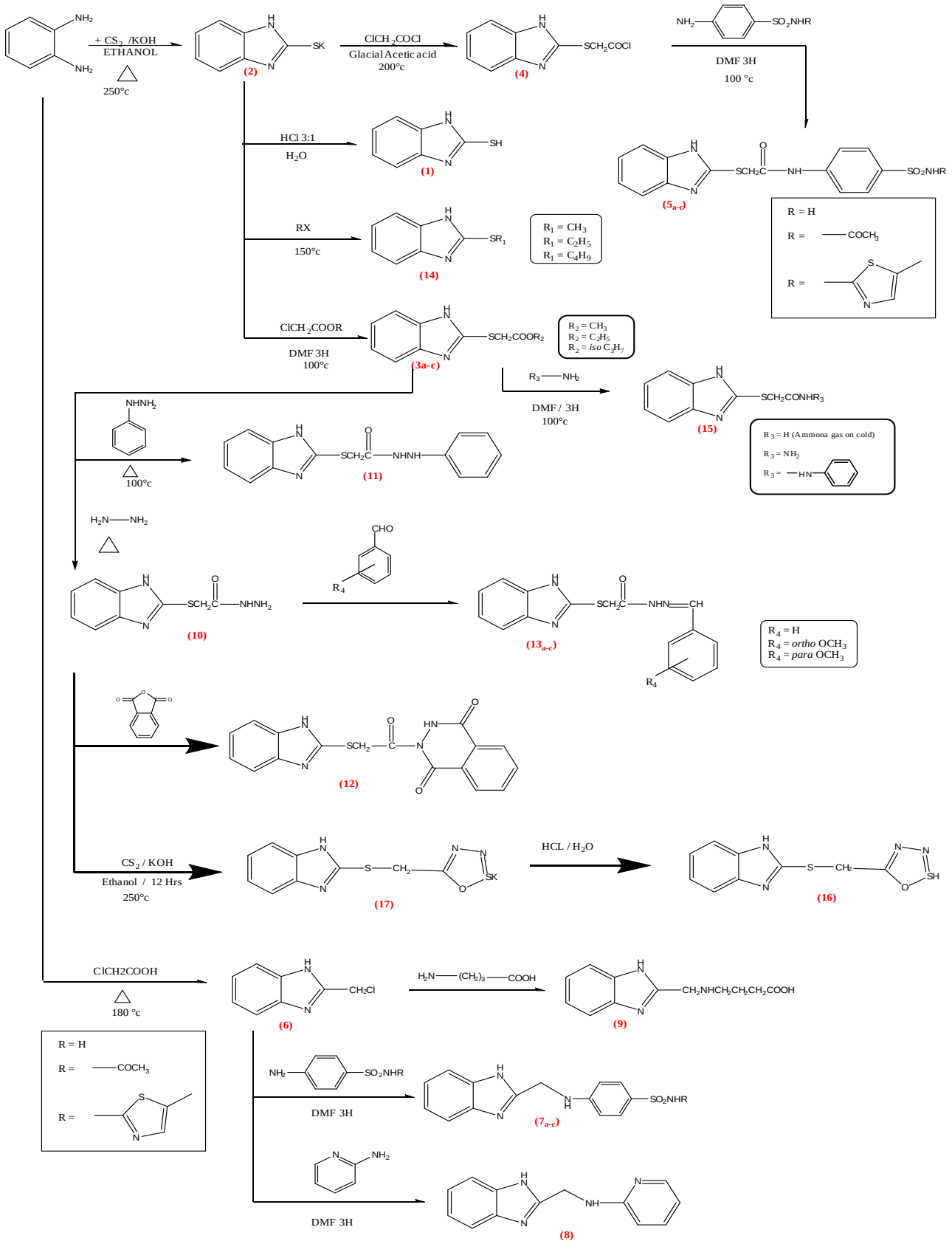
+ CS2 /KOH
ETHANOL
250°c
ClCH2COCl
Glacial Acetic acid
200°c
DMF 3H
100 °c
HCl 3:1
H2O
RX
150°c
ClCH2COOR
DMF 3H
100°c
DMF / 3H
100°c
100°c
H2N—NH2
ClCH2COOH
180 °c
DMF 3H
DMF 3H
CS2 / KOH
Ethanol / 12 Hrs
250°c
HCL / H2O
R1 = CH3
R1 = C2H5
R1 = C4H9
R2 = CH3
R2 = C2H5
R2 = iso C3H7
R3 = H (Ammona gas on cold)
R3 = NH2
R4 = H
R4 = ortho OCH3
R4 = para OCH3
R = H
R = COCH3
(1)
(2)
(4)
(5a-c)
(6)
(7a-c)
(8)
(9)
(10)
(11)
(12)
(13a-c)
(14)
(15)
(16)
(17)
(3a-c)

Scheme 1:

% protection = (1-Vt/Vc) X 100, where:

Vt is the mean increase in the paw volume in the test animals group,

Vc is the mean increase in the paw volume in the control group (in anti-inflammatory study).

Evaluation of edema, and percentage increase in paw volume in saline, carrageenan, standards and tested compounds (Table 1-23, Figure 1-24).

The anti-inflammatory effects of saline, diclofenac sodium, indomethacin, Celecoxib and tested compounds administration in comparison to carrageenan injected rats

Data in Table 24 showed that injection of 0.1ml saline in right hind paw (S.C) caused no significant increase in right paw volume by about (31.2%, 40.4%, 19.1% 12.7% 10.6% and 6.3% after 1, 2, 3, 6, 12 and 24 h, respectively, when compared to volume of left leg, while injection of 0.1ml carrageenan in right hind paw (S.C) in a dose of 1% w/v caused a significant increase in right paw volume by about (58.5%, 258%, 231% 204% 228% and 132% after 1, 2, 3, 6, 12 and 24 h, respectively, when compared to volume of left leg, while pretreatment with diclofenac sodium (I.P) in a dose of 7mg/kg caused a significant reduction in right paw volume by (49.3%, 65.8%, 82.3%, 104.4%, 129.7% and 85.4%), after 1, 2, 3, 6, 12 and 24 h, respectively,. when compared to volume of left leg The pretreatment with indomethacin (orally) in a dose of 10mg/kg caused a significant reduction in right paw volume by, (33.1%, 46.4%, 64.4%, 76.0%, 90.2% and 42.3%),

Table 1: Saline group.

Rat no.	Left leg	1 h	2 h	3 h	6 h	12 h	24 h
1	0.31	0.36	0.32	0.31	0.31	0.31	0.31
2	0.29	0.33	0.34	0.3	0.3	0.3	0.3
3	0.19	0.24	0.31	0.3	0.26	0.26	0.23
4	0.24	0.32	0.34	0.24	0.24	0.24	0.24
5	0.19	0.28	0.32	0.24	0.22	0.22	0.2
6	0.19	0.32	0.35	0.29	0.26	0.23	0.22
Total	141	185	198	168	159	156	150
Mean	23.5	30.8	33	28	26.5	26	25
% of S.D	------	31.2	40.4	19.1	12.7	10.6	6.3

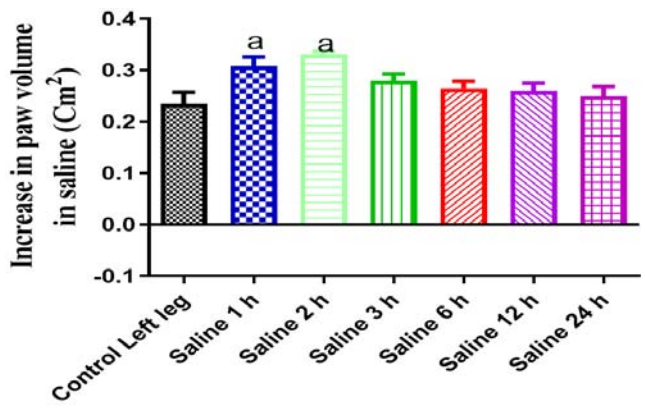

Figure 1: Saline group.

Table 2: Carrageenan group.

Rat no	Left leg	1 h	2 h	3 h	6 h	12 h	24 h
1	0.3	0.6	0.85	0.85	0.89	0.94	0.6
2	0.25	0.75	1.12	0.91	0.8	0.85	0.63
3	0.24	0.8	0.99	0.9	0.71	0.88	0.65
4	0.32	0.82	0.98	0.95	0.91	0.96	0.74
5	0.26	0.81	0.96	0.9	0.85	0.88	0.58
6	0.27	0.82	0.97	0.92	0.84	0.88	0.61
Total	1.64	2.6	5.87	5.43	5	5.39	3.81
Mean	0.273	0.766	0.978	0.905	0.833	0.898	0.635
% of S.D	-------	58.5	258	231	204	228	132

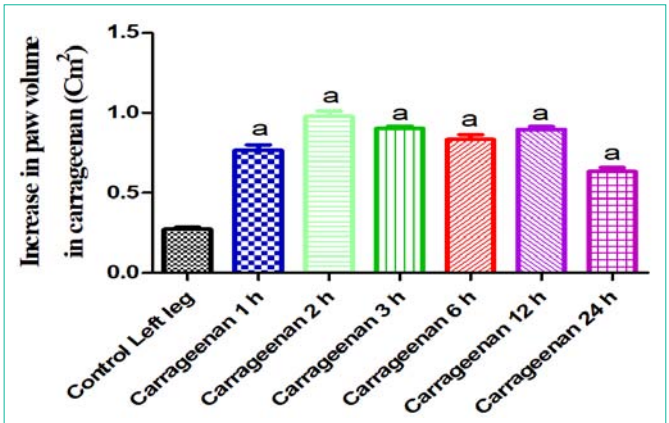

Figure 2: Carrageenan group.

Table 3: Diclofenac sodium group.

Rat no	Left leg	1 h	2 h	3 h	6 h	12 h	24 h
1	0.3	0.42	0.5	0.6	0.7	0.77	0.62
2	0.29	0.35	0.4	0.5	0.54	0.52	0.44
3	0.24	0.4	0.45	0.4	0.38	0.49	0.45
4	0.25	0.38	0.41	0.44	0.52	0.61	0.55
5	0.24	0.41	0.42	0.46	0.53	0.61	0.44
6	0.26	0.4	0.44	0.48	0.56	0.63	0.43
Total	1.58	2.36	2.62	2.88	3.23	3.63	2.93
Mean	0.263	0.393	0.436	0.48	0.538	0.605	0.488
% of S.D	------	49.3	65.8	82.3	104.4	129.7	85.4

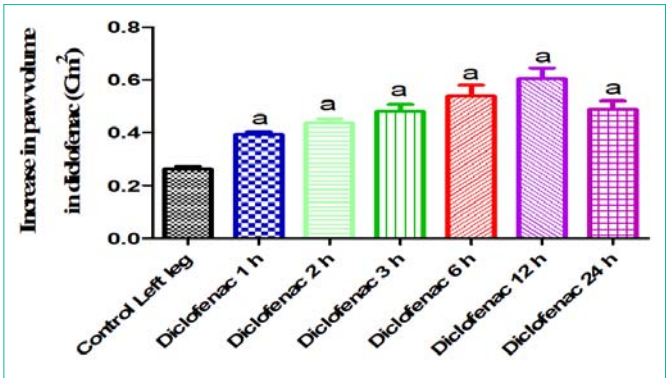

Figure 3: Diclofenac sodium group.

Table 4: Indomethacin group.

Rat no	Left leg	1 h	2 h	3 h	6 h	12 h	24 h
1	0.26	0.48	0.54	0.6	0.65	0.59	0.44
2	0.28	0.33	0.36	0.4	0.41	0.46	0.3
3	0.24	0.35	0.38	0.54	0.6	0.48	0.35
4	0.3	0.32	0.35	0.36	0.39	0.51	0.4
5	0.28	0.34	0.37	0.38	0.4	0.52	0.41
6	0.27	0.35	0.39	0.4	0.42	0.54	0.42
Total	1.63	2.17	2.39	2.68	2.87	3.1	2.32
Mean	0.271	0.361	0.398	0.446	0.478	0.516	0.386
% of S.D	------	33.1	46.6	64.4	76	90.2	42.3

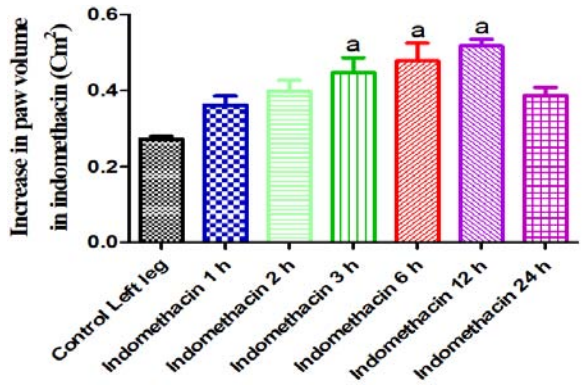

Figure 4: Indomethacin group.

Table 5: Celecoxib group.

Rat no	Left leg	1 h	2 h	3 h	6 h	12 h	24 h
1	0.28	0.47	0.56	0.49	0.4	0.35	0.35
2	0.26	0.43	0.54	0.49	0.49	0.39	0.38
3	0.27	0.43	0.55	0.45	0.35	0.35	0.35
4	0.28	0.51	0.58	0.49	0.39	0.33	0.33
5	0.24	0.5	0.56	0.46	0.4	0.4	0.34
6	0.25	0.5	0.58	0.45	0.38	0.38	0.36
Total	1.6	2.84	3.37	2.83	2.41	2.2	2.11
Mean	0.266	0.47	0.56	0.47	0.4	0.36	0.35
% of S.D	------	77	111	77	51	37	32

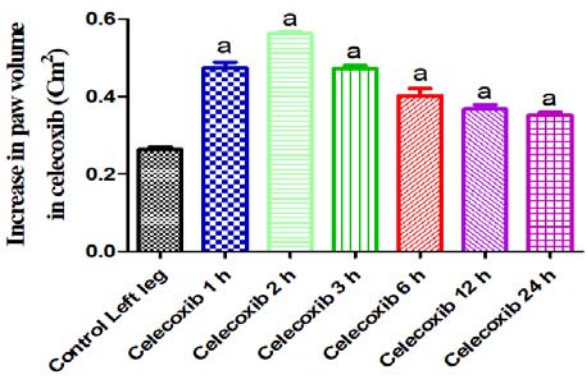

Figure 5: Celecoxib group.

Table 6: IX treated group.

Rat no	Left leg	1 h	2 h	3 h	6 h	12 h	24 h
1	0.28	0.6	0.7	0.8	0.9	0.75	0.6
2	0.23	0.55	0.7	0.84	0.84	0.76	0.66
3	0.26	0.6	0.75	0.8	0.75	0.7	0.64
4	0.24	0.56	0.68	0.74	0.77	0.75	0.54
5	0.27	0.55	0.7	0.75	0.7	0.7	0.46
6	0.29	0.5	0.65	0.68	0.74	0.6	0.44
Total	1.57	3.36	4.19	4.61	4.7	4.26	3.34
Mean	0.262	0.56	0.7	0.77	0.78	0.71	0.56
% of S.D	------	114	167	193	199	171	112

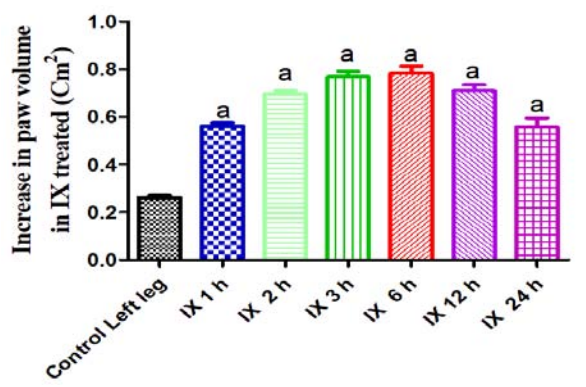

Figure 6: IX treated group.

Table 7: XVI treated group.

Rat no	Left leg	1 h	2 h	3 h	6 h	12 h	24 h
1	0.26	0.7	0.83	0.8	0.665	0.6	0.518
2	0.26	0.68	0.8	0.87	0.787	0.7	0.646
3	0.28	0.7	0.93	0.95	0.92	0.85	0.77
4	0.27	0.7	0.91	0.92	0.71	0.65	0.565
5	0.28	0.65	0.9	0.85	0.7	0.63	0.55
6	0.27	0.66	0.95	0.88	0.72	0.68	0.65
Total	1.62	4.09	5.32	5.27	4.5	4.11	3.68
Mean	0.27	0.68	0.88	0.87	0.75	0.68	0.61
% of S.D	------	147	220	216	172	147	121

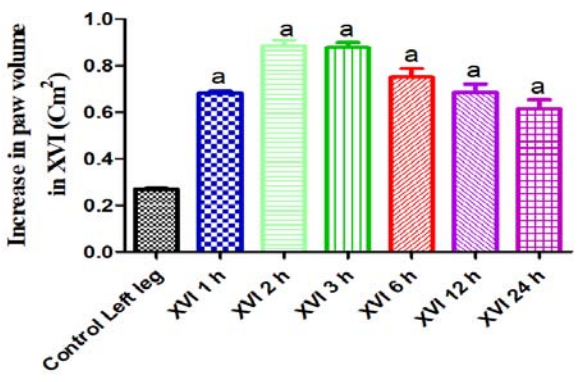

Figure 7: XVI treated group.

Table 8: Vb treated group.

Rat no	Left leg	1 h	2 h	3 h	6 h	12 h	24 h
1	0.27	0.6	0.7	0.9	0.96	0.8	0.74
2	0.28	0.6	0.7	0.92	0.97	0.8	0.7
3	0.24	0.61	0.66	0.92	0.92	0.7	0.59
4	0.27	0.6	0.68	0.78	0.8	0.68	0.6
5	0.28	0.56	0.7	0.8	0.8	0.67	0.6
6	0.26	0.66	0.76	0.82	0.85	0.7	0.61
Total	1.6	3.63	4.2	5.14	5.27	4.35	4.04
Mean	26.6	0.605	0.7	0.856	0.878	0.725	0.673
% of S.D	------	127	163	222	230	177	153

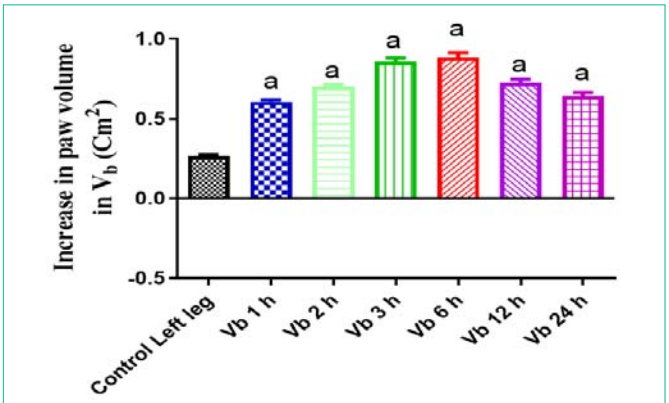

Figure 8: Vb treated group.

Table 9: XV treated group.

Rat no	Left leg	1 h	2 h	3 h	6 h	12 h	24 h
1	0.26	0.65	0.83	0.8	0.665	0.6	0.618
2	0.26	0.68	0.8	0.87	0.787	0.7	0.646
3	0.28	0.63	0.82	0.95	0.82	0.75	0.67
4	0.29	0.65	0.81	0.92	0.71	0.62	0.565
5	0.29	0.6	0.8	0.85	0.7	0.63	0.5
6	0.27	0.56	0.85	0.88	0.72	0.68	0.65
Total	1.65	3.77	4.91	5.27	4.39	3.98	3.63
Mean	0.275	0.63	0.82	0.88	0.73	0.66	0.6
% of S.D	------	129	198	220	165	140	118

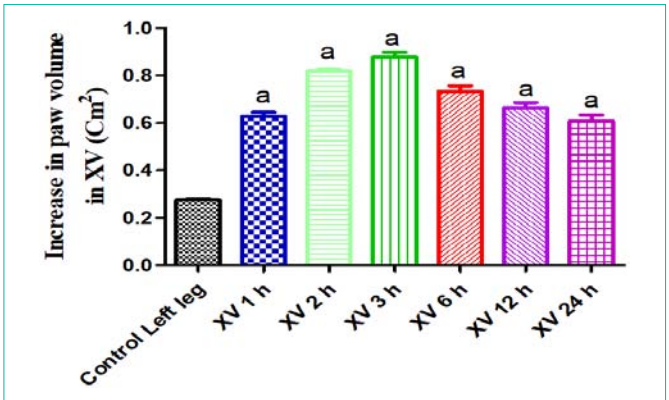

Figure 9: XV treated group.

Table 10: XIVa treated group.

Rat no	Left leg	1 h	2 h	3 h	6 h	12 h	24 h
1	0.26	0.65	0.73	0.88	0.98	0.9	0.84
2	0.27	0.62	0.7	0.9	0.98	0.55	0.51
3	0.3	0.61	0.66	0.9	0.98	0.64	0.6
4	0.3	0.54	0.68	0.83	0.9	0.8	0.74
5	0.28	0.52	0.72	0.75	0.8	0.7	0.54
6	0.3	0.56	0.68	0.88	0.93	0.7	0.68
Total	1.71	3.5	4.17	5.14	5.57	4.29	3.91
Mean	0.285	0.58	0.69	0.86	0.93	0.72	0.65
% of S.D	------	105	144	202	226	151	129

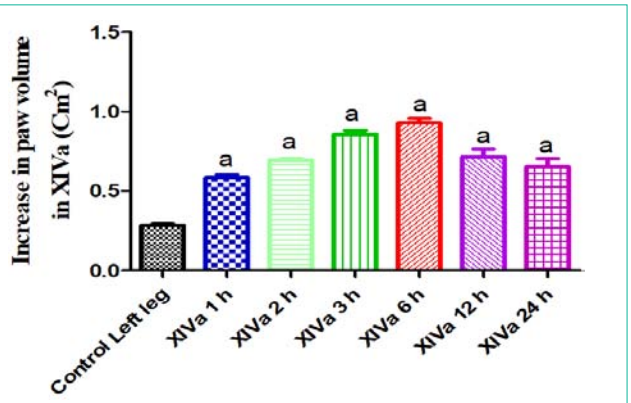

Figure 10: XIVa treated group.

Table 11: XIVb treated group.

Rat no	Left leg	1 h	2 h	3 h	6 h	12 h	24 h
1	0.28	0.5	0.79	0.9	0.89	0.8	0.65
2	0.28	0.68	0.74	0.89	0.93	0.83	0.72
3	0.25	0.6	0.7	0.7	0.7	0.7	0.6
4	0.28	0.55	0.88	0.78	0.79	0.75	0.63
5	0.27	0.65	0.8	0.85	0.8	0.74	0.7
6	0.26	0.58	0.75	0.88	0.84	0.76	0.72
Total	1.62	3.56	4.66	5	4.95	4.58	4.02
Mean	0.27	0.59	0.78	0.83	0.82	0.76	0.67
% of S.D	------	120	187	208	206	182	148

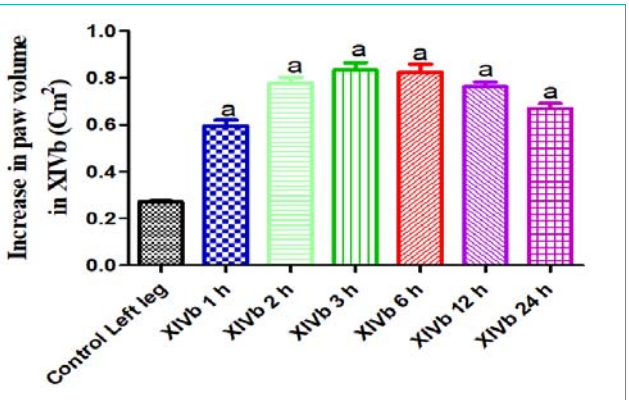

Figure 11: XIVb treated group.

Table 12: VIIb treated group.

Rat no	Left leg	1 h	2 h	3 h	6 h	12 h	24 h
1	0.26	0.66	0.75	0.9	0.9	0.7	0.6
2	0.26	0.65	0.8	0.99	0.84	0.72	0.67
3	0.29	0.55	0.85	0.99	0.9	0.8	0.71
4	0.28	0.66	0.83	0.9	0.9	0.75	0.62
5	0.28	0.5	0.9	0.95	0.8	0.72	0. 600
6	0.26	0.6	0.85	0.88	0.78	0.7	0.64
Total	1.63	3.63	4.98	5.62	5.12	4.39	3.84
Mean	0.271	0.61	0.83	0.94	0.85	0.73	0.64
% of S.D	------	123	206	246	215	170	136

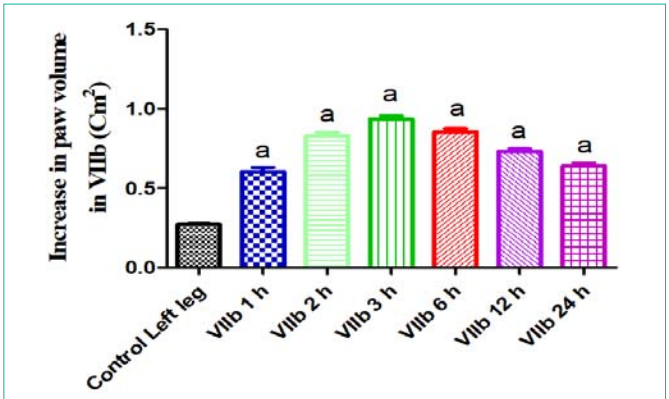

Figure 12: VIIb treated group.

Table 13: Vc treated group.

Rat no	Left leg	1 h	2 h	3 h	6 h	12 h	24 h
1	0.26	0.55	0.7	0.84	0.9	0.8	0.7
2	0.27	0.53	0.66	0.82	0.787	0.78	0.72
3	0.28	0.6	0.76	0.95	0.82	0.82	0.77
4	0.27	0.5	0.68	0.92	0.8	0.78	0.565
5	0.28	0.55	0.7	0.85	0.8	0.76	0.55
6	0.26	0.5	0.67	0.88	0.82	0.8	0.65
Total	1.62	3.23	4.17	5.26	4.92	4.74	3.95
Mean	0.27	0.54	0.7	0.88	0.82	0.79	0.66
% of S.D	------	100	159	226	203	193	144

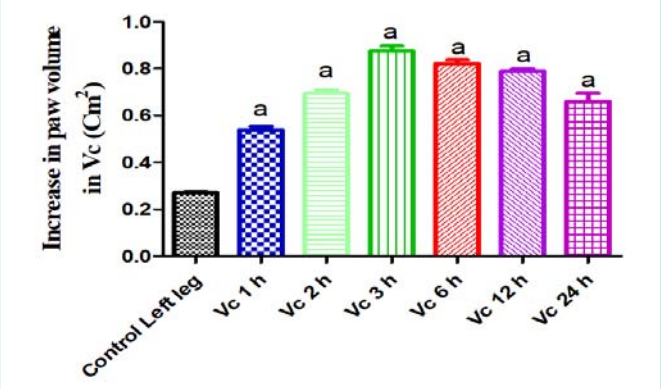

Figure 13: Vc treated group.

Table 14: VIII treated group.

Rat no	Left leg	1 h	2 h	3 h	6 h	12 h	24 h
1	0.28	0.6	0.79	0.9	0.8	0.75	0.7
2	0.23	0.68	0.84	0.89	0.9	0.83	0.72
3	0.26	0.6	0.75	0.9	0.75	0.7	0.6
4	0.24	0.65	0.88	0.98	0.79	0.75	0.63
5	0.27	0.55	0.7	0.85	0.7	0.74	0.6
6	0.29	0.58	0.75	0.88	0.64	0.7	0.58
Total	1.57	3.66	4.71	5.4	4.58	4.47	3.83
Mean	0.261	0.61	0.785	0.9	0.76	0.74	0.64
% of S.D	------	100	201	245	191	184	145

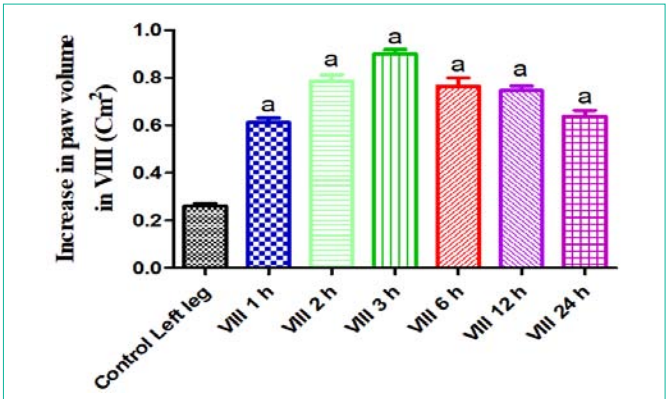

Figure 14: VIII treated group.

Table 15: XIIIa treated group.

Rat no	Left leg	1 h	2 h	3 h	6 h	12 h	24 h
1	0.28	0.7	0.83	0.99	0.95	0.85	0.57
2	0.26	0.6	0.8	0.97	0.84	0.75	0.68
3	0.25	0.63	0.76	0.8	0.85	0.7	0.57
4	0.24	0.65	0.78	0.83	0.83	0.72	0.6
5	0.29	0.6	0.72	0.77	0.8	0.67	0.6
6	0.28	0.56	0.6	0.67	0.72	0.6	0.58
Total	1.6	3.74	4.49	5.03	4.99	4.29	3.6
Mean	0.266	0.62	0.75	0.838	0.83	0.715	0.6
% of S.D	------	133	182	212	212	166	77

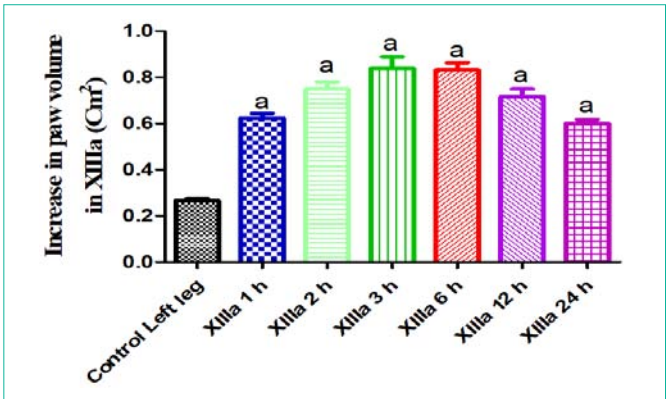

Figure 15: XIIIa treated group.

Table 16: X treated group.

Rat no	Left leg	1 h	2 h	3 h	6 h	12 h	24 h
1	0.28	0.7	0.83	0.88	0.7	0.65	0.57
2	0.26	0.6	0.7	0.8	0.71	0.55	0.48
3	0.27	0.63	0.66	0.8	0.66	0.54	0.46
4	0.26	0.56	0.78	0.83	0.7	0.6	0.54
5	0.29	0.58	0.72	0.75	0.7	0.6	0.54
6	0.28	0.56	0.58	0.66	0.6	0.6	0.58
Total	1.64	3.63	4.27	4.72	4.07	3.54	3.17
Mean	0.273	0.6	0.71	0.78	0.68	0.59	0.53
% of S.D	------	121	160	188	148	116	100

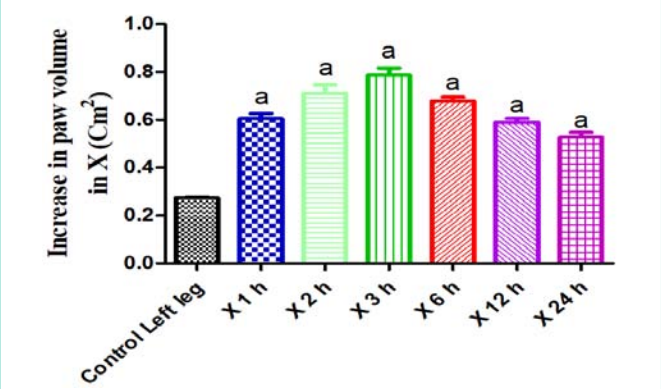

Figure 16: X treated group.

Table 17: XIIIb treated group.

Rat no	Left leg	1 h	2 h	3 h	6 h	12 h	24 h
1	0.26	0.5	0.6	0.74	0.59	0.5	0.36
2	0.27	0.53	0.66	0.82	0.77	0.7	0.52
3	0.28	0.6	0.76	0.85	0.72	0.7	0.6
4	0.27	0.6	0.68	0.82	0.7	0.68	0.55
5	0.28	0.55	0.7	0.8	0.72	0.6	0.55
6	0.26	0.55	0.66	0.82	0.7	0.7	0.6
Total	1.62	3.33	4.06	4.85	4.2	3.88	3.18
Mean	0.27	0.55	0.68	0.81	0.7	0.65	0.53
% of S.D	------	98	151	199	159	140	96

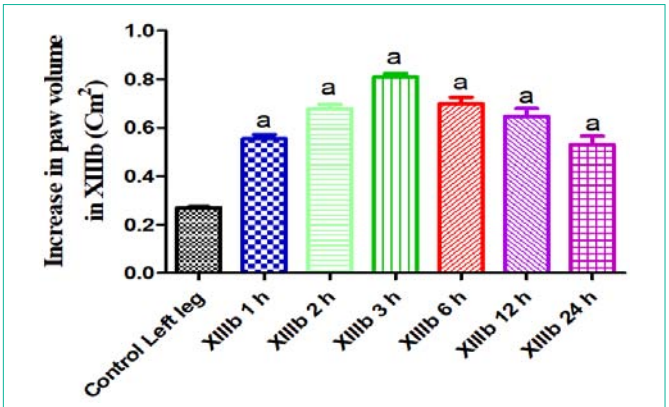

Figure 17: XIIIb treated group.

Table 18: XIVc treated group.

Rat no	Left leg	1 h	2 h	3 h	6 h	12 h	24 h
1	0.28	0.7	0.83	0.88	0.7	0.65	0.57
2	0.26	0.6	0.7	0.8	0.71	0.55	0.48
3	0.27	0.63	0.66	0.8	0.66	0.54	0.46
4	0.26	0.56	0.78	0.83	0.7	0.6	0.54
5	0.29	0.58	0.72	0.75	0.7	0.6	0.54
6	0.28	0.56	0.58	0.66	0.6	0.6	0.58
Total	1.64	3.63	4.27	4.72	4.07	3.54	3.17
Mean	0.273	0.6	0.71	0.78	0.68	0.59	0.53
% of S.D	------	121	160	188	148	116	100

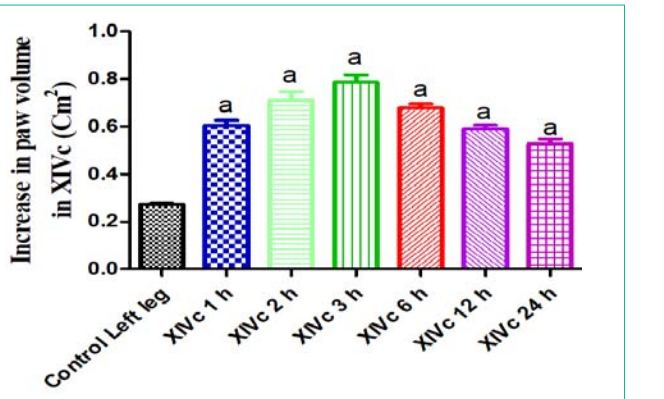

Figure 18: XIVc treated group.

Table 19: XIIIc treated group.

Rat no	Left leg	1 h	2 h	3 h	6 h	12 h	24 h
1	0.26	0.5	0.6	0.72	0.57	0.48	0.34
2	0.27	0.53	0.66	0.8	0.75	0.68	0.5
3	0.28	0.58	0.78	0.83	0.7	0.68	0.58
4	0.27	0.62	0.7	0.8	0.68	0.66	0.53
5	0.26	0.55	0.7	0.78	0.7	0.58	0.53
6	0.28	0.55	0.66	0.8	0.68	0.68	0.58
Total	1.62	3.33	4.06	4.73	4.08	3.76	3.06
Mean	0.27	0.55	0.68	0.78	0.68	0.62	0.51
% of S.D	------	98	151	191	152	132	89

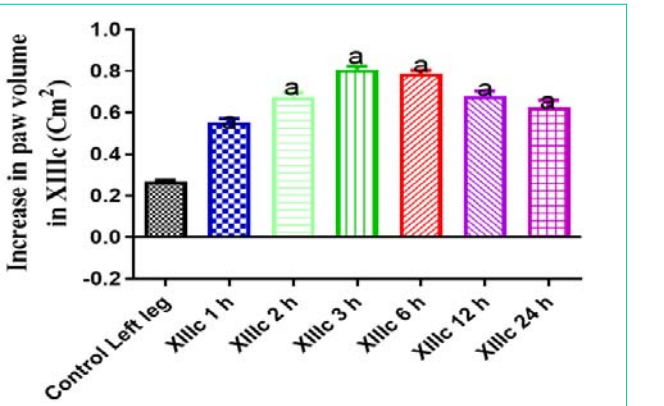

Figure 19: XIIIc treated group.

Table 20: Va treated group.

Rat no	Left leg	1 h	2 h	3 h	6 h	12 h	24 h
1	0.26	0.6	0.78	0.8	0.69	0.6	0.5
2	0.24	0.55	0.8	0.8	0.63	0.56	0.54
3	0.28	0.55	0.85	0.82	0.44	0.4	0.38
4	0.27	0.55	0.78	0.74	0.45	0.45	0.34
5	0.28	0.5	0.8	0.8	0.5	0.44	0.36
6	0.26	0.55	0.85	0.85	0.62	0.48	0.35
Total	1.59	3.3	4.86	4.81	3.33	2.93	2.47
Mean	0.265	0.55	0.81	0.8	0.555	0.49	0.41
% of S.D	------	108	205	202	109	84	55

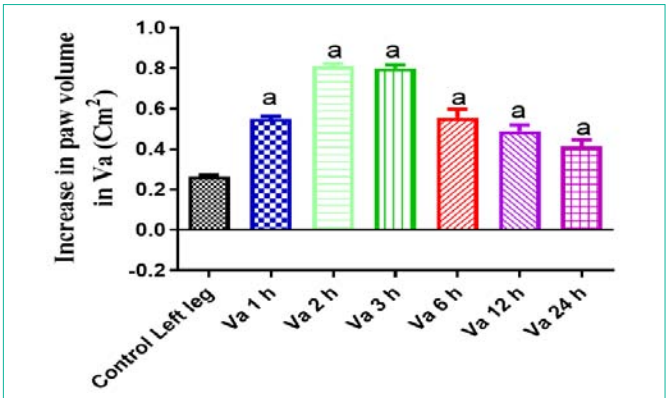

Figure 20: Va treated group.

Table 21: VIIa treated group.

Rat no	Left leg	1 h	2 h	3 h	6 h	12 h	24 h
1	0.27	0.5	0.5	0.52	0.45	0.4	0.29
2	0.35	0.63	0.76	0.87	0.62	0.55	0.4
3	0.24	0.61	0.76	0.8	0.58	0.5	0.41
4	0.27	0.54	0.68	0.85	0.55	0.5	0.38
5	0.28	0.55	0.75	0.78	0.6	0.55	0.42
6	0.26	0.56	0.76	0.85	0.65	0.6	0.48
Total	1.68	3.39	4.21	4.67	3.45	3.1	2.38
Mean	0.28	0.565	0.7	0.78	0.575	0.52	0.396
% of S.D	------	102	151	179	105	85	42

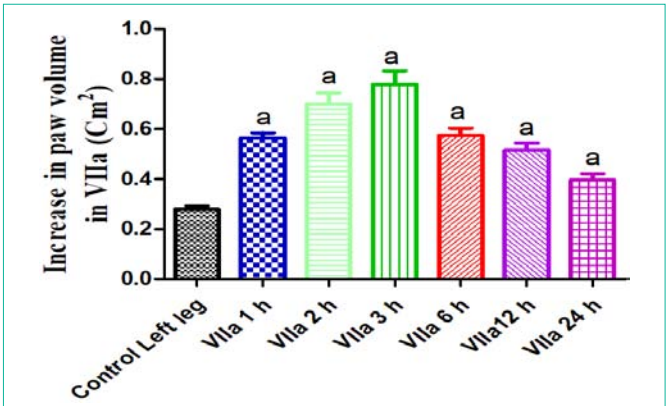

Figure 21: VIIa treated group.

Table 22: IIIc treated group.

Rat no	Left leg	1 h	2 h	3 h	6 h	12 h	24 h
1	0.3	0.55	0.5	0.6	0.55	0.45	0.4
2	0.36	0.65	0.76	0.87	0.62	0.55	0.4
3	0.24	0.61	0.8	0.8	0.58	0.5	0.41
4	0.27	0.54	0.68	0.85	0.65	0.5	0.38
5	0.27	0.55	0.75	0.78	0.6	0.55	0.42
6	0.26	0.56	0.76	0.85	0.65	0.6	0.48
Total	1.7	3.46	4.25	4.75	3.65	3.15	2.49
Mean	0.283	0.58	0.71	0.792	0.61	0.525	0.415
% of S.D	------	103	150	179	115	86	47

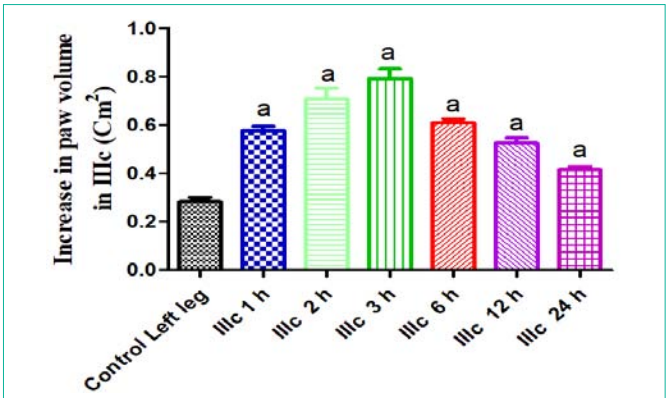

Figure 22: IIIc treated group.

Table 23: IIIb treated group.

Rat no	Left leg	1 h	2 h	3 h	6 h	12 h	24 h
1	0.26	0.65	0.73	0.88	0.53	0.4	0.34
2	0.27	0.62	0.7	0.71	0.62	0.55	0.49
3	0.3	0.61	0.66	0.71	0.53	0.44	0.34
4	0.3	0.74	0.78	0.94	0.51	0.5	0.44
5	0.28	0.52	0.72	0.81	0.6	0.55	0.4
6	0.3	0.66	0.8	0.88	0.63	0.6	0.46
Total	1.71	3.8	4.39	4.93	3.42	3.04	2.47
Mean	0.285	0.633	0.73	0.822	0.57	0.506	0.41
% of S.D	------	122	157	188	100	78	44

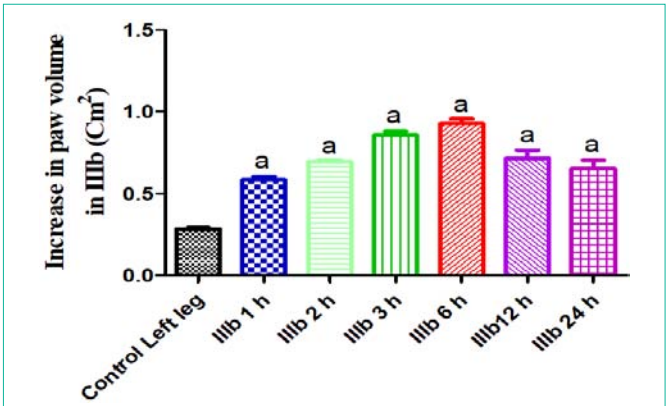

Figure 23: IIIb treated group.

Table 24: VIIc treated group.

Rat no	Left leg	1 h	2 h	3 h	6 h	12 h	24 h
1	0.26	0.65	0.7	0.88	0.63	0.5	0.3
2	0.27	0.6	0.7	0.81	0.62	0.55	0.49
3	0.24	0.61	0.66	0.77	0.63	0.54	0.34
4	0.25	0.7	0.78	0.9	0.81	0.6	0.44
5	0.28	0.5	0.76	0.86	0.76	0.55	0.4
6	0.27	0.6	0.8	0.88	0.63	0.6	0.46
Total	1.57	3.66	4.4	5.1	4.08	3.34	2.43
Mean	0.262	0.61	0.73	0.85	0.68	0.556	0.405
% of S.D	------	133	180	224	160	112	55

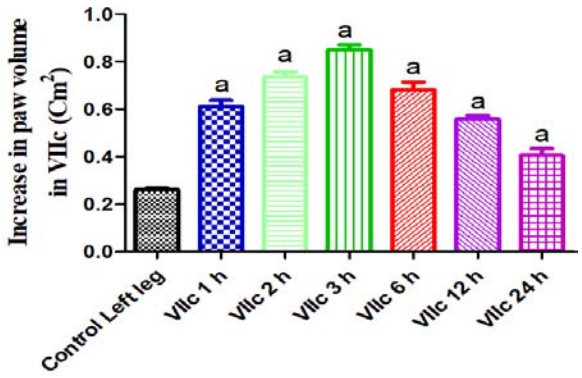

Figure 24: VIIc treated group.

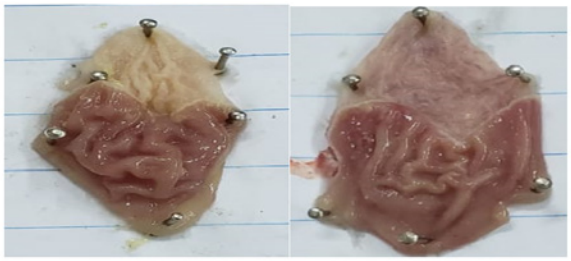

Figure 25: Normal stomach.

after 1, 2, 3, 6, 12 and 24 h, respectively, when compared to volume of left leg. While pretreatment with Celecoxib (orally) in a dose of 100mg/kg caused a significant reduction in right paw volume by (77%, 111%, 77%, 51%, 37% and 32%), after 1, 2, 3, 6, 12 and 24 h, respectively, when compared to volume of left leg. The compounds XIIIa, X, XIIIb, IX, VIII, Vc, VIIb, XVI, XV, XIVb and XIVa at a dose of (200mg/kg orally) shows no significant reduction in right hind paw volume after 1, 2, 3, 6, 12 and 24 h, respectively, when compared to volume of left leg .taken in consideration that this compounds had no activity when compared to three standards (diclofenac sodium, indomethacin and Celecoxib). The compounds Vb, and Va) at a dose of (200mg/kg orally) were failed to finish the experiment and most of animals were died and the remaining number not sufficient to carry out the statistics, taken in consideration that this compounds had no activity when compared to three standards (diclofenac sodium, indomethacin and Celecoxib). The use of tested compounds (XIVc and XIIIc) at (200mg/kg orally) had mild reduction in right paw volume when compared to standard treated groups after 1, 2, 3, 6, 12 and 24 h, respectively, The use of tested compound (IIIb) at (200mg/kg orally) had mild to moderate reduction in right paw volume after 1, 2, 3, 6, 12 and 24 h, respectively, when compared to standard treated groups. The use of tested compounds (VIIa, IIIc and VIIc) at (200mg/kg orally) had a remarkable promising reduction in right paw volume after 1, 2, 3, 6, 12 and 24 h, respectively, when compared to standard treated groups.

Discussion

The present study showed that, the selected dose (200 mg/kg) of tested compounds were evaluated for their in-vivo anti-inflammatory activity and compared to diclofenac sodium, indomethacin and Celecoxib as a references were measured before and 1, 2, 3, 6, 12, and 24 h, after carrageenan injection. Percent of the oedema inhibition was calculated as a regard to carrageenan control group and potency was calculated as a regard to the percentage of the change of the diclofenac sodium, indomethacin and Celecoxib and tested compounds, it was observed that the 200mg/kg dose of compounds (XIIIa, X, XIIIb, IX, VIII, Vc, VIIb, XVI, XV, XIVb and XIVa) had shown no anti-inflammatory activity at all-time points. The compounds (Vb and Va) at a dose of (200mg/kg orally) were failed to finish the experiment. It was observed that the 200mg/kg dose of compounds (XIVc and XIIIc) had shown mild anti-inflammatory activity at all-time points, while tested compounds, it was observed that the 200mg/kg dose of compound (IIIb) had shown mild to moderate anti-inflammatory activity at all-time points, it was observed that the 200mg/kg dose of compounds (VIIa, IIIc and VIIc) had shown highest anti-inflammatory activity at all-time points.

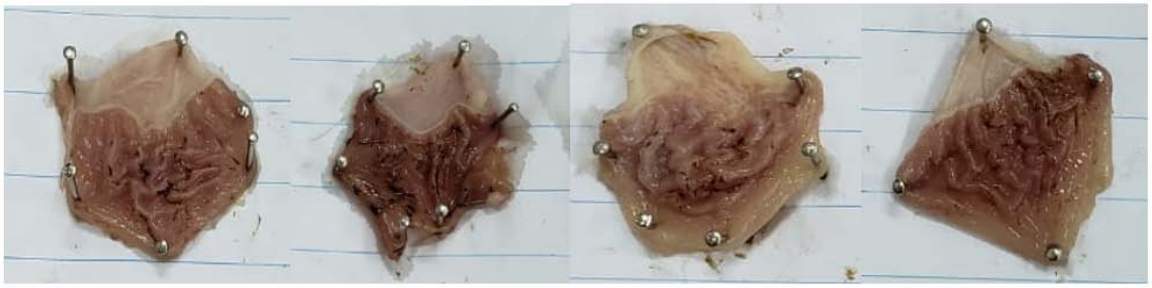

Figure 26: Indomethacin treated group.

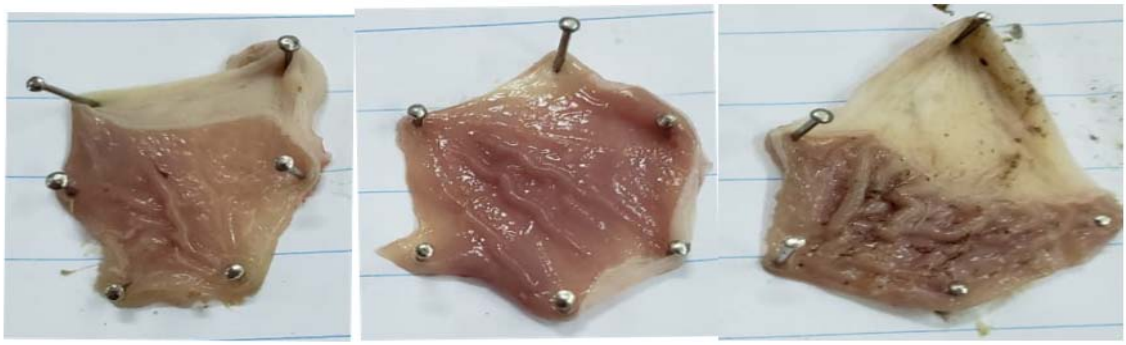

Figure 27: Compound VIIc.

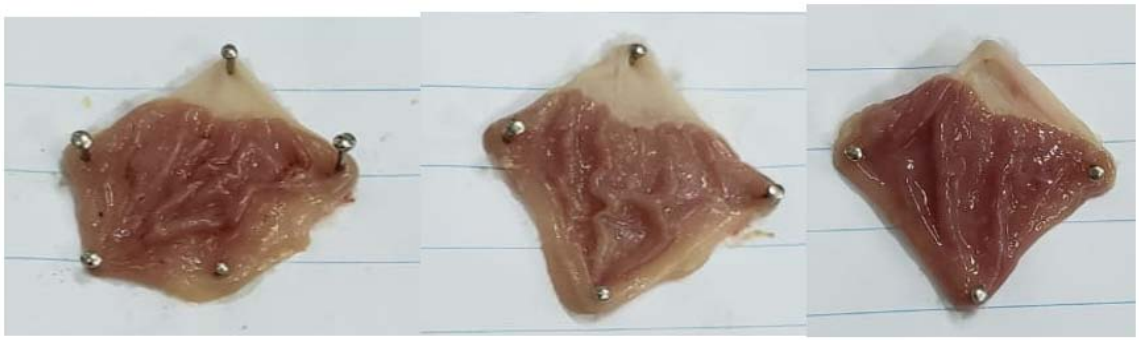

Figure 28: Compound VIII.

Figure 29: Compound XIVC.

Ulcer Index Experiment

Materials and methods

Drugs: Fresh solution of indomethacin 25 mg/kg was purchased from (El-Nile Co. for Pharmaceuticals and chemical industries, Cairo, Egypt), VIIc, VIII, IX, X, Va, Vb, XIVa, XIVb, XIVc, XIIIa and XIIIb the tested compounds were dissolved in DMSO while indomethacin in sterile water was prepared.

Animals: Seventy-eight female Sprague Dawly rats weighing between 150 ± 10 gm were used for the study. Prior to the experiments, the rats were kept in the animal house for one week for acclimatization in rat cages and were given standard rat feed with water ad libitum.

The animals were kept fasting for 24 hours before carrying out the experiments.

Experimental procedures

Rats were divided into 13 groups of 6 each:

Group I: Control (1ml/kg DMSO solvent orally).

Group II: Indomethacin (25mg/kg orally).

Group III: VIIc (600mg/kg orally in DMSO solvent).

Group IV: VIII (600mg/kg orally in DMSO solvent).

Group V: IX (600mg/kg orally in DMSO solvent).

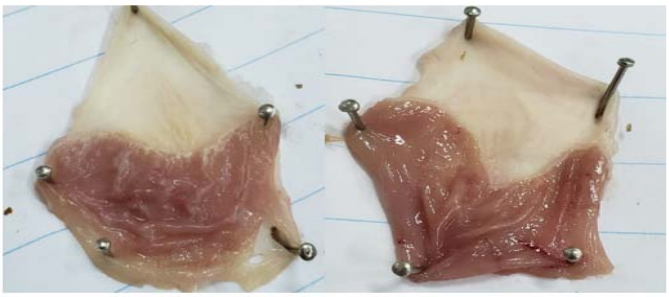

Figure 30: Compound XII.

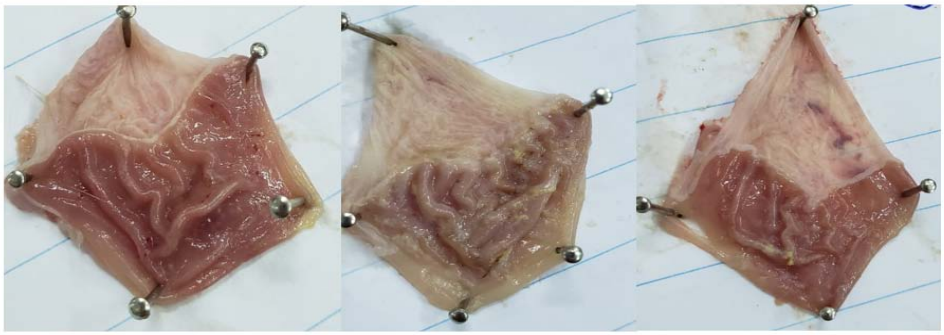

Figure 31: Compound XIVa.

Figure 32: Compound XIVb.

Group VI: X (600mg/kg orally in DMSO solvent).

Group VII: Va (600mg/kg orally in DMSO solvent).

Group VIII: Vb (600mg/kg orally in DMSO solvent).

Group IX: XIVa (600mg/kg orally in DMSO solvent).

Group X: XIVb (600mg/kg orally in DMSO solvent).

Group XI: XIVc, (600mg/kg orally in DMSO solvent).

Group XII: $XIII_a$ (600mg/kg orally in DMSO solvent).

Group XIII: $XIII_b$ (600mg/kg orally in DMSO solvent).

The rats were sacrificed by cervical dislocation 3 hours after indomethacin and tested compounds administration. The abdomen was dissected to retrieve stomach, analyzed for ulcer index. With small nick, fundus of stomach was perforated on greater curvature of stomach. The greater curvature of stomach was opened. Gastric mucosa was observed under magnifying glass to calculate the ulcer index.

Measurement of gastric lesions

Measurement of gastric ulcerations following their induction is done by first dissecting the stomach along its greater curvature and fixing on a board or transparent glass.

Examination can be carried out macroscopically with a hand lens and by tracing on a transparent paper after which the transparent paper is placed onto a graph sheet and sizes of ulcers are measured.

According to the method by Kulkarni, the ulcer index can be measured or registered using the following scores involving the number and severity of ulcers:

0.0 = normal colored stomach,

0.5 = red coloration,

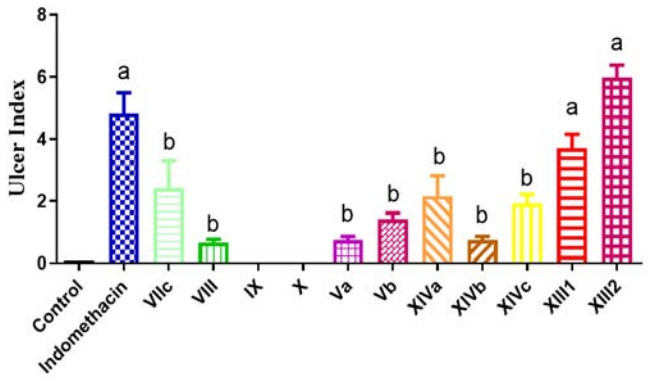

Figure 33: Effects of indomethacin and VIIc, VIII, IX, X_1, Va, Vb, XIVa, XIVb, XIVc, $XIII_1$ and $XIII_2$, on gastric mucosa of rats compared to control group. Data are expressed as means ± SEM of six rats per group. Indomethacin (25mg/kg), VIIc, VIII, IX, X_1, Va, Vb, XIVa, XIVb, XIVc, $XIII_1$ and $XIII_2$ (600mg/kg) were given orally single dose.
[a]Significantly different from control group.
[b]Significantly different from indomethacin-administered group using one-way analysis of variance (ANOVA) followed by Tukey-Kramer test for multiple comparison at P ≤0.05.

1.0 = spot ulcers,

1.5 = hemorrhagic streaks,

2.0 = ulcers with area >3 but ≤5mm^2,

$$\text{Ulcer Index (UI)} = \frac{\text{Total Ulcer Score}}{\text{No. of Animals Ulcerated}}$$

Statistical analysis

All the data are expressed as mean ± standard error of mean. One-way analysis of variance (ANOVA) test (SPSS Version no. 15) unpaired one tailed '*t*' test was also used considering P ≤0.05 to be statistically significant (Figure 29-36).

Effects of indomethacin and VIIc, VIII, IX, X, Va, Vb, XIVa, XIVb, XIVc, $XIII_a$ and $XIII_b$, on gastric mucosa of rats compared to control group

The results in Figure 29 illustrate that the effects of indomethacin and VIIc, VIII, IX, X1, Va, Vb, XIVa, XIVb, XIVc, XIII1 and XIII2, on incidence of ulcer in rats compared to control group.

Apparently, oral administration with indomethacin in a dose of 25 mg/kg body weight significantly increase the incidence of ulcer index (100%) compared to the control group. Similarly, oral administration with compounds VIIc, XIVa and XIVc significant decrease the incidence of ulcer index to 50% in comparison with indomethacin-administered group, while compounds, VIII, Va, Vb, and XIVb significant decrease the incidence of ulcer index to 75% in comparison with indomethacin-administered group. In addition, the compound XIIIa showed non-significant reduction in ulcer index in comparison with indomethacin-administered group, finally the compound XIIIb induced ulcer index greater than that occurs with indomethacin treated group. Unfortunately, the animals that treated with compounds IX and X were died during the carried out of the experiments (Figure 37).

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