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Research Article

EVALUATION OF ANTI-INFLAMMATORY ACTIVITY OF JOINTS KARE A POLYHERBAL FORMULATION - EXPERIMENTAL STUDY

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ABSTRACT

The Anti-inflammatory activity of JOINTS KARE, a poly herbal ayurvedic formulation developed and studied in the Jammu Institute of Ayurveda and Research Pharmacy with the technical assistance from Indian Institute of Integrative Medicine, the study was performed on albino rats of carrageenan induced model. 20 healthy albino rats were selected randomly and divided into five groups. The inflammatory reaction is readily produced in rats in the form of paw oedema with the help of irritants. Here the rats of treated Group II, III, IV and V were administered orally with Ibuprofen solution, Joints Kare 125mg/Kg Body wt, Joints Kare 250mg/Kg Body wt, Joints Kare 500mg/Kg Body wt, respectively one hour before injecting 1% w/v suspension of carrageenan (0.1 ml) into the sub plantar region of left hind paw of all the five groups.

Paw volume of all 20 rats were measured soon after injecting carrageenan. The volume was again measured after 1, 2, 3, 4 & 24 hrs in all the five groups of rats. The change in paw volume of Group I was compared with Group II, III, IV & V. Also the treated Group II, III, IV, V were also compared in between and expressed as percentage oedema inhibition by the drug. Results of the present study are based on the oedema of hind paw of rats of all five groups measured after 1hr, 2hrs, 3hrs, 4hrs and 24hrs after carrageenan injection. The maximum activity of all trial Groups were observed during first and second hr. and the results are significant (P <0.005) and are comparable to standard Ibuprofen. Highest percentage oedema Inhibition was seen after 1 & 2 hrs. Summarizing the above it is concluded that Joints Kare has showed its extreme utility or significance on the inflammation probably because of its excellent activity of inhibiting the both early released and late released mediators which is rarely seen in any anti-inflammatory formulation.

KEYWORDS: Joints Kare, Polyherbal Formulation, Anti-Inflammatory Activity.

INTRODUCTION

Inflammation is very normal effect in the body to rid the body of all its ill effects, our body creates inflammation under different situations, as soon as it has done its share of work, the body produces anti-inflammatory process and inflammation settles down. In normal healthy person this process is in perfect balance, however where there is an imbalance our body does not stop producing inflammation, instead inflammation will often simmer for long time and turning into a serious problems. The newer concept of the anti-inflammatory therapy originated with the discovery of salicylates from the plant source in the mid eighteenth century, when Reverend Edmound Stone, in England, accidentally tasted the bark of the common willow, Salix alba vulgaris. The active ingredient in this was a bitter glycoside Salicin which was first isolated in pure form by Leroux in 1829, who evaluated its antipyretic activity also later on plenty of Anti-inflammatory were discovered but having serious side effects.

There are millions of people around the world is affecting with this pathological condition and are totally dependent on multiple of anti-inflammatory drugs, which are abundantly available in modern medical science, but with maximum side effects and complications[1]. On an average 1 in 1200 patients taking NSAIDs for at least two months die from Gastrodudenal complications who would not have died had they not taken NSAIDs[2]. Similarly eight to ten percent of the 50,000 annual kidney failures in the United States are caused by Acetaminophen[3]. So

development of a new Ayurvedic anti-inflammatory agent is the present day need in the world which has gathered momentum from the last two decades because of the fact that currently available synthetic drugs show side effects as mentioned above.

MATERIALS AND METHODS

This Study was performed on albino rats of carrageenan induced model for evaluating its Anti-inflammatory activity[4], twenty healthy albino rats were selected randomly and divided into five groups for the experimental study to evaluate the anti-inflammatory activity of different doses of Joints Kare poly herbal formulation. Animals were divided into five groups each group containing four rats, which are kept in separate cages and labeled as

Group I - control, placebo given-saline water (1ml/100gm Body wt.),

Group II - Standard, Medicine given Ibuprofen Sol. (100mg/Kg Body wt.)

Group III – Trial, Joints Kare will be given. (125mg/Kg Body wt)

Group IV – Trial, Joints Kare will be given. (250mg/Kg Body wt)

Group V – Trial, Joints Kare will be given. (500mg/Kg Body wt)

The inflammatory reaction is readily produced in rats in the form of paw oedema with the help of irritant Carrageenan. Here the rats of treated Group II, III, IV, V are administered orally with drugs as mentioned above in the table before one hour injecting 1% w/v suspension of carrageenan (0.1 ml).

Paw volume of all rats were measured soon after injecting carrageenan. The volume was again measured after 1, 2, 3, 4 & 24 hrs in all the groups of rats. The change in paw volume of Group I was compared with Group II, III, IV & V Group. Also the treated Group II, III, IV, V were also compared in between and expressed as percentage oedema inhibition by the drug.

RESULTS AND DISCUSSION

The result of the present study are based on the oedma of hind paw of rats of all five groups measured after 1hr,2hrs,3hrs,4hrs and 24hrs after administration of Carrageenin Injection.

Table 1: Showing data of Anti-inflammatory activity after 1 hr of carrageenin Inj.

S. No	Group 1	Body Wt	Drug and Dose	Initial paw volume ml	Final paw volume ml	Oedema Vol. ml	Mean Oedema Vol. ml/SEM.	% Inhibition
1	Control	133gms	Saline water 1ml/100gm body Wt.	0.7	1.8	1.1	-	-
2	Control	150gms	Saline water 1ml/100gm body Wt.	0.8	1.8	1.0	-	-
3	Control	140gms	Saline water 1ml/100gm body Wt.	0.6	1.3	0.7	-	-
4	Control	133gms	Saline water 1ml/100gm body Wt.	0.8	1.6	0.8	0.9 ±0.0912	0%
	Group 2							
5	Standard	137gms	Ibuprofen100mg/ Kg Body Wt.	0.7	1.4	0.7	-	-
6	Standard	145gms	Ibuprofen100mg/ Kg Body Wt.	0.8	1.4	0.6	-	-
7	Standard	140gms	Ibuprofen100mg/ Kg Body Wt.	0.9	1.2	0.3	-	-
8	Standard	147gms	Ibuprofen100mg/ Kg Body Wt.	1.0	1.5	0.5	0.52±0.0853	42.22 %
	Group 3							
9	Trial (A)	130gm	JOINTS KARE 125mg/Kg Body Wt.	0.8	1.3	0.5	-	-
10	Trial (A)	135gm	JOINTS KARE 125mg/Kg Body Wt.	0.7	1.3	0.6	-	-

11	Trial (A)	148gm	JOINTS KARE 125mg/Kg Body Wt.	0.9	1.4	0.5	-	-
12	Trial (A)	130gm	JOINTS KARE 125mg/Kg Body Wt.	0.9	1.4	0.5	0.52±0.0250	42.2%
	Group 4							
13	Trial (B)	135gm	JOINTS KARE 250mg/Kg Body Wt	0.8	1.4	0.6	-	-
14	Trial (B)	140gm	JOINTS KARE 250mg/Kg Body Wt.	0.8	1.1	0.3	-	-
15	Trial (B)	138gm	JOINTS KARE 250mg/Kg Body Wt.	0.9	1.4	0.5	-	-
16	Trial (B)	140gm	JOINTS KARE 250mg/Kg Body Wt.	0.9	1.6	0.7	0.52±0.0853	42.22%
	Group 5							
17	Trial (C)	140gms	JOINTS KARE 500mg/Kg Body Wt.	0.9	1.5	0.6	-	-
18	Trial (C)	140gms	JOINTS KARE 500mg/Kg Body Wt.	0.7	1.2	0.5	-	-
19	Trial (C)	139gms	JOINTS KARE 500mg/Kg Body Wt.	0.7	1.1	0.4	-	-
20	Trial (C)	136gms	JOINTS KARE 500mg/Kg Body Wt.	0.7	1.0	0.3	0.45±0.0645	50.0%

Table 2: Showing Summary of Data (after 1hr)

Group	N	Mean (Oedema vol. ml)	Std Dev	SEM
1	4	0.9	0.1826	0.09129
2	4	0.525	0.1708	0.08539
3	4	0.525	0.05	0.025
4	4	0.525	0.1708	0.08539
5	4	0.45	0.1291	0.06455

Table 3: Showing Intermediate calculation of Anova table (after 1 hr)

Source of Variation	SS	DF	Variance Est (MS)
Between Groups	0.513	4	0.1282
Within Groups	0.3325	15	0.02217
Total	0.8455	19	

$$F = \frac{s2\_bet}{s2\_wit} = \frac{MSbet}{MSwit} = \frac{0.1282}{0.02217} = 5.789 \qquad P = 0.005$$

Table 4: Bonferroni : Showing comparison of each group with Control - 1st group. (after 1 hr)

Comparison	Difference of means	t	P<.05
5 vs 1:	0.45 - 0.9 = -0.45	4.274	Yes
3 vs 1:	0.525 - 0.9 = -0.375	3.562	Yes
2 vs 1:	0.525 - 0.9 = -0.375	3.562	Yes
4 vs 1:	0.525 - 0.9 = -0.375	3.562	Yes

Degrees of freedom: 15

It is evident from Table No 1 & Graph A, that the volume(ml) of Oedema in Group1 (Control) is 0.9± 0.0912, Group 2 (Standard) 0.52±0.0853, Group 3 (Joints Kare125mg/Kg) 0.52±0.0250, Group 4 (Joints Kare 250mg/Kg) 0.52±0.0853 and Group 5 (Joints Kare 500mg/Kg) 0.45±0.0645. The results are statistically significant by ANOVA test. When compared with control group all treatment groups showed more significant by multiple comparisons as mentioned above in Table – 4.

Table 5: Showing data of Anti-inflammatory activity after 2 hrs of carrageenin Inj.

S. No	Group 1	Body Wt	Drug and Dose	Initial paw volume ml	Final paw volume ml	Oedema Vol. ml	Mean Oedema Vol. ml/SEM.	% Inhibition
1	Control	133gms	Saline water 1ml/100gm body Wt.	0.7	2.0	1.3	-	-
2	Control	150gms	Saline water 1ml/100gm body Wt.	0.8	2.2	1.4	-	-
3	Control	140gms	Saline water 1ml/100gm body Wt.	0.6	1.7	1.1	-	-
4	Control	133gms	Saline water 1ml/100gm body Wt.	0.8	2.1	1.3	1.27±0.0629	0%
	Group 2							
5	Standard	137gms	Ibuprofen100mg/Kg Body Wt.	0.7	1.6	0.9	-	-
6	Standard	145gms	Ibuprofen100mg/Kg Body Wt.	0.8	1.6	0.8	-	-
7	Standard	140gms	Ibuprofen100mg/Kg Body Wt.	0.9	1.3	0.4	-	-
8	Standard	147gms	Ibuprofen100mg/Kg Body Wt.	1.0	1.6	0.6	0.67±0.0750	47.24%
	Group 3							
9	Trial (A)	130gm	J.K 125mg/Kg Body Wt	0.8	1.9	1.1	-	-
10	Trial (A)	135gm	J.K. 125mg/Kg Body Wt	0.8	1.3	0.5	-	-
11	Trial (A)	148gm	J.K. 125mg/Kg Body Wt	0.9	1.8	0.9	-	-
12	Trial (A)	130gm	J.K. 125mg/Kg Body Wt	0.9	2.2	1.3	0.95±0.1708	27.19%
	Group 4							
13	Trial (B)	135gm	J.K 250mg/Kg Body Wt	0.8	1.8	1.0	-	-
14	Trial (B)	140gm	J.K 250mg/Kg Body Wt	0.7	1.5	0.8	-	-
15	Trial (B)	138gm	J.K 250mg/Kg Body Wt	0.9	1.9	1.0	-	-
16	Trial (B)	140gm	J.K 250mg/Kg Body Wt	0.9	1.6	0.7	0.875±0.075	31.49%
	Group 5							
17	Trial (C)	140gms	J.K 500mg/Kg Body Wt	0.9	1.6	0.7	-	-
18	Trial (C)	140gms	J.K 500mg/Kg Body Wt	0.7	1.4	0.7	-	-
19	Trial (C)	139gms	J.K 500mg/Kg Body Wt	0.7	1.1	0.4	-	-
20	Trial (C)	136gms	J.K 500mg/Kg Body Wt	0.7	1.1	0.4	0.55±0.0866	56.69%

It is evident from Table No 5 and Graph C That the volume (ml) of Oedema in Group1 (Control) is 1.27±0.0629, Group2 (Standard) 0.67±0.0750, Group 3 (J.K. 125mg/Kg) 0.95±0.1708, Group 4 (J.K. 250mg/Kg) 0.875±0.075 and Group 5 (J.K. 500mg/Kg) 0.55±0.0866. The results are statistically significant by ANOVA test. When compared with control group, Group 5 (J.K.500mg/Kg) and Group 2 (Standard) showed more significant by multiple comparisons.

Table 6: Showing data of Anti-inflammatory activity after 3 hrs of carrageenin Inj.

S. No	Group 1	Body Wt	Drug and Dose	Initial paw volume ml	Final paw volume ml	Oedema Vol. ml	Mean Oedema Vol. ml/SEM.	% Inhibition
1	Control	133gms	Saline water 1ml/100gm body Wt.	0.7	2.1	1.4	-	-
2	Control	150gms	Saline water 1ml/100gm body Wt.	0.8	2.3	1.5	-	-
3	Control	140gms	Saline water 1ml/100gm body Wt.	0.6	2.0	1.4	-	-
4	Control	133gms	Saline water 1ml/100gm body Wt.	0.8	2.1	1.3	1.4±0.0408	O%
	Group 2							
5	Standard	137gms	Ibuprofen100mg/Kg Body Wt.	0.7	1.8	1.1	-	-
6	Standard	145gms	Ibuprofen100mg/Kg Body Wt.	0.8	1.9	1.1	-	-
7	Standard	140gms	Ibuprofen100mg/Kg Body Wt.	0.9	1.3	0.4	-	-
8	Standard	147gms	Ibuprofen100mg/Kg Body Wt.	1.0	1.8	0.8	0.85±0.1658	39.28%
	Group 3							
9	Trial (A)	130gm	J.K 125mg/Kg Body Wt	0.8	2.0	1.2	-	-
10	Trial (A)	135gm	J.K 125mg/Kg Body Wt	0.7	1.8	1.1	-	-
11	Trial (A)	148gm	J.K 125mg/Kg Body Wt	0.9	2.1	1.2	-	-
12	Trial (A)	130gm	J.K 125mg/Kg Body Wt	0.9	1.9	1.0	1.12±0.0478	20%
	Group 4							
13	Trial (B)	135gm	J.K 250mg/Kg Body Wt	0.8	2.0	1.2	-	-
14	Trial (B)	140gm	J.K 250mg/Kg Body Wt	0.8	1.6	0.8	-	-
15	Trial (B)	138gm	J.K 250mg/Kg Body Wt	0.9	2.0	1.1	-	-
16	Trial (B)	140gm	J.K 250mg/Kg Body Wt	0.9	2.2	1.3	1.1±0.1080	21.42%
	Group 5							
17	Trial (C)	140gms	J.K 500mg/Kg Body Wt	0.9	1.9	1.0	-	-
18	Trial (C)	140gms	J.K 500mg/Kg Body Wt	0.7	1.7	1.0	-	-
19	Trial (C)	139gms	J.K 500mg/Kg Body Wt	0.7	1.4	0.7	-	-
20	Trial (C)	136gms	J.K 500mg/Kg Body Wt	0.7	1.3	0.6	0.82±0.1031	41.42%

It is evident from Table No 6 and Fig E That the volume(ml) of Oedema in Group1 (Control) is 1.4±0.0408, Group2 (Standard) 0.85±0.1658, Group 3(J.K.125mg/Kg) 1.12±0.0478, Group 4 (J.K. 250mg/Kg) 1.1±0.1080 and Group 5 (J.K. 500mg/Kg) 0.82±0.1031. The results are statistically significant by ANOVA test. When compared with control group, Group 5 (J.K.500mg/Kg) and Group 2 (Standard) showed more significant by multiple comparisons.

Table 7: Showing data of Anti-inflammatory activity after 4 hrs of carrageenin Inj.

S. No	Group 1	Body Wt	Drug and Dose	Initial paw volume ml	Final paw volume ml	Oedema Vol. ml	MeanOedema Vol. ml/SEM.	% Inhibition
1	Control	133gms	Saline water 1ml/100gm body Wt.	0.7	2.0	1.3	-	-
2	Control	150gms	Saline water 1ml/100gm body Wt.	0.8	2.2	1.4	-	-

3	Control	140gms	Saline water 1ml/100gm body Wt.	0.6	1.9	1.3	-	-
4	Control	133gms	Saline water 1ml/100gm body Wt.	0.8	2.0	1.2	1.3±0.04083	0%
	Group 2							
5	Standard	137gms	Ibuprofen100mg/Kg Body Wt.	0.7	1.8	1.1	-	-
6	Standard	145gms	Ibuprofen100mg/Kg Body Wt.	1.0	1.9	0.9	-	-
7	Standard	140gms	Ibuprofen100mg/Kg Body Wt.	0.9	1.2	0.3	-	-
8	Standard	147gms	Ibuprofen100mg/Kg Body Wt.	1.0	1.9	0.9	0.8± 0.1732	38.46%
	Group 3							
9	Trial (A)	130gm	J.K. 125mg/Kg Body Wt	0.8	1.8	1.0	-	-
10	Trial (A)	135gm	J.K. 125mg/Kg Body Wt	0.8	1.5	0.7	-	-
11	Trial (A)	148gm	J.K. 125mg/Kg Body Wt	0.9	1.9	1.0	-	-
12	Trial (A)	130gm	J.K. 125mg/Kg Body Wt	0.9	2.1	1.2	0.97±0.1031	25.38%
	Group 4							
13	Trial (B)	135gm	J.K. 250mg/Kg Body Wt	0.8	1.8	1.0	-	-
14	Trial (B)	140gm	J.K. 250mg/Kg Body Wt	0.7	1.7	1.0	-	-
15	Trial (B)	138gm	J.K. 250mg/Kg Body Wt	0.9	1.8	0.9	-	-
16	Trial (B)	140gm	J.K. 250mg/Kg Body Wt	0.9	1.7	0.8	0.92±0.0478	29.22%
	Group 5							
17	Trial (C)	140gms	J.K. 500mg/Kg Body Wt	0.9	1.7	0.8	-	-
18	Trial (C)	140gms	J.K. 500mg/Kg Body Wt	0.7	1.8	1.1	-	-
19	Trial (C)	139gms	J.K. 500mg/Kg Body Wt	0.7	1.4	0.7	-	-
20	Trial (C)	136gms	J.K. 500mg/Kg Body Wt	0.7	1.2	0.5	0.77±0.1250	40.76%

It is evident from Table No 7 and Graph G That the volume (ml) of Oedema in Group1(Control) is 1.3±0.04083, Group2 (Standard) 0.8±0.1732, Group 3 (J.K.125mg/Kg) 0.97±0.1031, Group 4 (J.K.250mg/Kg) 0.92±0.0478 and Group 5 (J.K. 500mg/Kg) 0.77±0.1250. The results are statistically significant by ANOVA test. When compared with control group, Group 5 (J.K. 500mg/Kg) and Group 2 (Standard) showed more significant by multiple comparisons.

Table 8: Showing data of Anti-inflammatory activity after 24 hrs of carrageenin Injection

Sr. No	Group 1	Body Wt	Drug and Dose	Initial paw volume ml	Final paw volume ml	Oedema Vol. ml	Mean Oedema Vol. ml/SEM.	% Inhibition
1	Control	133gms	Saline water 1ml/100gm body Wt.	0.7	1.3	0.6		
2	Control	150gms	Saline water 1ml/100gm body Wt.	0.9	1.6	0.7		
3	Control	140gms	Saline water 1ml/100gm body Wt.	0.7	1.3	0.6		
4	Control	133gms	Saline water 1ml/100gm body Wt.	0.7	1.3	0.6	0.62±0.0250	0%
	Group 2							
5	Standard	137gms	Ibuprofen100mg/Kg	0.9	1.4	0.5		

			Body Wt.					
6	Standard	145gms	Ibuprofen100mg/Kg Body Wt.	1.0	1.6	0.6		
7	Standard	140gms	Ibuprofen100mg/Kg Body Wt.	0.7	1.2	0.5		
8	Standard	147gms	Ibuprofen100mg/Kg Body Wt.	0.9	1.7	0.8	0.6±0.0707	3.22%
	Group 3							
9	Trial (A)	130gm	J.K. 125mg/Kg Body Wt	0.8	1.2	0.4		
10	Trial (A)	135gm	J.K. 125mg/Kg Body Wt	0.7	1.2	0.5		
11	Trial (A)	148gm	J.K. 125mg/Kg Body Wt	0.8	1.5	0.7		
12	Trial (A)	130gm	J.K. 125mg/Kg Body Wt	0.8	1.6	0.8	0.6±0.0912	3.22%
	Group 4							
13	Trial (B)	135gm	J.K. 250mg/Kg Body Wt	0.7	1.3	0.6		
14	Trial (B)	140gm	J.K. 250mg/Kg Body Wt	0.7	1.2	0.5		
15	Trial (B)	138gm	J.K. 250mg/Kg Body Wt	0.8	1.3	0.5		
16	Trial (B)	140gm	J.K. 250mg/Kg Body Wt	0.7	1.1	0.4	0.5±0.0408	19.35%
	Group 5							
17	Trial (C)	140gms	J.K. 500mg/Kg Body Wt	0.9	1.4	0.5		
18	Trial (C)	140gms	J.K. 500mg/Kg Body Wt	0.8	1.4	0.6		
19	Trial (C)	139gms	J.K. 500mg/Kg Body Wt	0.8	1.2	0.4		
20	Trial (C)	136gms	J.K. 500mg/Kg Body Wt	0.8	1.1	0.3	0.45±0.0645	27.41%

It is evident from Table No 7 and Graph I That the volume(ml) of Oedema in Group1 (Control) is 0.62±0.0250, Group2 (Standard) 0.6±0.0707, Group 3 (J.K.125mg/Kg) 0.6±0.0912, Group 4 (J.K.250mg/Kg 0.5±0.0408 and Group 5 (J.K. 500mg/Kg) 0.45±0.0645 and the results are statistically insignificant by ANOVA test.

DISCUSSION

If we compare all the three doses of trial drug (Joints Kare), we conclude that J.K. 500mg/kg Body wt has more activity in all 1,2,3,4 and 24 hrs after carrageenin injection, even it shows higher effectively than the Standard (Ibuprofen).

If we compare the J.K. 500mg/kg with Standard (Ibuprofen) as graph L we find that pattern of effectively after 1,2,3,4 and 24 hours of above mentioned both groups are almost same which indicates that there may be a resemblance in mode of action in these two groups as Ibuprofen which is believed to work through inhibition of cyclooxygenase (COX), thus inhibiting prostaglandin synthesis. There are at least 2 variants of cyclooxygenase (COX-1 and COX-2). Ibuprofen inhibits both COX-1 and COX-2. It appears that it's analgesic, antipyretic, and anti-inflammatory activities are achieved principally through COX-1 inhibition; whereas COX-2 inhibition is responsible for its unwanted effects on platelet aggregation and the GI mucosa.

CONCLUSION

Summarizing the above it is concluded that Joints Kare has showed its extreme utility or significance on the inflammation probably because of its excellent activity of inhibiting the both early released and late released mediators which is rarely seen in any anti-inflammatory formulation. The present study revealed that the Joints Kare showed better anti-inflammatory activity. The maximum activity was observed during first and second hr. and the results are significant and are comparable to standard Ibuprofen. The anti-inflammatory activity may be due to the inhibition of release of histamine, serotonin and kinins in first hour after the injection of carrageenan, and this also retarded the release of prostaglandin like late phase mediators showing anti-inflammatory potential of Joints Kare.

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Experimental Study

Fig.23 - Different Concentrations

Fig.24 - Weighing of Rat

Fig.25- Oral Administration of Standard Drug (Ibuprofen)

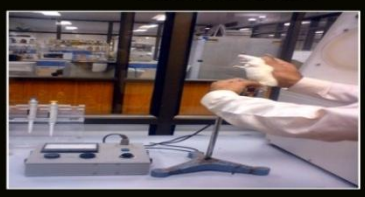
Fig.26 -Oral Administration of Trial Drug

Fig.27 - Grouping of Animals

Experimental Study

Fig.28 - Injecting Carrageenan to Hind Paw of Rat

Fig.29 - Hind Paw of Rat after Inducing Inflammation



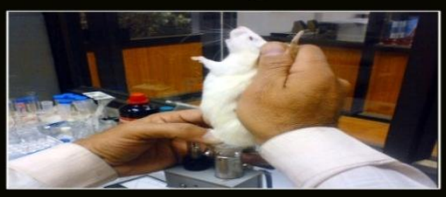
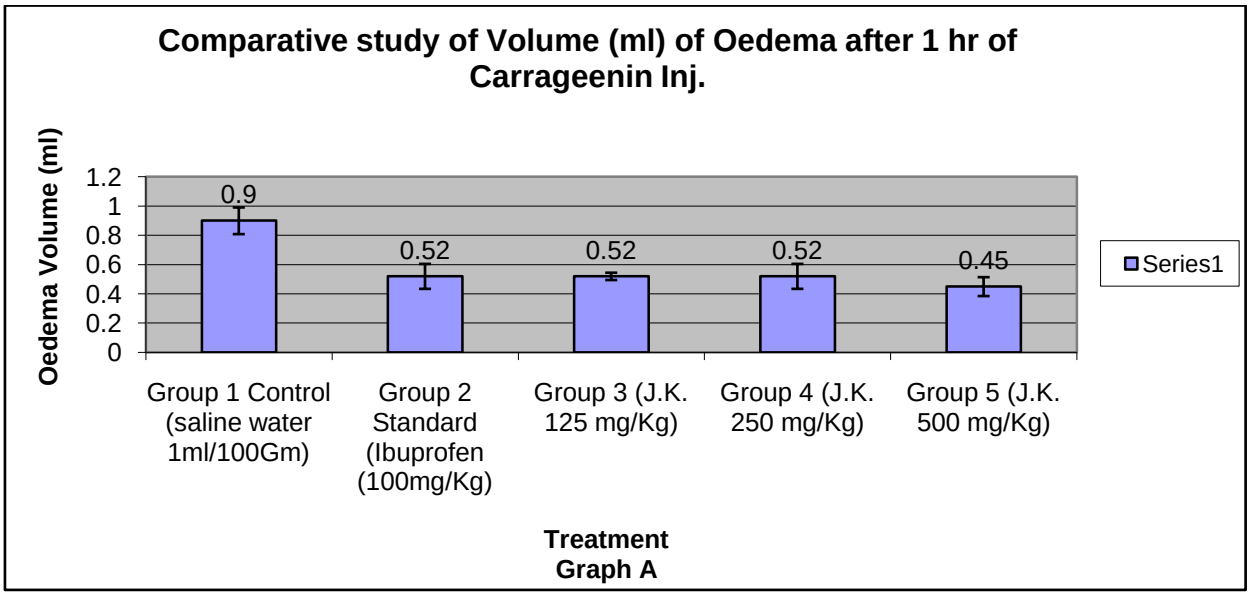
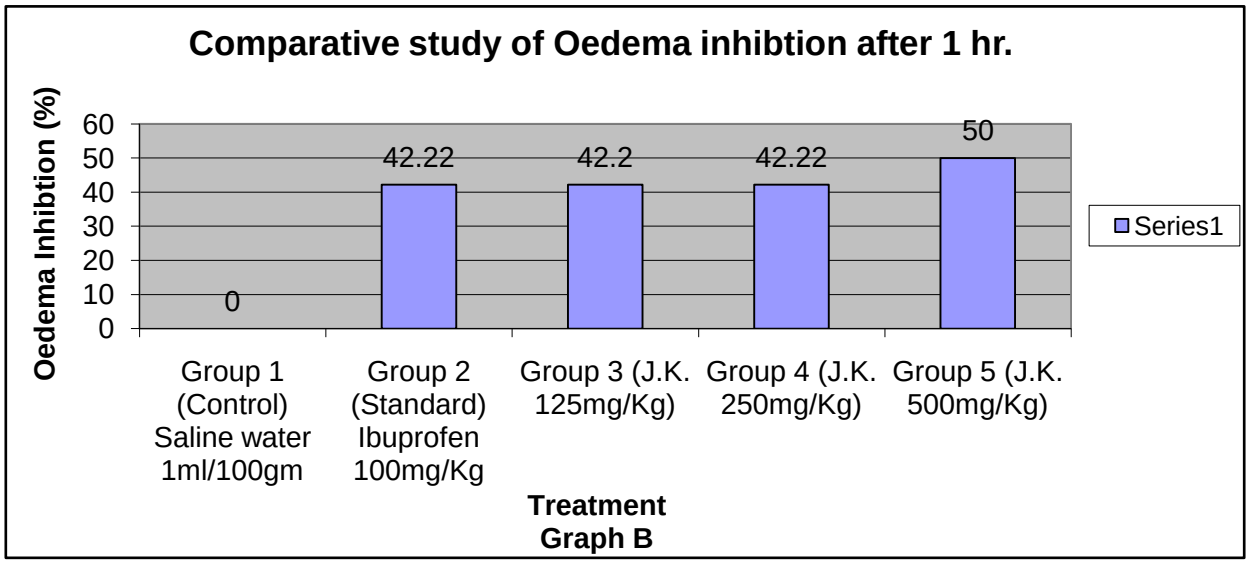


Fig.30,31- Measurment of Inflammation using Volume Differential Meter

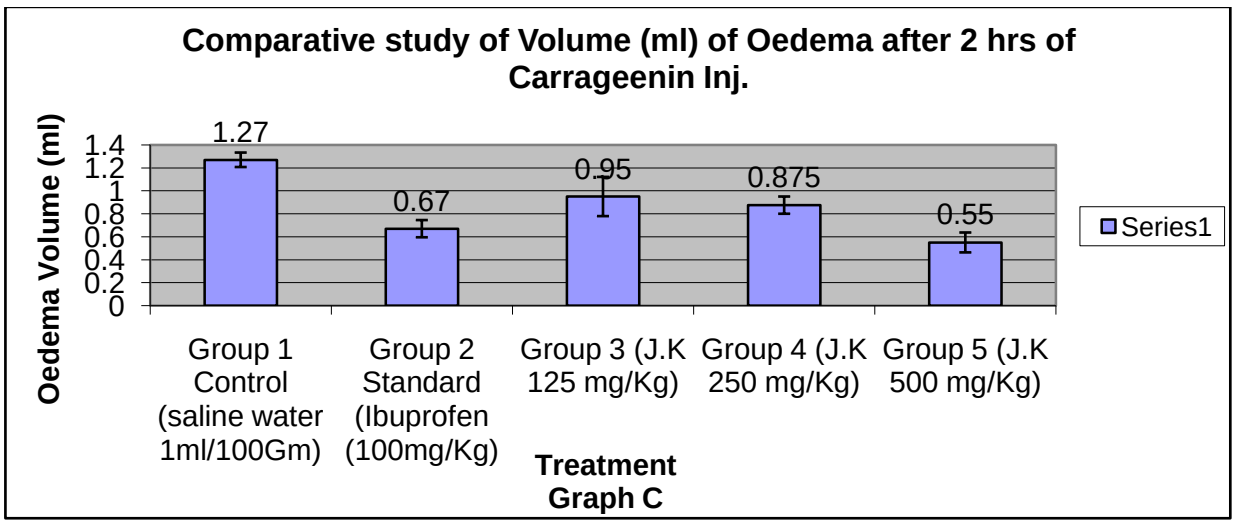
Fig.32 - Volume Differential Meter
(Model No- 7140 Ugo Basile,Italy)



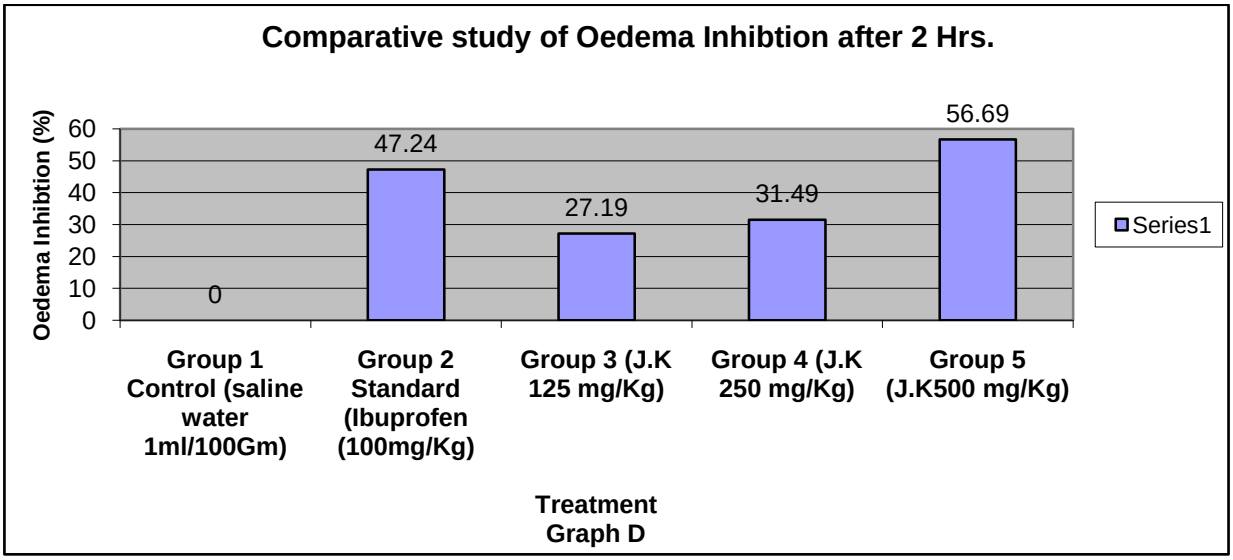
Graph A



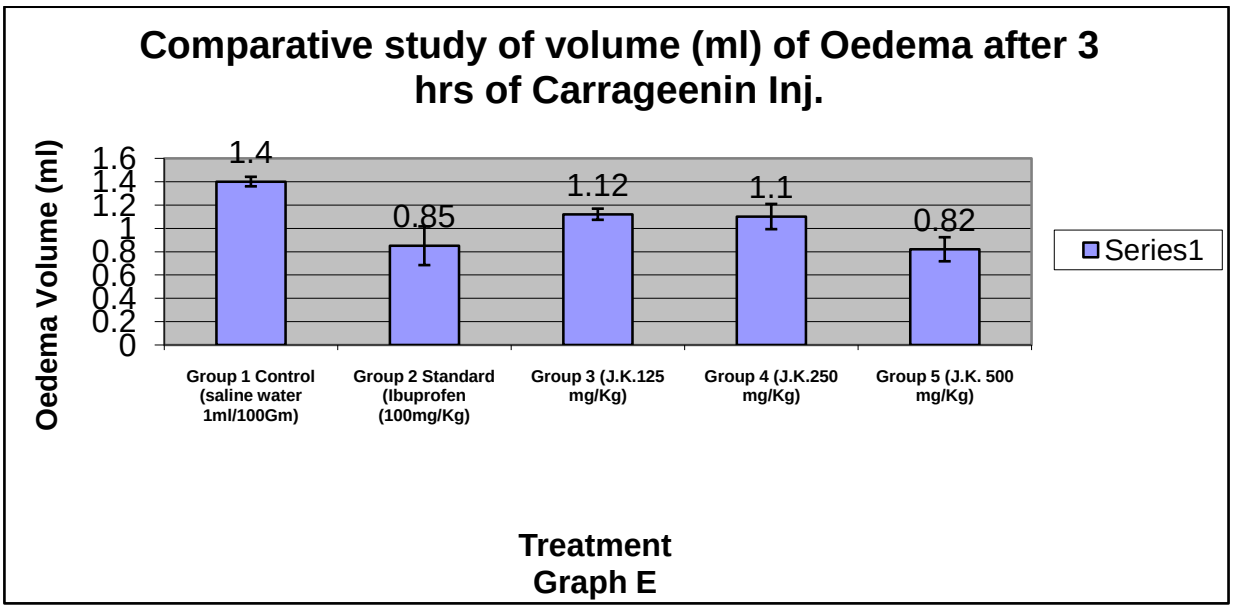
Graph B



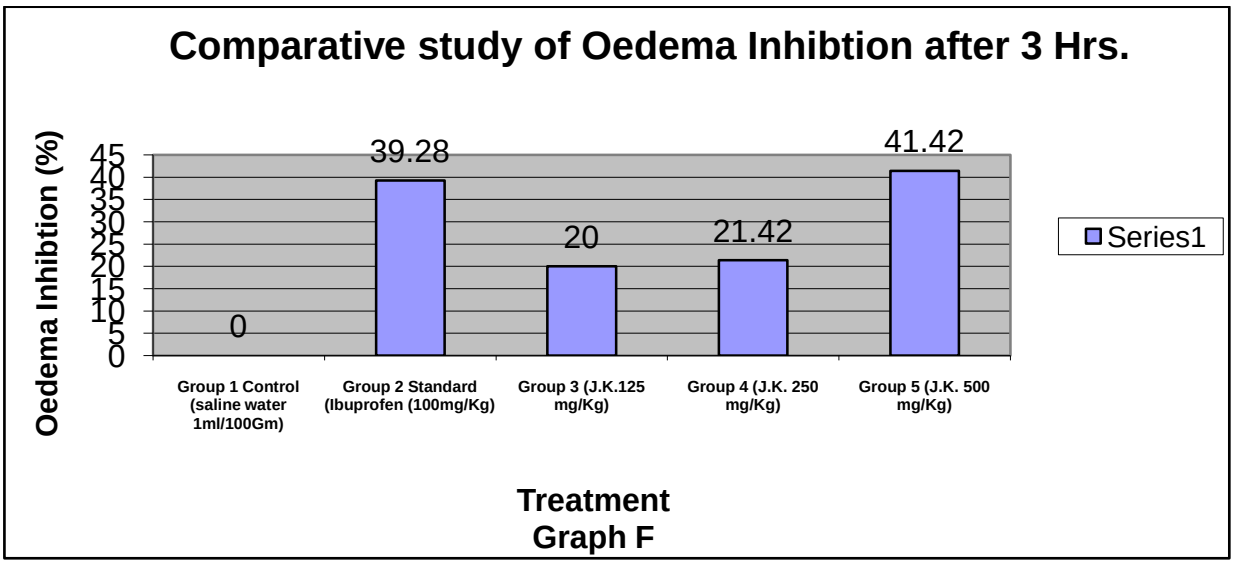
Graph C



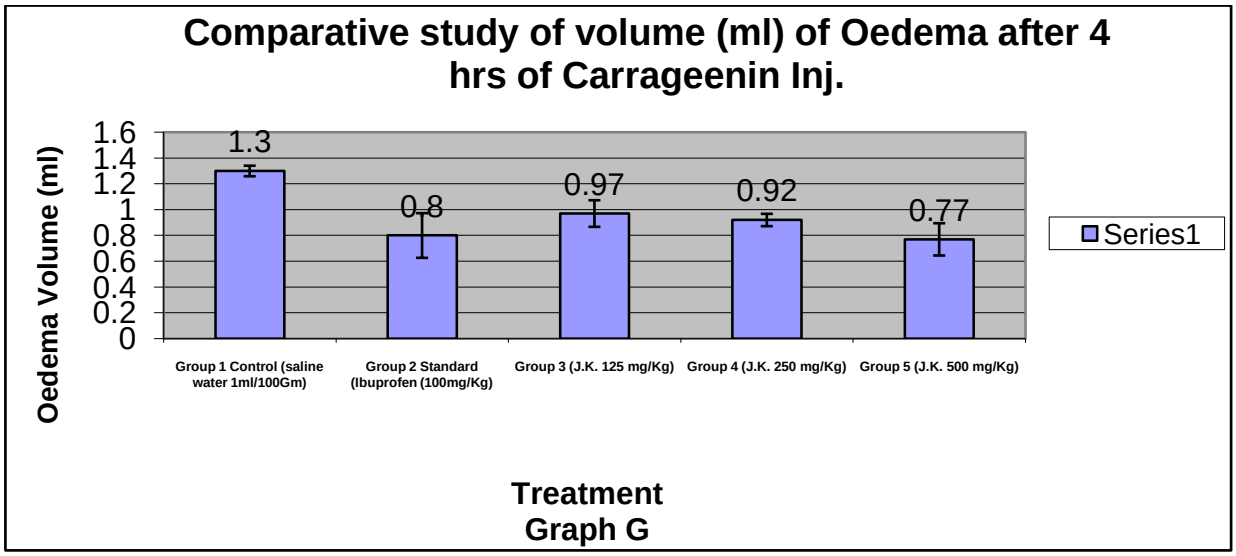
Graph D



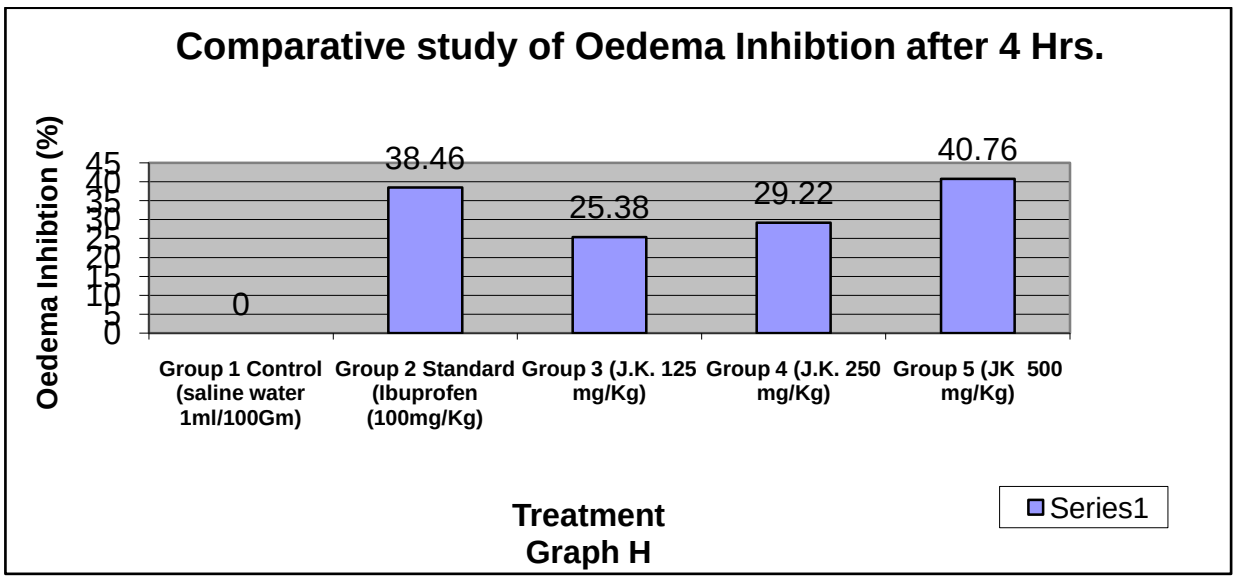
Graph E



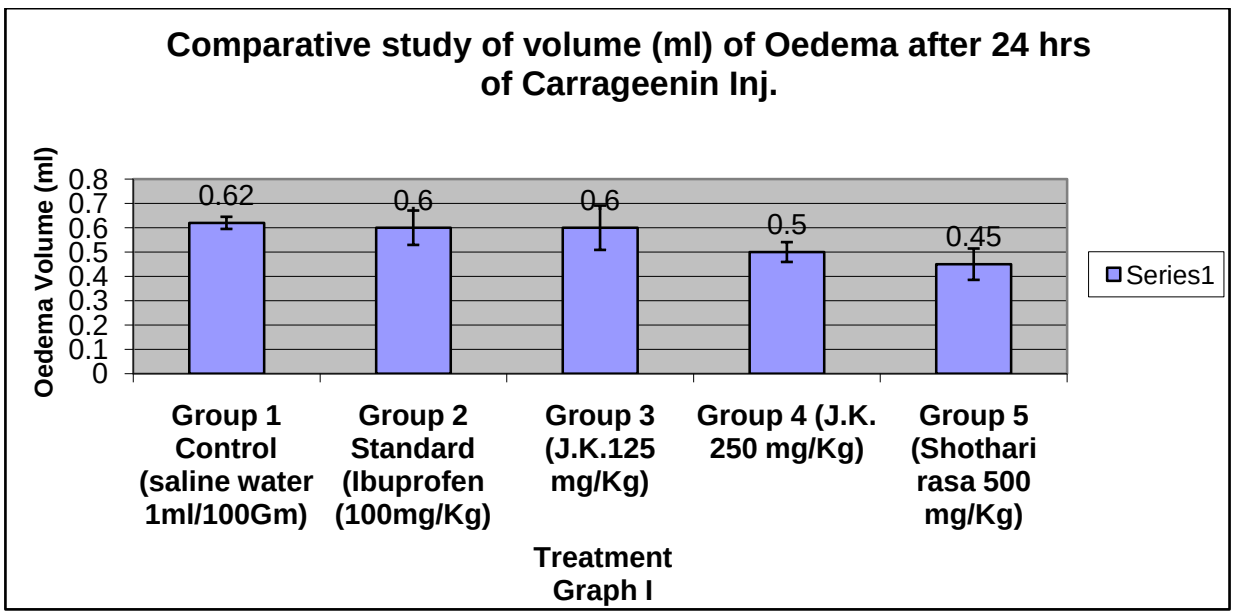
Graph F



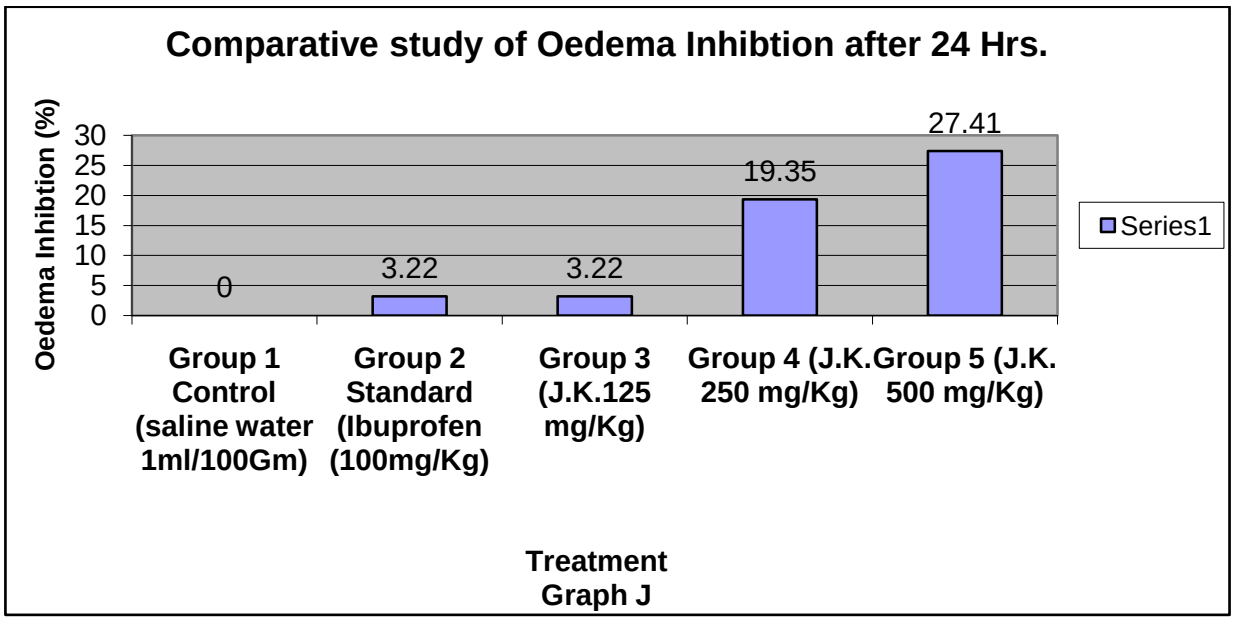
Graph G



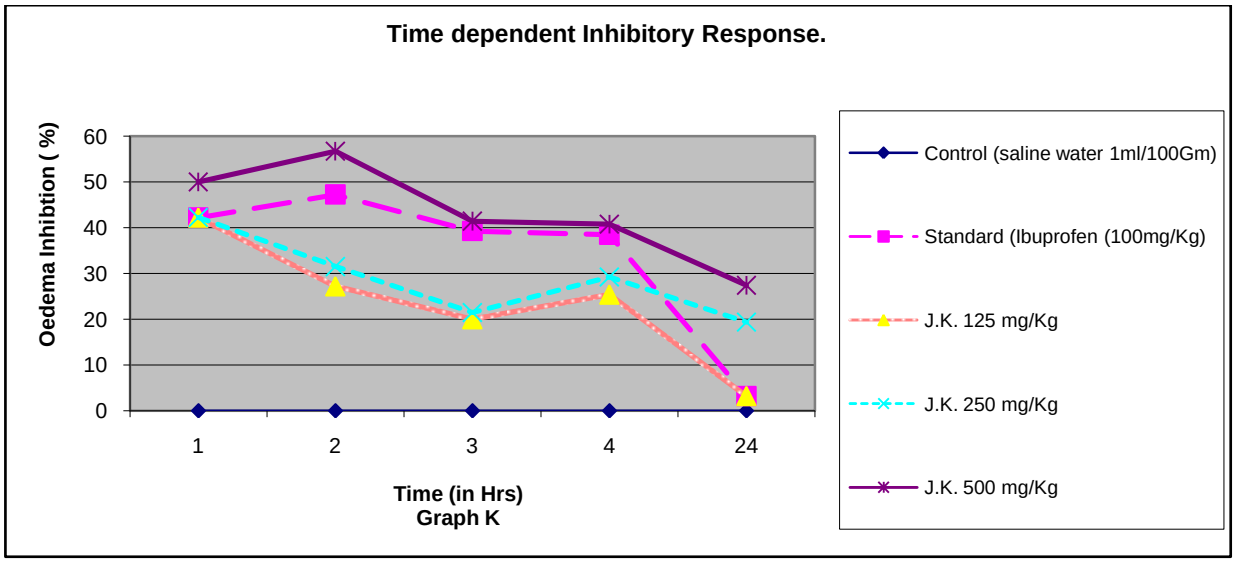
Graph H



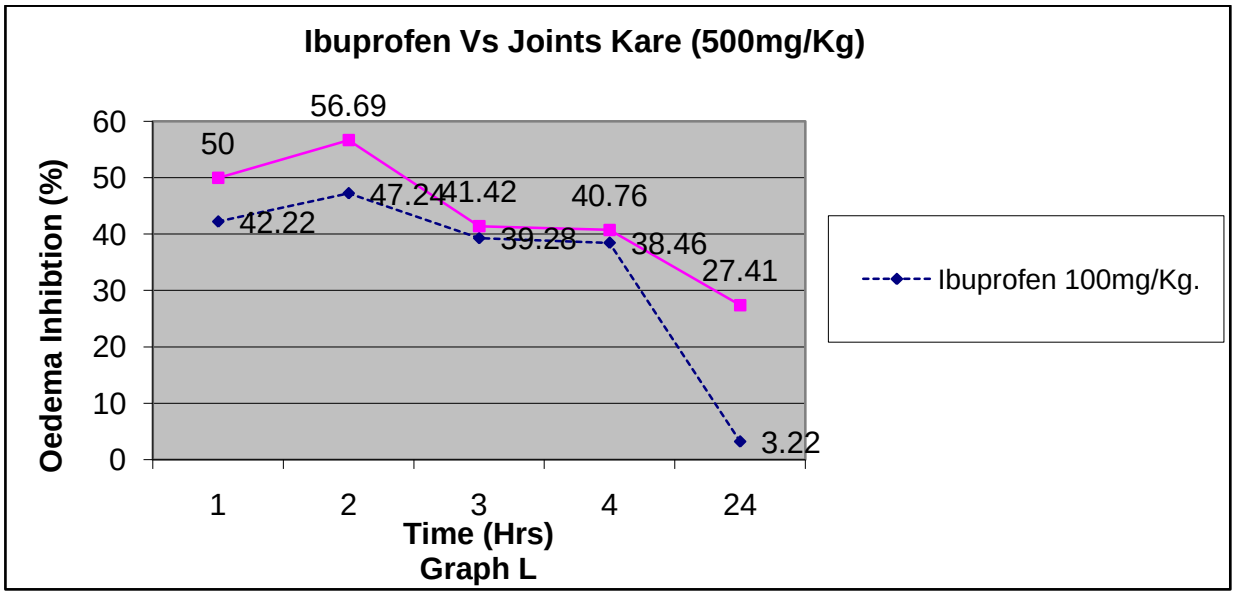
Graph I



Graph J



Graph K



Graph L