4. RESULTS

4.1 Biochemical-

Cyclooxygenase inhibition assays (*in vitro)*

4.1.1 *In vitro* Assay(COX-1 and COX-2 Inhibition Assays)

(ALL MCR series synthetic compounds, Semi synthetic compound BCOV and All herbal extracts are incorporated in this study)

Amongst all the compounds, methoxy(-OMe) substituted compounds MCR-207, MCR-292, MCR-320, MCR-322, MCR-363 showed COX-2 enzyme inhibition higher than that shown by compounds with other substitutes. 3,4-Di(4-methoxyphenyl)-1,2,5-oxadiazole *N*-oxide (MCR-363) showed COX-2 enzyme inhibition of 100% at 22 µM and COX-1 enzyme inhibition of 18% at 156 µM concentration. However, its deoxygenated analog MCR-207 showed lower COX-2 enzyme inhibition (98% at 22 µM) and higher COX-1 enzyme inhibition (84% at 88 µM). This preliminary study suggests that the methoxy (-OMe) group at 4-position of one of the phenyl rings may be a suitable pharmacophore for COX-2 enzyme binding in this series of compounds. Replacement of one of the -OMe groups of compound MCR-207 by an electron withdrawing -NO2 group resulted in increase COX-2 enzyme affinity. Compounds MCR-162, MCR-189, MCR-302, MCR-290 and MCR-345 with the well known COX-2 enzyme pharmacophore (methylsulfonyl, -SO2Me) failed to show COX-2 enzyme inhibition at a 22 µM concentration. Compound MCR-363 was found to be the most active compound in the series. The organometallic compound BCOV showed slightly better activity than Curcumin, its gave 82 % COX-2 enzymatic inhibition at 22 µM for BCOV and 64% 22 µM inhibition for Curcumin. BCOV exhibited COX-2 selective inhibition, its IC_{50} for COX-2 and COX-1 inhibition were 21.23 and 71.93 respectively. This results suggests that BCOV showed some COX-2 selective inhibition with selectivity ratio 3.38. MCR 363, MCR 364, MCR 207 demonstrated COX-2 inhibition activity with IC_{50} value 0.47, 13.58 and 3.46 µM respectively and COX-1 IC_{50} value were 310.7, 238.49 and 12.3 µM respectively. MCR 363, MCR 364, MCR 207 demonstrated COX-2 selectivity and there COX-1/COX-2 ratio were 658, 17.56 and 3.55 µM respectively. (Table10, 12, figures-21, 22)

Amongst the herbal drugs BNB and PME have shown COX-2 inhibition activity with IC_{50} values 32µg/ml and 98.9 µg/ml respectively, IC_{50} value for COX-1 inhibition were 64.4 and 147.2 µg/ml respectively. The calculated COX-1/COX-2 ratio for BNB and PME were 2.04 and 1.48 respectively (Table10,11,12)

Out of 93 synthetic compounds only 32 compounds have shown some good COX-2 over COX-1 inhibition. Semi synthetic compound BCOV has also shown better COX-2 inhibition compared to its base Curcumin. Out of 9 herbal drugs BNB and PME have shown also shown some selective COX-2 inhibition. All herbal drugs were studied for the acute *in vivo* assay. Those compounds which shown promising *in vitro* results were further studied for acute *in vivo* anti-inflammatory model of carrageenan induced rat paw edema. They are as follows:

Synthetic compounds:
MCR-95, MCR-101, MCR-163, MCR-175, MCR-207, MCR-189, MCR-192, MCR-93,MCR-190, MCR-179, MCR-180, MCR-240, MCR-241, MCR-243, MCR-242, MCR-262,MCR-263, MCR-264, MCR-265, MCR-273, MCR-292, MCR-293, MCR-295
MCR-296, MCR-316, MCR-320, MCR-322, MCR-323, MCR-327, MCR-333, MCR-349
MCR-364, MCR-363.

Semi synthetic compound: BCOV

Herbal Drugs Extracts:
Banaba-*Lagerstroemia speciosa L*— leaf extract(BNB),
Pomegranate- *Punica Granatum*-Fruit extract(PME),

4.12 Compounds screening chart

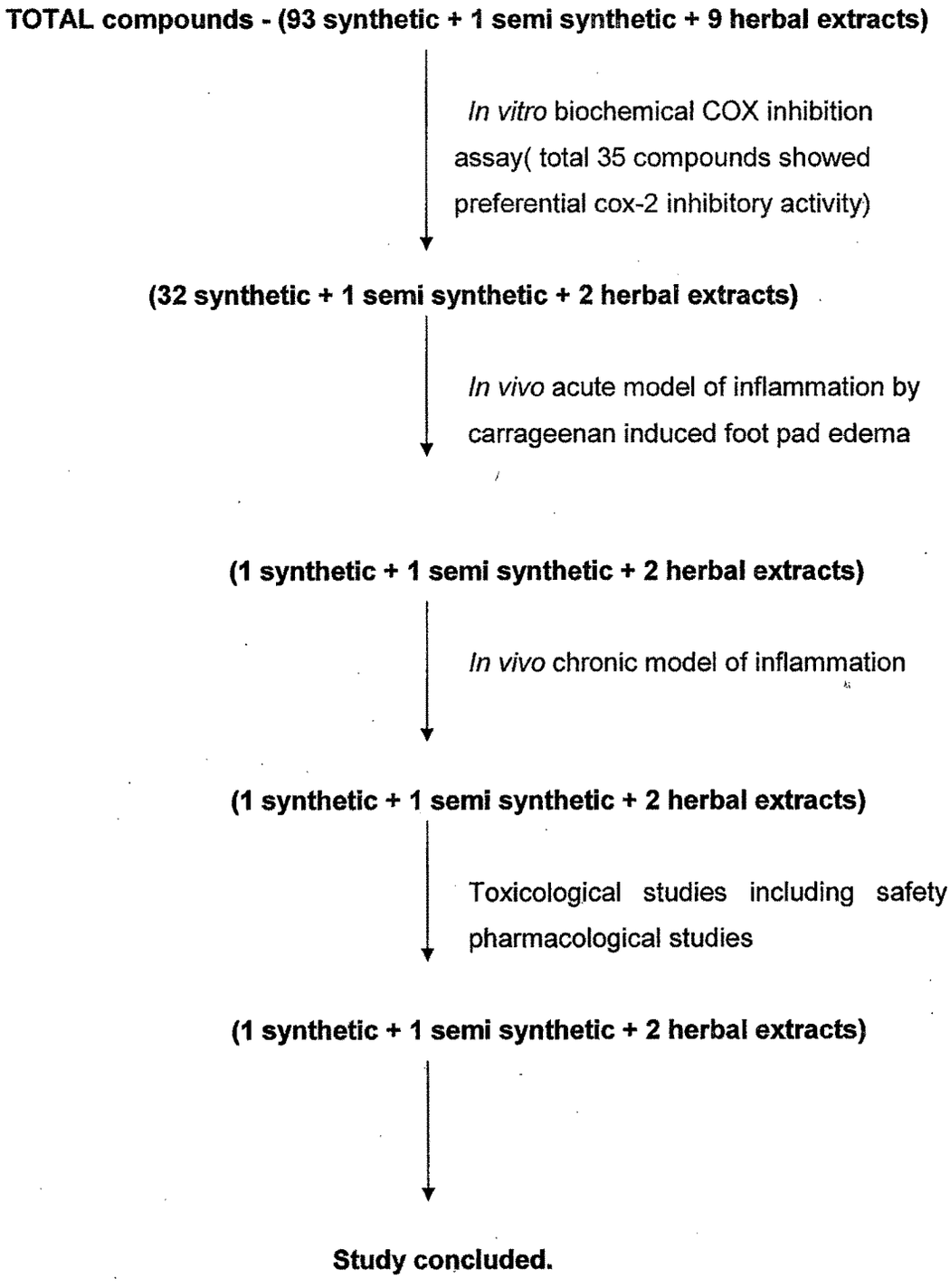


Table 10. *In vitro* COX-1 and COX-2 enzyme inhibition assay for all MCR compounds(93), semi synthetic compound BCOV(1) and herbal drugs(9). Percent inhibition of COX-2 (at 22 µM) and COX-1 (at 88 µM) determine by using Cayman colorimetric kit. Assays were performed in triplicate. Data shows average % inhibition using triplicate. Values are means of three determinations acquired using an ovine COX-1/COX-2 Cayman Chemicals kit and the deviation from the mean is <10% of the mean value.

*32 highlighted compounds showed preferential COX-2 inhibition.

	Cox-2		**Cox-1**	
Test	**Conc.**	**% Inhibition**	**Conc.**	**% Inhibition**
Substance	**µM**		**µM**	
MCR-49	22	5.1	--	--
MCR-50	22	0.0	--	--
MCR-127	22	0.0	--	--
MCR-102	22	0.0	--	--
MCR-166	22	10.2	88	16.67
MCR-167	22	0.0	--	--
MCR-95	**22**	**26.1**	**22**	**19.00**
			2.25	14.10
***MCR-101**	**22**	**53.9**	**88**	**24.53**
	5.5	52.9	--	--
MCR-161	22	17.2	88	98.25
			5.5	90.65
MCR-163	**22**	**20.7**	**88**	**33.71**
MCR-169	22	0.0	--	--
MCR-165	22	0.0	--	--
MCR-162	22	0.0	--	--
MCR-164	22	2.6	--	--
MCR-125	22	7.9	88	42.11
MCR-168	22	0.0	--	--
MCR-172	22	0.0	--	--
MCR-173	22	0.0	--	--
MCR-178	22	0.0	--	--
MCR-174	22	4.3	88	82.46
			5.5	5.04
MCR-175	**22**	**20.8**	**88**	**53.13**
MCR-176	22	0.0	--	--
MCR-177	22	0.0	--	--
MCR-206	22	4.6	88	79.82
MCR-217	22	3.7	--	--

	Cox-2		Cox-1	
Test substance	**Conc. µM**	**% Inhibition**	**Conc. µM**	**% Inhibition**
MCR-207	**22**	**87.2**	**88**	**84.38**
	5.5	57.0	22	77.34
	1.375	22.8	5.5	25.36
	0.344	7.2	--	--
	0.086	2.7	--	--
MCR-218	22	9.0	88	88.60
			5.5	0.00
MCR-219	22	6.0	--	--
MCR-220	22	4.8	88	83.33
			5.5	72.66
MCR-221	22	10.5	--	--
MCR-170	22	13.2	--	--
MCR-189	**22**	**20.0**	**88**	**10.9**
MCR-171	22	0.0	--	--
MCR-194	22	0.0	--	--
MCR-192	**22**	**22.7**	**88**	**15.3**
MCR-193	**22**	**25.4**	**88**	**17.5**
MCR-190	**22**	**20.4**	**88**	**10.5**
MCR-191	22	0.0	--	--
MCR-179	22	8.2	--	--
MCR-180	**22**	**27.4**	**88**	**5.7**
MCR-181	22	31.8	88	92.97
			22	69.53
MCR-184	22	4.3	88	36.84
MCR-182	22	11.1	88	53.27
MCR-230	22	0.0	--	--
MCR-231	22	8.6	88	17.19
MCR-222	22	5.3	88	10.53
MCR-232	22	0.0	--	--
MCR-233	22	0.0	--	--
MCR-234	22	0.0	--	--
MCR-223	22	0.0	--	--
MCR-235	22	0.0	--	--
Valdecoxib	**22**	**92.7**	**22**	**8.33**
	5.5	77.1	88	25.64
	1.375	80.1	--	--
	0.344	76.7	--	--
	0.086	62.9	--	--
	0.0276	30.9	--	--
	0.00532	14.2	--	--
Celecoxib	**22**	**94.9**	**22**	**20.31**
	1.375	74.2	44	17.81
	0.344	71.8	--	--
	0.086	61.0	--	--

	Cox-2		Cox-1	
Test	**Conc.**	**% Inhibition**	**Conc.**	**% Inhibition**
Substance	**µM**		**µM**	
	0.0276	39.4	--	--
	0.00532	29.2	--	--
Indomethacin	22	86.0	1.375	94.23
	5.5	51.8	0.344	87.82
Indomethacin	1.375	29.1	0.086	62.82
	0.334	7.6	0.086	45.77
			0.0215	20.42
Rofecoxib	22	91.2	88	23.48
MCR-240	**22**	**26.1**	**88**	**13.9**
MCR-241	**22**	**21.5**	**88**	**4.5**
MCR-243	**22**	**23.8**	**88**	**2.5**
MCR-242	**22**	**27.3**	**88**	**7.9**
			22	15.21
MCR-263	**22**	**23.2**	**88**	**6.8**
MCR-264	**22**	**33.4**	**88**	**25.7**
MCR-265	**22**	**29.3**	**88**	**13.4**
MCR-273	**22**	**28.05**	**88**	**15.6**
MCR-267	22	12.00	88	21.93
MCR-268	22	8.82	88	42.98
MCR-270	22	19.76	88	23.11
MCR-271	22	27.72	88	0.00
MCR-289	22	0	--	--
MCR-290	22	0	--	--
MCR-285	22	0	--	--
MCR-286	22	0	--	--
MCR-287	22	0	--	--
MCR-291	22	0	--	--
MCR-292	**22**	**83.03**	**88**	**71.20**
	5.5	10.02	22	48.33
MCR-293	**22**	**59.2**	**88**	**58.29**
	5.5	23.9	22	19.71
MCR-294	22	3.8	--	--
MCR-295	**22**	**63.54**	**88**	**56.30**
	5.5	19.27	22	41.89
MCR-296	**22**	**24.3**	**88**	**34.89**
MCR-296	--	--	22	27.46
MCR-297	22	0	--	--
MCR-301	22	0	--	--
MCR-302	22	0	--	--
MCR-303	22	0	--	--
MCR-313	22	0	--	--
MCR-314	22	6.94	--	--
MCR-315	22	0	--	--
MCR-316	**22**	**30.07**	**88**	**35.71**

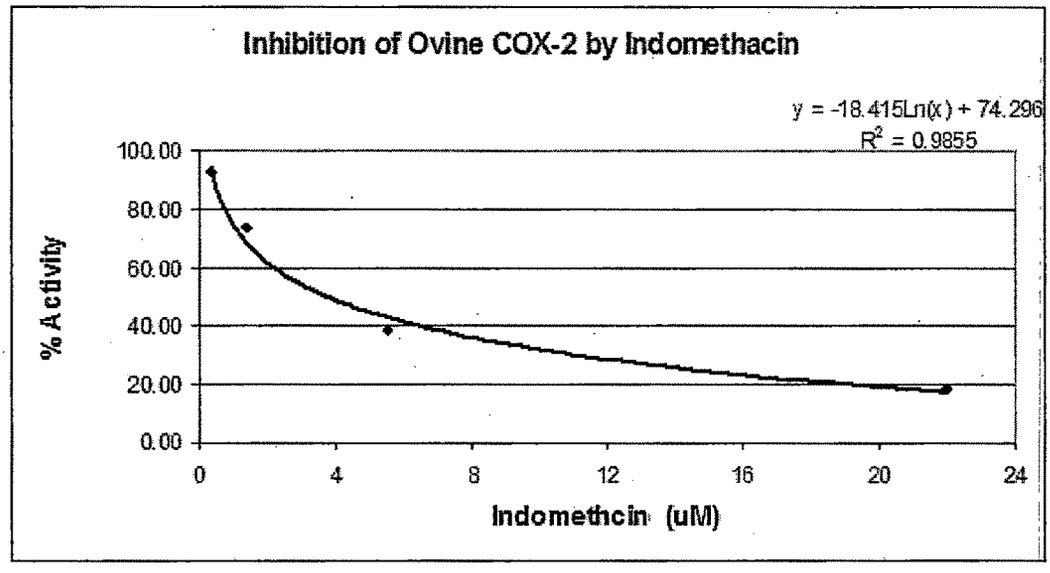
	Cox-2		Cox-1	
Test	**Conc.**	**% Inhibition**	**Conc.**	**% Inhibition**
Substance	**µM**		**µM**	
MCR-317	22	0	--	--
MCR-318	22	10.02	88	0.00
MCR-319	22	3.14	88	98.25
MCR-320	**22**	**86.75**	**88**	**53.28**
MCR-320	22	23.9	22	29.71
MCR-321	22	2.295	--	--
MCR-322	**22**	**87.66**	**88**	**49.81**
	11	45.71	22	25.54
	2.75	30.08	88	23.89
MCR-323	**22**	**59.28**	**88**	**78.95**
MCR-324	22	0	--	--
MCR-326	22	0	--	--
MCR-327	**22**	**23.09**	**88**	**43.76**
MCR-327	---	---	22	11.00
MCR-328	22	0	--	--
MCR-329	22	10.93	88	16.67
MCR-304	22	0	--	--
MCR-305	22	0	--	--
MCR-306	22	0	--	--
MCR-307	22	4.18	--	--
MCR-308	22	0	--	--
MCR-333	**22**	**40.88**	**88**	**39.21**
MCR-333	5.5	23.91	22	17.89
MCR-335	22	0	--	--
MCR-336	22	0	--	--
MCR-334	22	0	88	0.00
MCR-344	22	0	--	--
MCR-345	22	13.59	--	--
MCR-349	**22**	**29.9**	**88**	**21.00**
MCR-349	--	--	5.5	76.98
MCR-352	22	0	--	--
MCR-362	22	25.82	--	--
MCR-364	22	40.78	--	--
MCR-363	**22**	**100**	**88**	**0.00**
	5.5	76.4	176	18.71
	1.375	61.3	--	--
	0.343	44.8	--	--
BCOV	**22**	**54.375**	**176**	**82.09**
	5.5	23.11	88	35.94
	1.375	15.09	22	24.73
	--	---	5.5	8.23
Curcumin	**88**	**62.39**	**88**	**64.04**
	22	34.25	22	26.37
	5.5	10.15	5.5	9.87

Table 11. Inhibitory effects of herbal drug extracts on COX-1 and COX-2 activity. Percent inhibition of COX-2 (100 µg/ml) and COX-1 (100 µg/ml) by Cayman colorimetric kit. Assays were performed in triplicate. Average % inhibition using triplicate. Values are means of three determinations acquired using an ovine COX-1/COX-2 Cayman Chemicals kit. The deviation from the mean is <10% of the mean value.

		Cox-2		Cox-1	
Sr.No	**Test Substance**	**Conc. µg/ml**	**% Inhi.**	**Conc. µg/ml**	**% Inhi.**
128	BNB(Banaba)	100	100	150	100.00
	BNB	**50**	**76.53**	**75**	**88.49**
		25	24.51	50	10.79
		6.25	9.91	6.25	0.00
129	Ashwagandha	100	0	100	0.00
130	Lodra	100	0	100	0.00
131	Wheatgrass	100	0	100	0.00
132	Sariva	100	0	100	0.00
133	Arjuna	100	41.57	100	51.38
134	**PME(Pomegranate)**	**150**	**76.77**	**200**	**61.49**
		100	51.23	100	21.23
		50	12.5	50	5.35
135	Bitter Melon	100	0	100	0
136	Tulsi	100	0	100	0.00

Figure 18. Concentration-response curve for the inhibition of COX-2 and COX-1 by Indomethacin. *In vitro* COX-1 and COX-2 enzyme inhibition assay was performed by using cayman colorimetric kit. Assays were performed in triplicate. Average % inhibition using triplicate(see Experimental). Values are means of two determinations acquired using an ovine COX-1/COX-2 Cayman Chemicals Inc., Ann Arbor, MI, USA) and the deviation from the mean is <10% of the mean value.

Calculated IC_{50} for COX-2 = 5.50 µM, for COX-1 = 0.08, COX-1/COX-2 =0.014



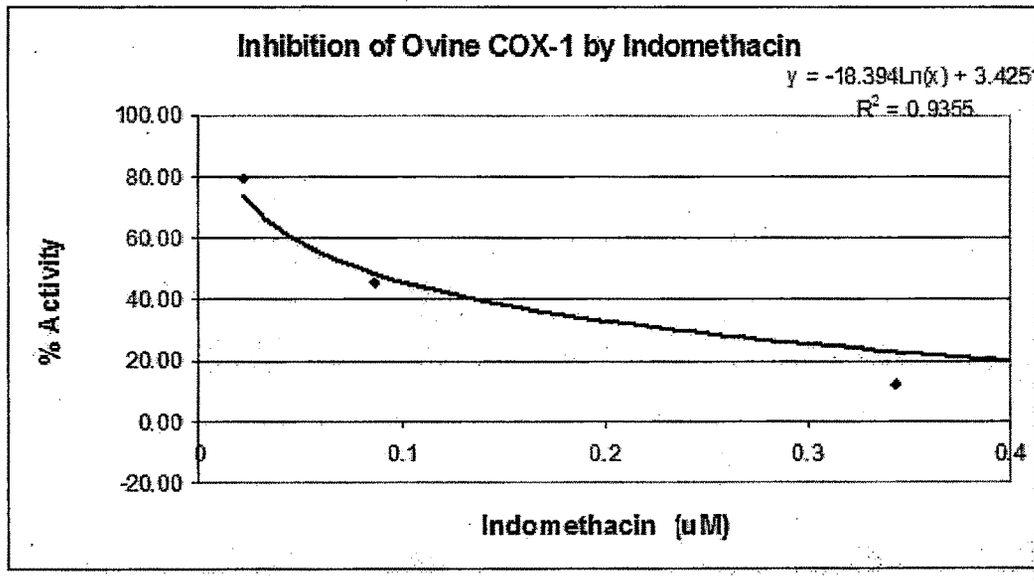
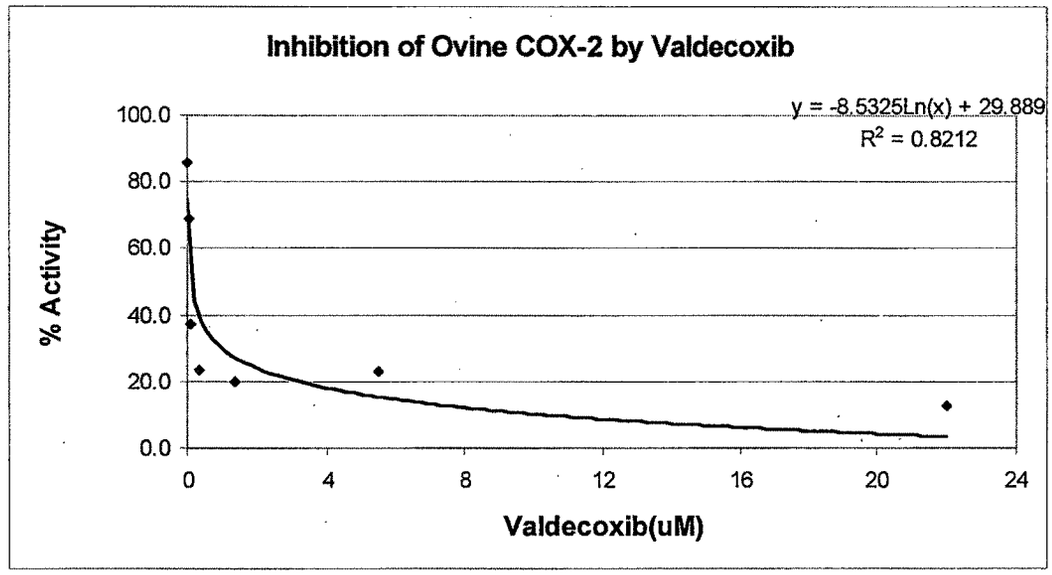


Figure 19. Concentration-response curve for the inhibition of COX-2 and COX-1 by Valdecoxib. *In vitro* COX-1 and COX-2 enzyme inhibition assay was performed by using Cayman colorimetric kit. Assays were performed in *triplicate*. Graph shows average % inhibition using triplicates. Values are means of three determinations acquired using an ovine COX-1/COX-2 Cayman Chemicals Inc., Ann Arbor, MI, USA) and the deviation from the mean is <10% of the mean value.

Calculated IC_{50} COX-2 = 0.08 µM, COX-1 = 46.45, COX-1/COX-2 =580.6



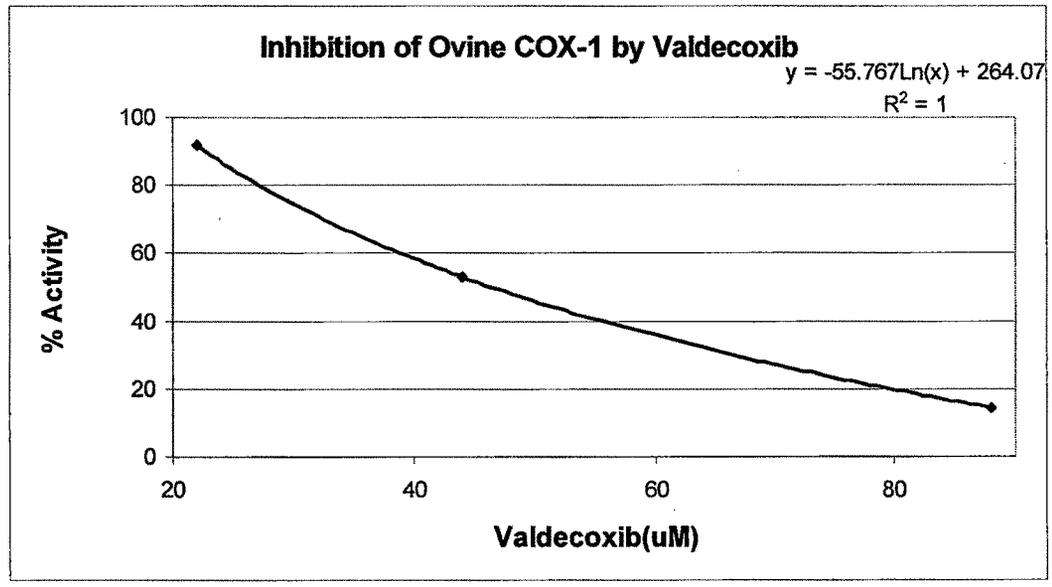
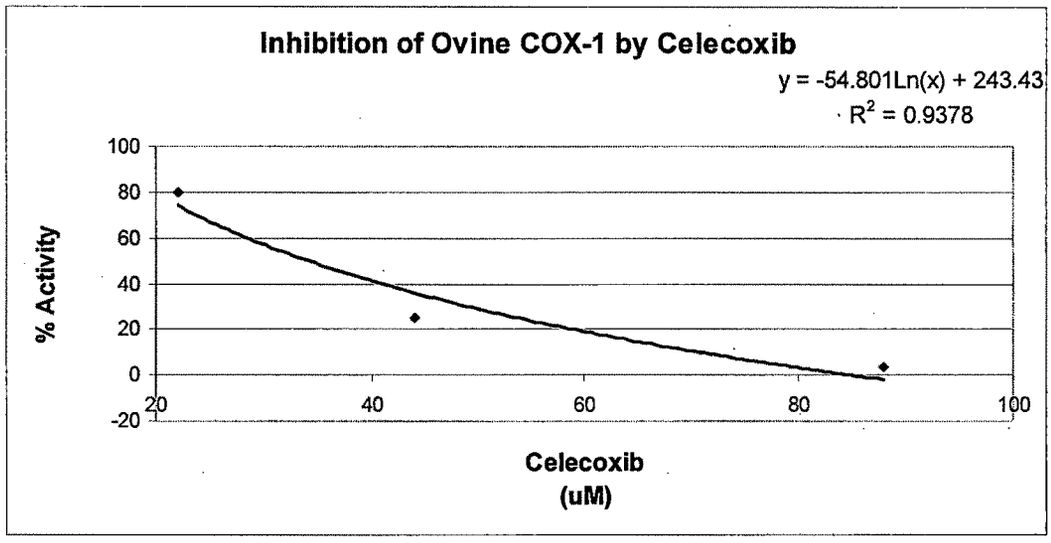


Figure 20. Concentration-response curve for the inhibition of COX-2 and COX-1 by Celecoxib. *In vitro* COX-1 and COX-2 enzyme inhibition assay was performed by using Cayman colorimetric kit. Assays were performed in triplicate. Graph shows average % inhibition using triplicates (see Experimental). Values are means of three determinations acquired using an ovine COX-1/COX-2 Cayman Chemicals Inc., Ann Arbor, MI, USA) and the deviation from the mean is <10% of the mean value.

Calculated IC_{50} COX-2 = 0.06 µM, COX-1 = 34.11, COX-1/COX-2 =532.9



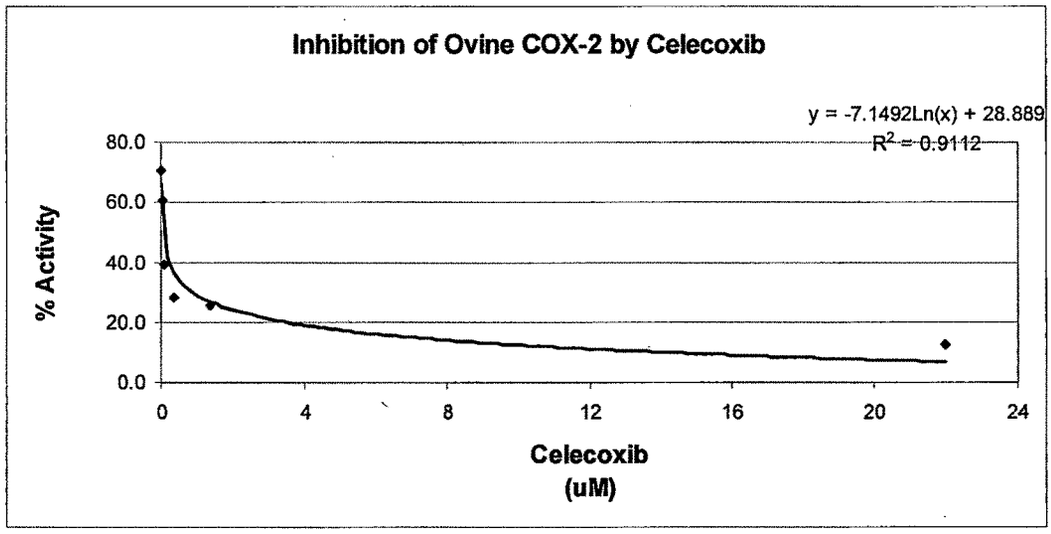
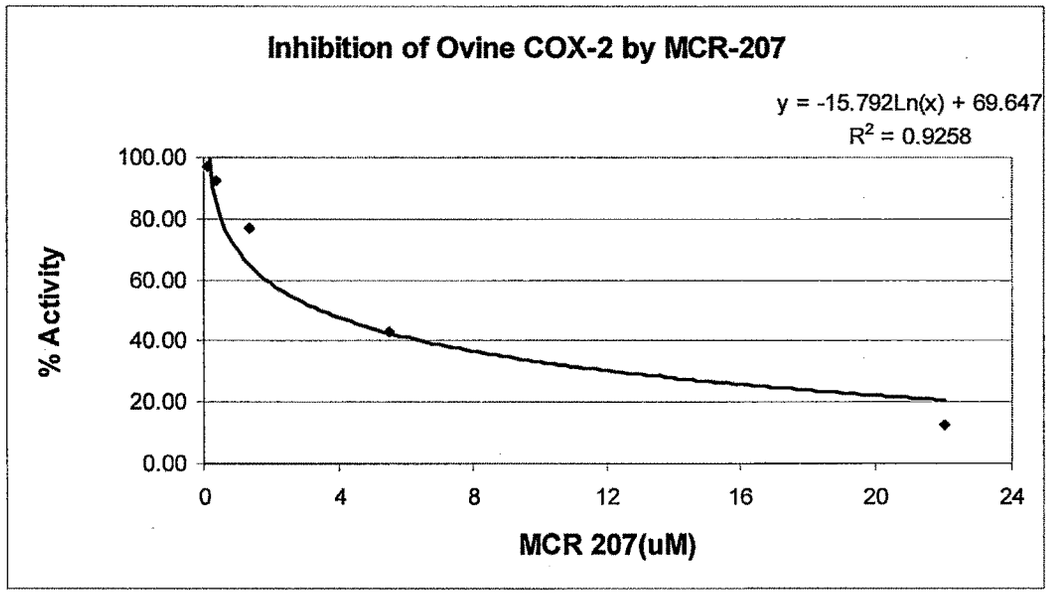


Figure 21. Concentration-response curve for the inhibition of COX-2 and COX-1 by MCR-207. *in vitro* COX-1 and COX-2 enzyme inhibition assay was performed by using Cayman colorimetric kit. Assays were performed in triplicate. Graph shows % inhibition using triplicates. Values are means of three determinations acquired using an ovine COX-1/COX-2 Cayman Chemicals kit The deviation from the mean is <10% of the mean value.

Calculated IC_{50} COX-2 = 3.46 µM, COX-1 = 12.3, COX-1/COX-2 =0.014



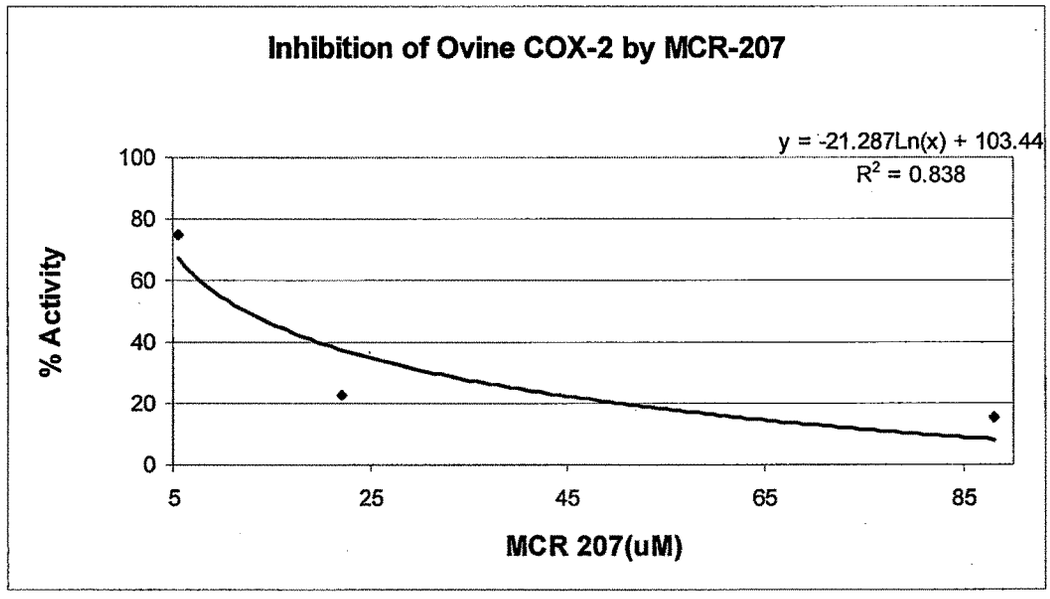
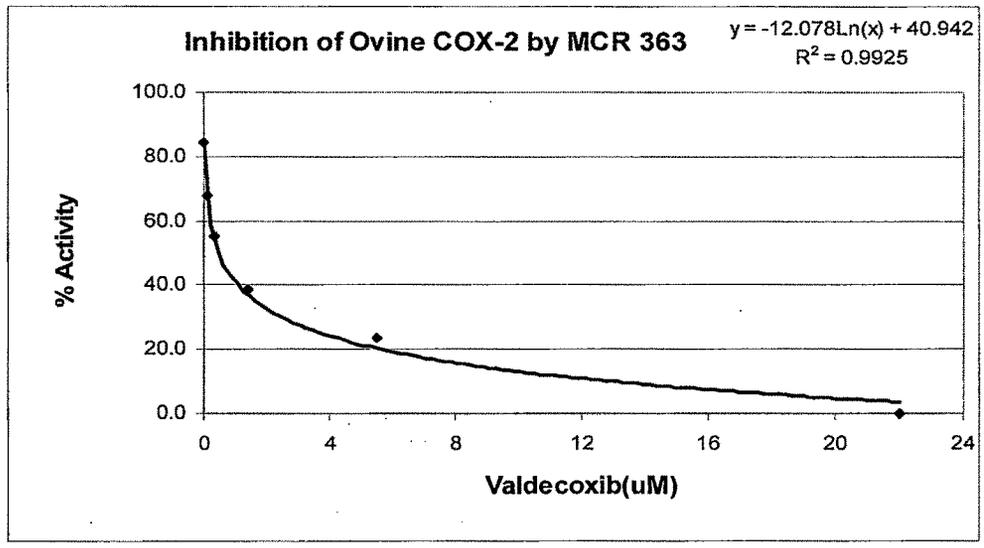


Figure 22. Concentration-response curve for the inhibition of COX-2 and COX-1 by MCR363 *in vitro* COX-1 and COX-2 enzyme inhibition assay was performed by using Cayman colorimetric kit. Assays were performed in triplicate. Graph shows average % inhibition. Values are means of three determinations acquired using an ovine COX-1/COX-2 Cayman Chemicals kit. The deviation from the mean is <10% of the mean value.

Calculated IC_{50} COX-2 = 0.47 µM, COX-1 = 310.7, COX-1/COX-2 =658.26



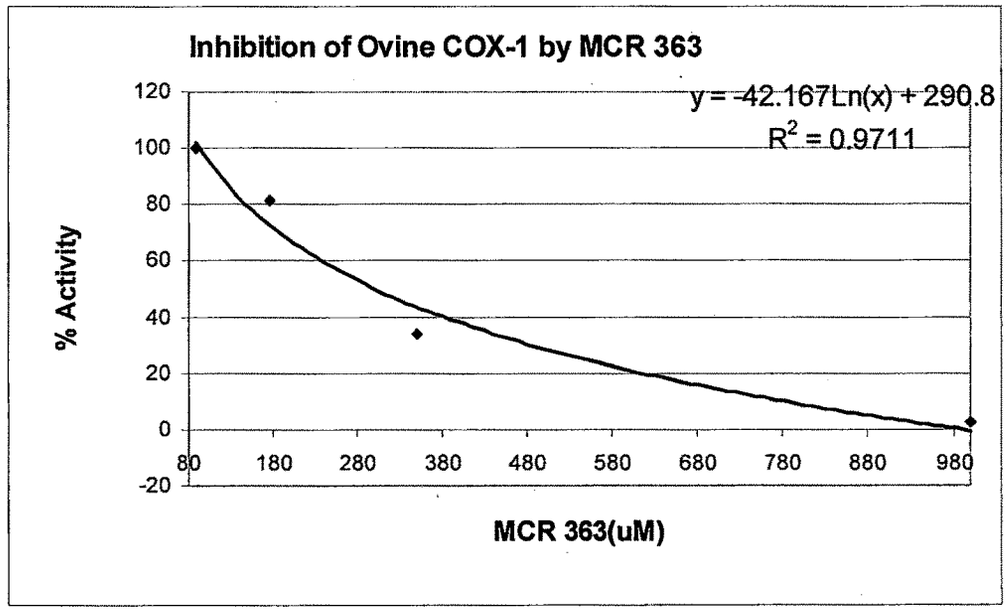
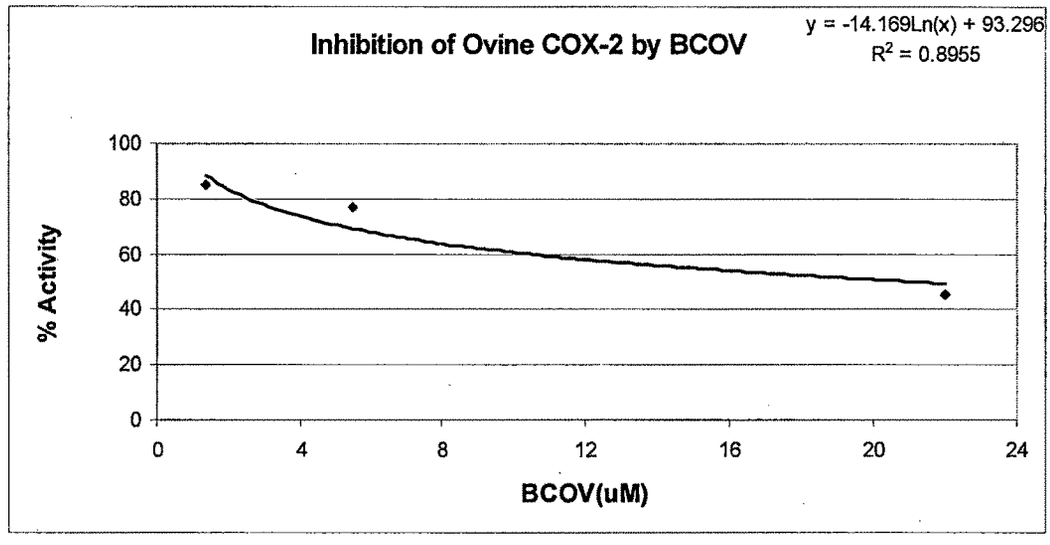


Figure 23. Concentration-response curve for the inhibition of COX-2 and COX-1 by BCOV. *In vitro* COX-1 and COX-2 enzyme inhibition assay was performed by using Cayman colorimetric kit. Assays were performed in triplicate. Graph shows average % inhibition. Values are means of three determinations acquired using an ovine COX-1/COX-2 Cayman Chem. Kit. The deviation from the mean is <10% of the mean value.

Calculated IC_{50} COX-2 = 21.23 µM, COX-1 =71.93, COX-1/COX-2 =3.388



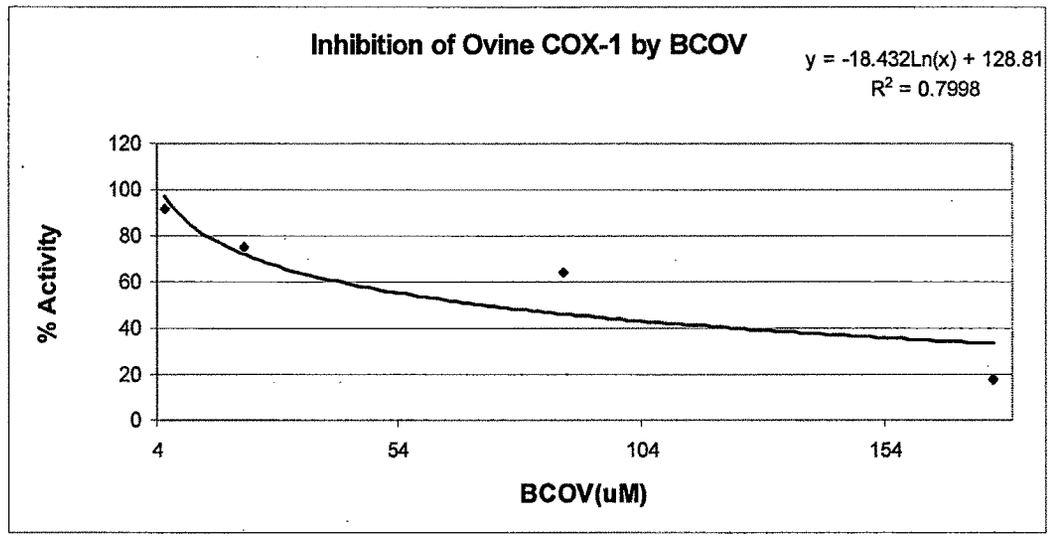
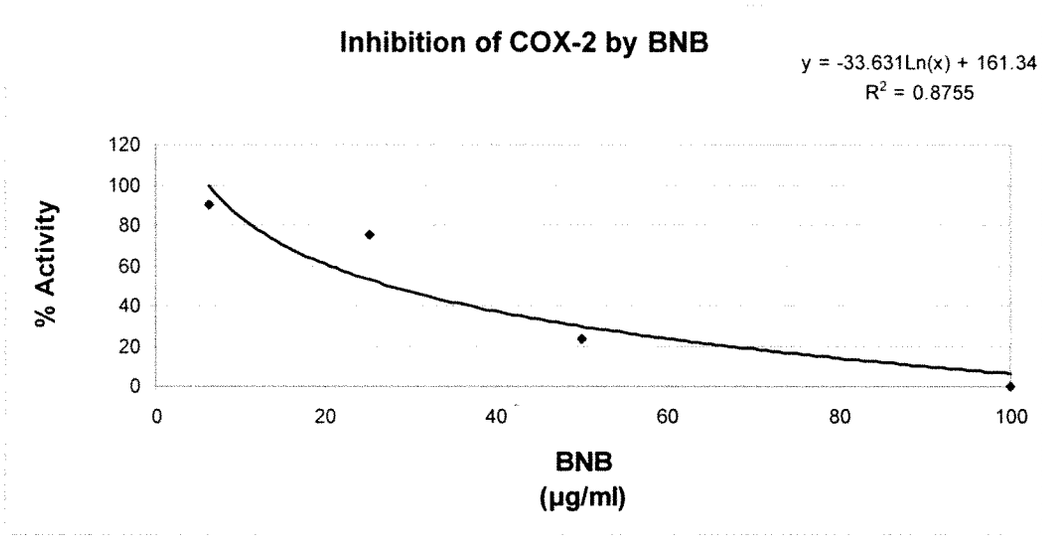


Figure 24. Concentration-response curve for the inhibition of COX-2 and COX-1 by BNB. *In vitro* COX-1 and COX-2 enzyme inhibition assay was performed by using Cayman colorimetric kit. Assays were performed in triplicate. Graph shows average % inhibition using triplicates. Values are means of three determinations acquired using an ovine COX-1/COX-2 Cayman Chemicals kits. The deviation from the mean is <10% of the mean value.

Calculated IC_{50} COX-2 = 32 µM, COX-1 = 65.5, COX-1/COX-2 =2.04



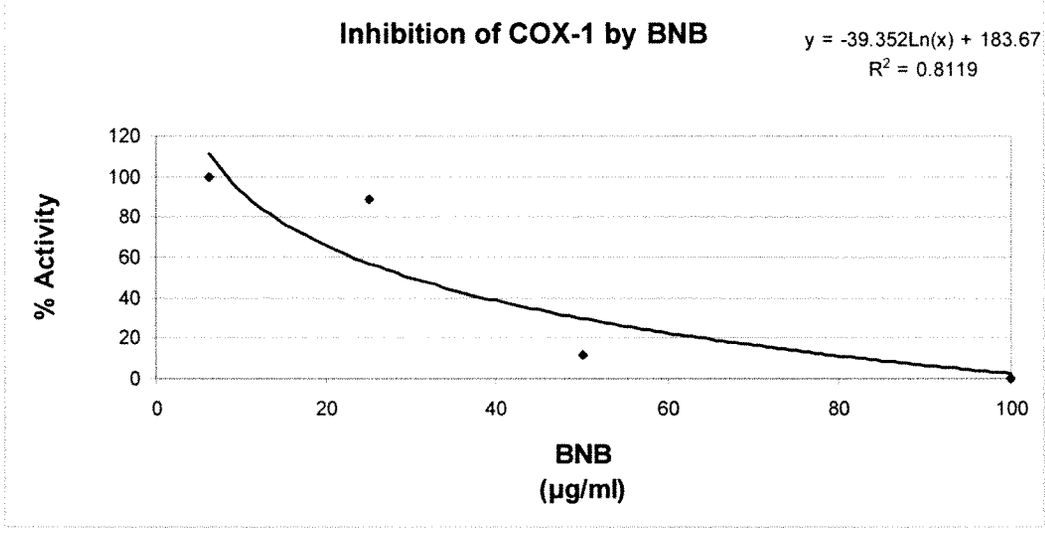
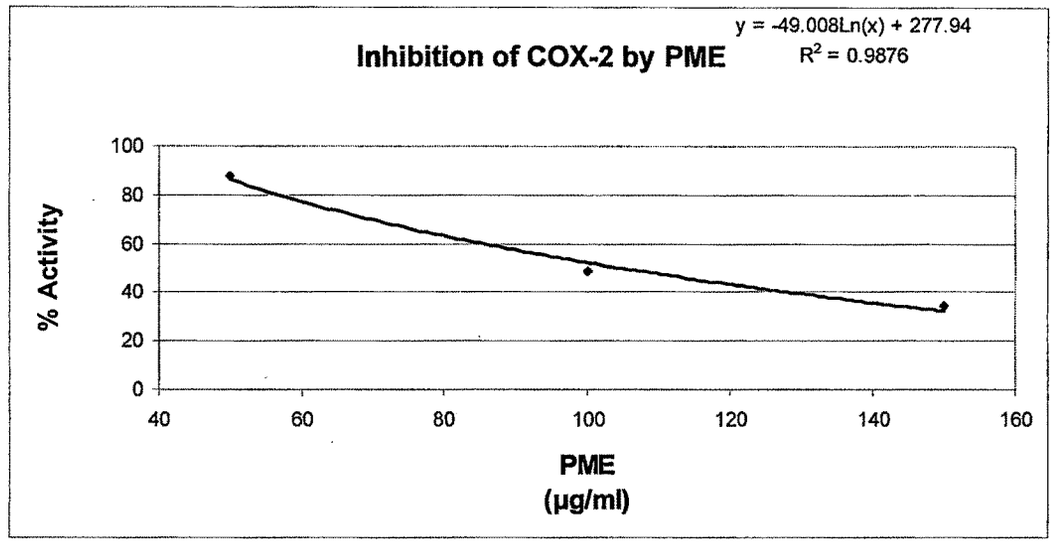


Figure 25. Concentration-response curve for the inhibition of COX-2 and COX-1 by PME. *In vitro* COX-1 and COX-2 enzyme inhibition assay was performed by using Cayman colorimetric kit. Assays were performed in triplicate. Graph shows % average inhibition using triplicates. Values are means of three determinations acquired using an ovine COX-1/COX-2 Cayman Chemicals kit. The deviation from the mean is <10% of the mean value.

Calculated IC_{50} COX-2 = 98.9 µM, COX-1 = 147.2, COX-1/COX-2 =1.48



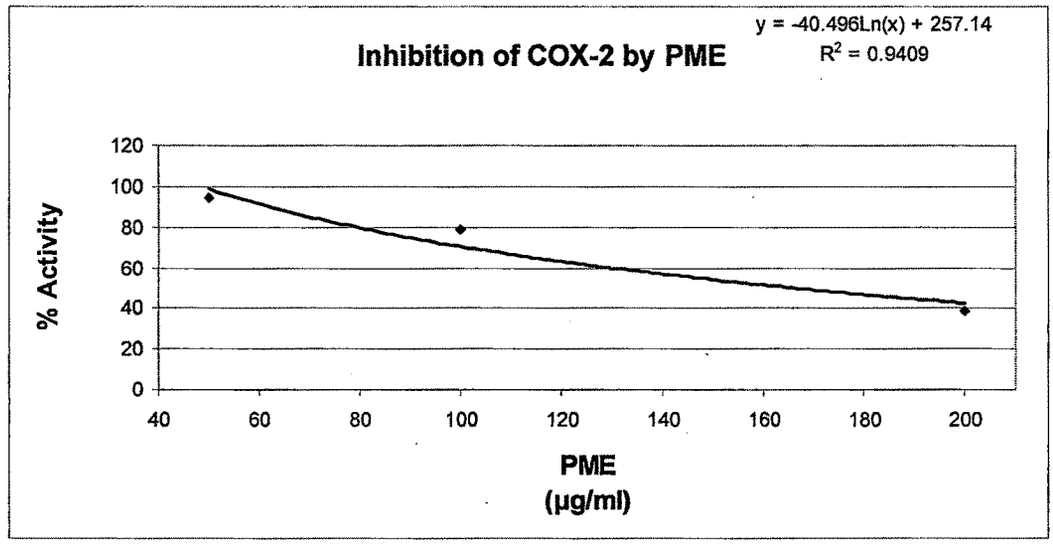


Table 12. Summary of inhibitory potencies towards COX-1 and COX-2 and IC_{50} values of test compounds, with selectivity at 50% inhibition (IC_{50} COX-1/IC_{50} COX-2). The assays were performed by using Cayman colorimetric kit. Assays were performed in triplicate. Data shows average % inhibition using triplicates. Values are means of three determinations acquired using an ovine COX-1/COX-2 Cayman Chemicals kit. The deviation from the mean is <10% of the mean value. Out of 93 synthetic compounds,1 semi synthetic compounds and 9 herbal drugs 4 synthetic, 1 Semisynthetic and 2 herbal drugs were studied for their detailed COX-IC_{50}, these drugs were showed potent activity in both preliminary *in vitro* and acute *in vivo* carrageenan induced paw edema.

Sr.No	Test	Solubility	Cox-2		Cox-1		Cox-2 IC_{50}	Cox-1 IC_{50}	Ratio COX-1 /COX-2
			Conc.	% Inhi.	Conc.	% Inhi.			
	Substance		µM		µM		µM	µM	
52	Valdecoxib	DMSO	22	92.7	22	8.33	0.08	46.45	580.6
			5.5	77.1	44	46.93			
			1.375	80.1	88	85.64			
			0.344	76.7					
			0.086	62.9					
			0.0276	30.9					
			0.00532	14.2					
53	Celecoxib	DMSO	22	94.9	22	20.31	0.06	34.11	532.9
			1.375	74.2	44	75.24			
			0.344	71.8	88	96.28			
			0.086	61.0					
			0.0276	39.4					
			0.00532	22.5					
54	Indomethacin	DMSO	22	86.0	1.375	94.23	5.50	0.08	0.014
			5.5	51.8	0.344	87.82			
			1.375	29.1	0.086	62.82			
			0.334	7.6	0.086	45.77			
					0.0215	20.42			
26	MCR-207	DMSO	22	87.2	88	84.38	3.46	12.3	3.554
			5.5	57.0	22	77.34			
			1.375	22.8	5.5	25.36			
			0.344	7.2					
			0.086	2.7					
124	MCR-364	DMSO	5.5	35.6	88	0.00	13.58	238.49	17.56
			22	48.62	176	25.30			
			88	98.72	352	75.23			

Sr.No	Test Substance	Solubility	Cox-2 Conc. µM		Cox-1 % Inhi.		Cox-2 IC_{50} Conc. µM	Cox-1 IC_{50} Conc. µM	Ratio COX-1 /COX-2
			µM	% Inhi.	µM	% Inhi			
125	MCR-363 .	DMSO	22	100	88	0.00	0.47	310.70	658.26
			5.5	76.4	176	18.71			
125	MCR-363	DMSO	1.375	61.3	352	65.79			
			0.343	44.8	1000	97.55			
128	BNB(Banaba)	Methanol	100	100	150	100.00	32	64.4	2.04
			50	76.53	75	88.49			
			25	24.51	50	10.79			
			6.25	9.91	6.25	0.00			
134	PME (Pomegranate)	Methanol	150	76.77	200	61.49	98.9	147.2	1.48
			100	51.23	100	21.23			
			50	12.5	50	5.35			
126	BCOV	DMSO	22	54.375	176	82.09	21.23	71.93	3.388
			5.5	23.11	88	35.94			
			1.375	15.09	22	24.73			
					5.5	8.23			
127	Curcumin	DMSO	88	62.39	88	64.04	47.25	51.38	1.087
			22	34.25	22	26.37			
			5.5	10.15	5.5	9.87			

4.2 Anti-inflammatory studies (*in vivo*)

4.2 *In vivo* assay for efficacy

4.2.1. Acute Inflammation Model

Carrageenan Induced Paw Edema:

(32 selected MCR series synthetic compounds, 1 semi synthetic compound BCOV and 2 herbal extracts included in this model)

Table 13 shows anti-inflammatory activity of test drugs on edema formation induced in rat paws by carrageenan. 1hr prior to carrageenan injection MCR series compounds were administered (25mg/kg/p.o.)p.o. and the herbal drugs were administered at (500mg/kg/p.o.) p.o.,. Data are presented % inhibition of edema as compared to vehicle treated group. The control animals received 1% CMC orally. The baseline for the various groups were very similar, with mean values of 1.0–1.1 mL. In the untreated control animals, swelling of the paw was evident by 1 hr after administration of carrageenan and reached a peak at 3 hr, with an increased volume of 0.9 mL, decreasing slowly thereafter. The anti-inflammatory effects of all the test compounds were finally compared with the reference standards Valdecoxib and Indomethacin. The test compounds when administered 1 hr before the injection of carrageenan, amongst the all MCR series compounds MCR-95, MCR-194, MCR-191, MCR-181, MCR-184, MCR-182, MCR-364, MCR-363 have shown some remarkable inhibition. BCOV has improved the activity as compared to Curcumin. In herbal drugs group BNB and PME showed good anti-inflammatory potential. The calculated ED_{50} values for synthetic compounds MCR 95, MCR 363, BCOV are 20.9$\pm$2, 13.9$\pm$2, 41.1$\pm$5 mg/kg/p.o. respectively. The ED_{50} for BNB and PME came 569$\pm$31 and 632$\pm$27 mg/kg/p.o. respectively. The ED_{50} value for standard drug Valdecoxib is 10.2$\pm$3.(Table 13, figures 26-31).From this study it was indicated that MCR-95 has shown good inhibition of carrageenan induced edema but the compound was rejected for further screening because of its poor COX inhibition profile(Table10). Compounds MCR 363, BCV, BNB, PME has shown good activity in acute *in vivo* model as well as *in vitro* cox-inhibition, These four test compounds 363, BCV, BNB, PME were taken for chronic anti-inflammatory and toxicological studies including safety pharmacology.

5.22.2 Chronic models of inflammation

a. Adjuvant-induced arthritis[204].

In the rat model of adjuvant-induced arthritis(AA), the oral administration of MCR-363, BCOV, BNB, PME at the doses 25 mg/kg/p.o., 25mg/kg/p.o., 500mg/kg/p.o. and 500 mg/kg/p.o. respectively from day 0 to day 21 showed inhibition of hind leg inflammation. Based on day 16 measurements inhibition of paw arthritic inflammation was 63% at 25 dose mg/kg/p.o./day of MCR-363, 64% at dose 25 mg /kg/day of BCOV and 59% of BNB at 500mg/kg/p.o. dose and 39% inhibition of PME at 1000mg/kg/p.o. (Table 14, Figure 33). This inhibition of arthritic paw inflammation (edema) was sustained, and by day 21 it was 63% at 25 mg/kg/p.o./day for MCR-363, 67% at 25 mg /kg/day for BCOV, and 67% for BNB at 500mg/kg/p.o. and 43% for PME at 500mg/kg/p.o.(Table 14, Figure 33). Effect of adjuvant administration and different treatment groups on rat body weight showed in figure 32. Results expressed as the mean body weight of rats in various treatment group. Treatment of indomethacin (5mg/kd/day/p.o.), MCR 363(100mg/kg/p.o.), BCOV(100mg/kg/p.o.) have shown significant recovery in weight compared to adjuvant induced control group. ($P < 0.05$).

Figure 34 shows picture of AA group control animal taken on day16 with inflammation in hind limb. Figure 35 shows radiograph inflamed hind left and right paws, there were marked periarticular soft tissue swelling. The tarsocrural joint is collapsed and the intertarsal articulations are narrow in AA control group . There were multiple sites of cortical and trabecular osteolysis. Relatively less damage can bee seen in x-rays of groups MCR 363(100mg/kg/p.o./day), BCOV(100mg/kg/p.o./day), BNB(500mg/kg/p.o./day), PME(500mg/kg/p.o./day),. On day 22 all rats were euthanized, the weights of the thymus, spleen, adrenals were examined, test drugs had no effect at all concentrations on these organ weights, but Indomethacin significantly atrophied them. ($P<0.05$, Table 15)

b. TNBS (trinitrobenzenesulfonic acid) induced colitis in rats[205]

Effect of compounds on body weight of animals: Induction of colitis in animals by TNBS administration resulted in loss weight at various intervals of observation. The animals treated with TNBS alone showed significant loss of weight as compared to untreated control animals, whereas the animals pretreated with MCR-363, BCOV, BNB, PME at the dose 25 mg/kg/p.o., 25 mg/kg/p.o., 500 mg/kg/p.o., 500mg/kg/p.o. respectively along with TNBS treatment did not show any significant loss of weight on 18th day of treatment. The loss of weight in TNBS animals correlates with the severity of the colitis and the recovery of weight in MCR-363, BCOV treated animals may be due to its healing effect. Herbal drugs BNB and PME treated animals no significant weight loss observed as compared to normal control group.

Relative weight of colon: The animals were sacrificed and colon segment (5 cm) washed with saline, adhering tissues were removed and weighed for the change in relative weight. Animals with TNBS alone showed significant ($P<0.05$) increase in relative weight of colon where as the animals pretreated with MCR-363, BCOV, BNB, PME at the doses 25 mg/kg/p.o., 25 mg/kg/p.o., 500mg/kg/p.o and 500 mg/kg/p.o. respectively, after colitis induction showed no significant change in relative weight of colon at the end of 18 days of treatment regimen in comparison with control animals. (Table no.17, Fig-36). The gain in relative weight signifies the deposition of fibrous tissues on the intestinal linings.

Effect on Myeloperoxidase (MPO) Activity: Myeloperoxidase enzyme extensively used as a leukocyte infiltration marker is located in cells of myeloid origin. Levels of myeloperoxidase enzyme activity were increased in animals subjected to colonic injury by TNBS treatment. This significant increase in MPO activity in TNBS treated animals as compared to control animals indicates leukocyte infiltration in inflamed tissues. This is again confirmed by the presence of brown to black spots indicating neutrophil presence in colon strip by histochemical demonstration in leukocyte peroxidation assay. (figure 36). The

animals treated with MCR-363, BCOV, BNB, PME at doses 25 mg/kg/p.o., 25 mg/kg/p.o., 500mg/kg/p.o and 500 mg/kg/p.o showed significant ($P<0.05$) prevention of neutrophil infiltration by reducing myeloperoxidase activity levels as compared to TNBS treated animals.(Table 18).

Morphological assessment of colon for ulcerations: The colon was visually observed for the severity of the ulcerogenic damage, TNBS treated animals showed significant increase in severity of the ulcers as compared to control animals, where as the pretreated with MCR-363, BCOV, BNB, PME ($P<0.05$) animals showed significant decrease in severity of colon damage in comparison to TNBS alone treated animals. (Table 19). Microscopic view of histological sections of colonic tissues for various treatment groups given in the figure 36, showed TNBS histological sections of colonic mucosa from colitis rats 4 weeks after trinitrobenzenesulphonic acid (TNBS) instillation. TNBS control group showing the persistence of wide areas of ulceration, inflammation and fibrosis with associated regenerative changes in the adjacent mucosa, MCR 363-treated group (100mg/kg/p.o.), BCOV -100/mg/kg/p.o., BNB- 500mg/kg/p.o., PME-500mg/kg/p.o., Sulphasalazine- showed the healing effect on intestinal wall with low patchy inflammatory infiltrate and submucosa brosis as compared to TNBS control animals.

4.3 Analgesic activity

Tail immersion method in mice

The analgesic activity of the test compounds are summarised in table 20. Results mice are reported as % inhibition of tail flick latency with respect to vehicle treated control. The positive control Tramadol has shown significant inhibition 75% at the dose 5mg/kg/p.o. as compared to control group($P<0.05$). While treatment groups MCR-363(100mg/kg/p.o.), BCOV(100mg/kg/p.o.), BNB(500mg/kg/p.o.) and PME (500mg/kg/p.o.) showed non significant effect as compared to vehicle treated control.(Table 20)

Peripheral analgesic activity-writhing tests

Intraperitoneal injection of acetic acid is presumed to cause a sensation of pain in mice, which leads to abdominal contractions (writhing). Writhings were counted for five minutes after i.p. injection of 1% acetic acid(10ml/kg). The analgesic effect of test compounds were compared to control that received 1% CMC. The analgesic effect of test compounds shown in table 21. The positive control group Diclofenac sodium 40mg/kg/p.o. showed significant analgesic activity at dose 40mg/kg/p.o. as compared to control group($P<0.05$). Newly synthesized moiety MCR363 at dose 25mg/kg/p.o. has shown 35% analgesic activity remained significant as compared to control($P<0.01$), while BCOV at same dose showed significant 33% analgesic activity as compared to vehicle treated control group($P<0.05$). The herbal drugs BNB and PME have shown 31.23 % and 17.23 % inhibition of analgesia respectively.(table 21)

4.4. Antipyretic effect: endotoxin induced pyresis in rats[208]:

NSAID, such as diclofenac, are antipyretic in both human and animal models of pyresis[208] The test compounds the oral treatment of MCR 363, BCOV, PME and standard drug paracetamol showed at least more than 25-50% of temperature reversal effect at doses 25, 25, 500, 500, 25 mg/kg/p.o. respectively which was significant effect as compared to vehicle treated control ($P<0.05$). (Table 22).

Acute model of Inflammation- Carrageenan induced rat paw edema

Table 13. Summary of anti-inflammatory activity of compounds in carrageenan induced paw edema in rats. (n=5, Data shows dose mg/kg/p.o. vs % inhibition of edema). 1% CMC or MCR compounds (1-25 mg/kg/p.o.) or herbal drugs (100-1000mg/kg/p.o.) in 1ml of vehicle p.o. at -1 hr. At 0 hr 1% carrageenan (50ul) given subplantarly in right paw, increase in paw volume was measured at 3hr. ED_{50} data were calculated based on the % inhibition of edema from three different doses.

Compd.	Test	Dose	% Inhibition	ED_{50}
No.	Substance	mg/kg/p.o.	Mean± SEM	mg/kg/p.o.
7	MCR-95	25	70.5±4	19.31±2
		12.5	20.7±1	
		6.25	16±2	
8	MCR-101	25	5.2±1	
10	MCR-163	25	25.3±4	
21	MCR-175	25	31.27±7	
26	MCR-207	25	0	
32	MCR-189	25	21.81±10	
35	MCR-192	25	38.34±14	
36	MCR-193	25	42.25±8	
37	MCR-190	25	36.6±12	
39	MCR-179	25	10.3±5	
40	MCR-180	25	5.2±3	
52	Valdecoxib	25	70± 10	10.2±3
		12.5	57.50±13	
		6.25	41.90±7	
53	Celecoxib	12.5	48.20± 9	
61	Rofecoxib	12.5	53.23±8	
62	MCR-240	25	52.6±13	
		12.5	20.5±5	
63	MCR-241	25	34.7±7	
64	MCR-243	25	13.86±8	
65	MCR-242	25	19.56±9	
66	MCR-262	25	30.7±9	
67	MCR-263	25	20.8±6	
68	MCR-264	25	14.68±4	
69	MCR-265	25	4.1±5	
70	MCR-273	25	8.1±3	
82	MCR-292	25	4.7±2	
83	MCR-293	25	12.8±6	
85	MCR-295	25	12.80±7	
86	MCR-296	25	27.7±9	

Compd.	Test	Dose	% Inhibition	ED_{50}
No.	**Substance**	**mg/kg/p.o.**	**Mean± SEM**	**mg/kg/p.o.**
94	MCR-316	25	52.6±11	
		12.5	16.42±6	
98	MCR-320	25	16±4	
100	MCR-322	25	3.37±4	
101	MCR-323	25	32.7±9	
104	MCR-327	25	30.61±11	
115	MCR-333	25	23.9±8	
121	MCR-349	25	21.34±5	
124	MCR-364	25	63.9±13	
125	MCR-363	25	69.2±15	13.9±2
		12.5	46.3±10	
		6.25	21.7±9	
126	BCOV	25	34.2±14	41.3±5
		50	59.2±11	
		12.5	10.6±9	
127	Curcumin	25	18.45±5	
128	BNB	100	24±6	578±31
		300	40±9	
		1000	69.5±11	
129	Ashwagandha extract	500	35.6±13	
130	Lodra extract	500	17.89±6	
131	Wheatgrass extract	500	0	
132	Sariva extract	500	0	
133	Arjuna extract	500	21.3±5	
134	PME	500	45.70±15	694±27
		100	20.34±4	
		1000	59.45±5	
135	Bitter Melon extract	500	0	
136	Tulsi extract	500	24.3± 6	

Figure 26. Effects of MCR-95 on rat paw edema induced by carrageenan. MCR-95 was dosed p. o. 1h before the intraplantar injection of carrageenan at the doses of MCR-95 (6-25 mg/kg/p.o.). Paw edema was evaluated by plethysmometry at 3hr post carrageenan injection. Following plot obtained from paw edema inhibition measured for the animals pre-treated three different doses 6.25, 12.5, 25 mg/kg/p.o. of MCR 95. ED_{50} value was calculated based on the % inhibition of edema from three different doses. ED_{50} 19.31±2 mg/kg/p.o.

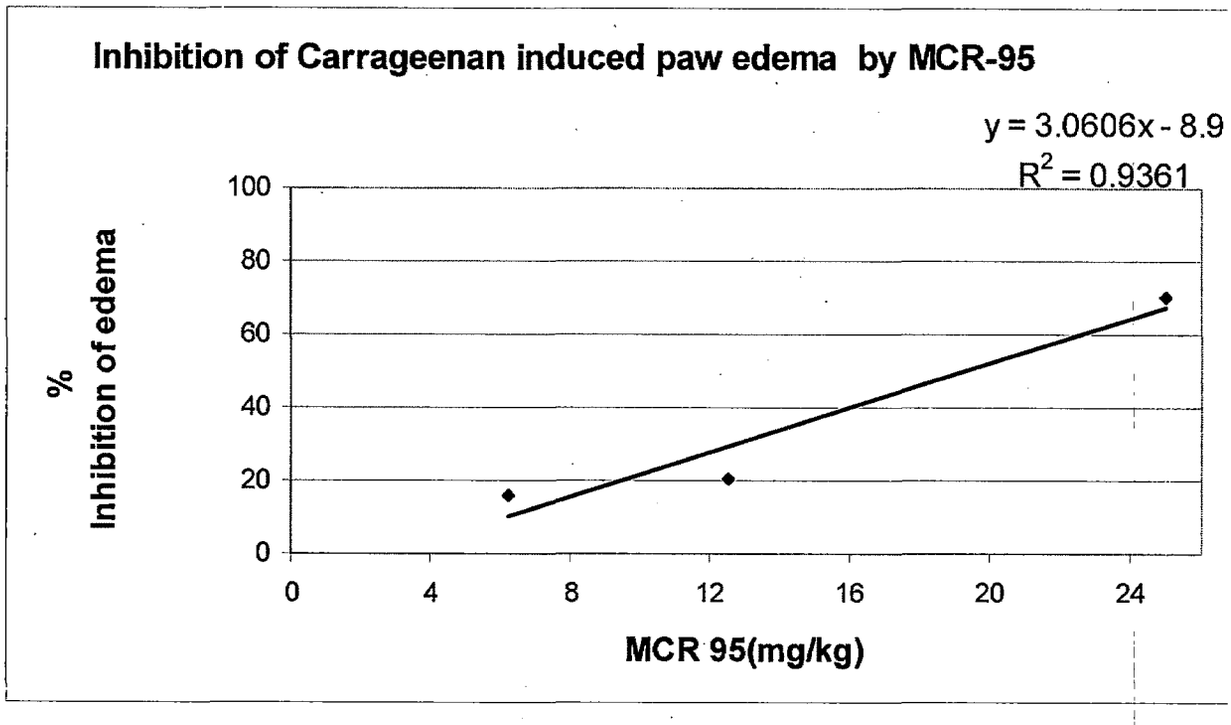


Figure 27. Effects of MCR-363 on rat paw edema induced by carrageenan. MCR-363 was dosed p.o. 1 h before the intraplantar injection of carrageenan at the doses of MCR-363(6-25 mg/kg/p.o.). Paw edema was evaluated by plethysmometry at 3hr post carrageenan injection. Following plot obtained from paw edema inhibition measured for the animals pre-treated with three different doses 6.25, 12.5, 25 mg/kg/p.o. of MCR-363. ED_{50} value was calculated based on the % inhibition of edema from three different doses. ED_{50} 19.31±2 mg/kg/p.o.

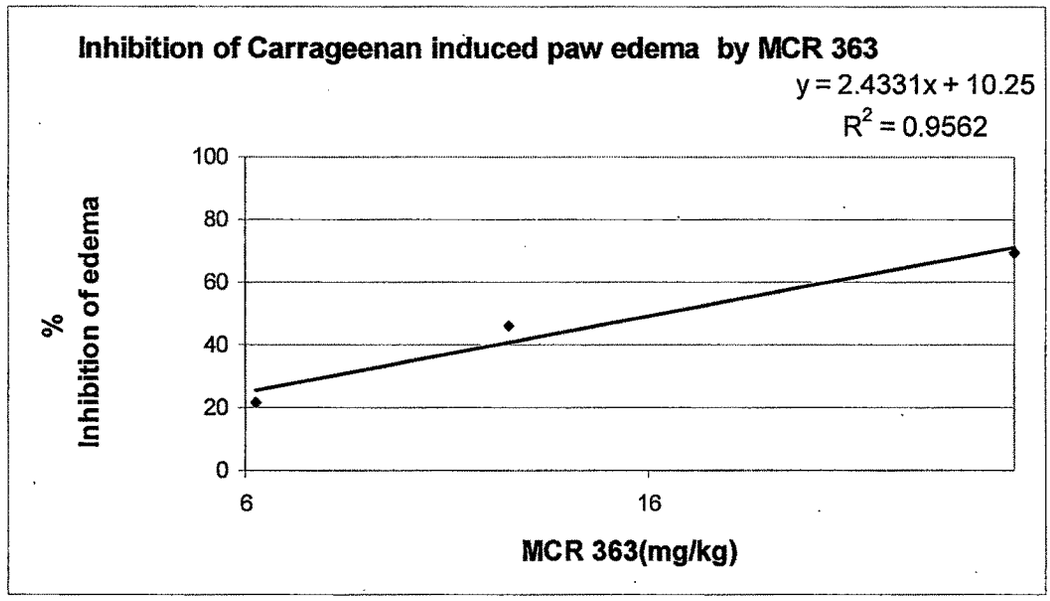


Figure 28. Effects of Valdecoxib on rat paw edema induced by carrageenan. Valdecoxib was dosed p.o. 4 h before the intraplantar injection of carrageenan at the doses of Valdecoxib (6-25 mg/kg/p.o.). Following plot obtained from paw edema inhibition measured for the animals pre-treated three different doses 6.25, 12.5, 25 mg/kg/p.o. of Valdecoxib. ED_{50} value was calculated based on the % inhibition of edema from three different doses. ED_{50} 10.2±2mg/kg/p.o.

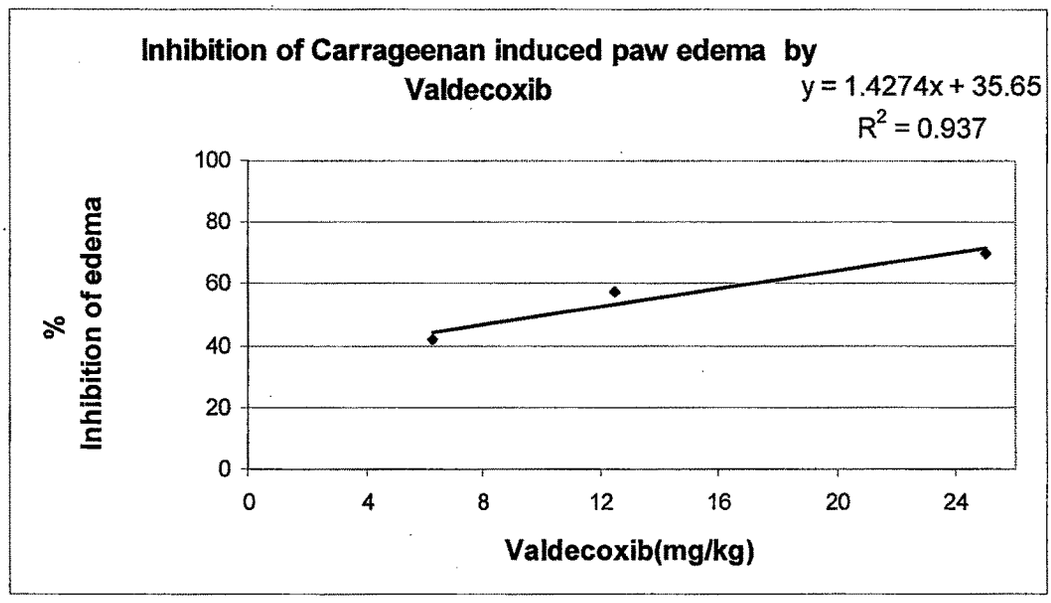


Figure 29. Effects of BCOV on rat paw edema induced by carrageenan. BCOV was dosed p. o. 1h before the intraplantar injection of carrageenan at three doses of BCOV (6-50 mg/kg/p.o.). Following plot obtained from paw edema inhibition measured for the animals pre-treated three different doses 6.25,25, 50 mg/kg/p.o. of BCOV. ED_{50} value was calculated based on the % inhibition of edema from three different doses. ED_{50} 41.3±5mg/kg/p.o.

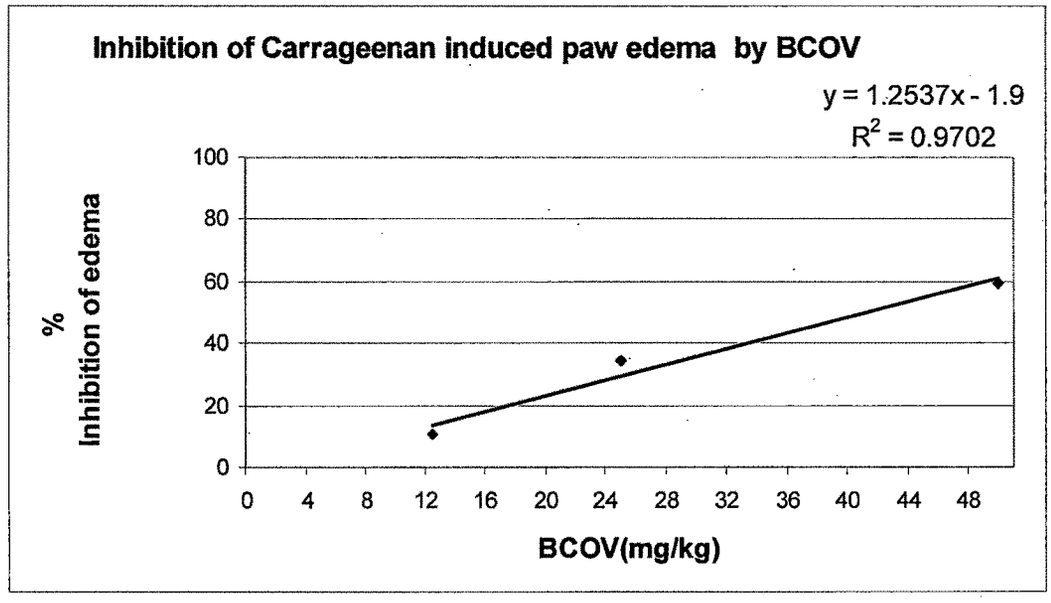


Figure 30. Effects of BNB on rat paw edema induced by carrageenan. BNB was dosed p.o. 1h before the intraplantar injection of carrageenan at three doses of BNB (100-1000 mg/kg/p.o.).). Following plot obtained from paw edema inhibition measured for the animals pre-treated three different doses 100, 300, 1000 mg/kg/p.o. of BNB. ED_{50} value was calculated based on the % inhibition of edema from three different doses. ED_{50} 578±31mg/kg/p.o.

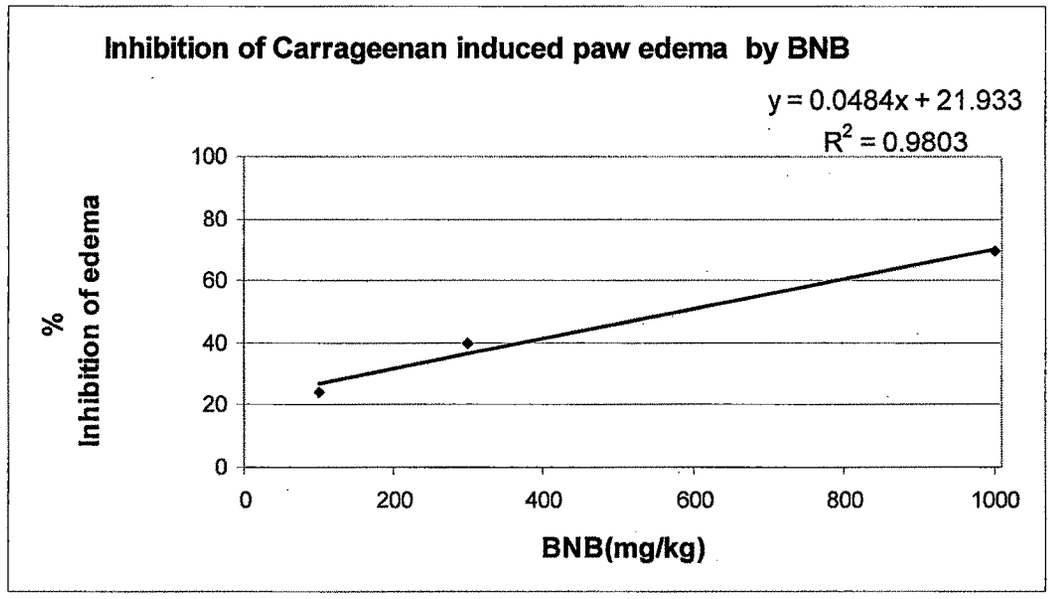
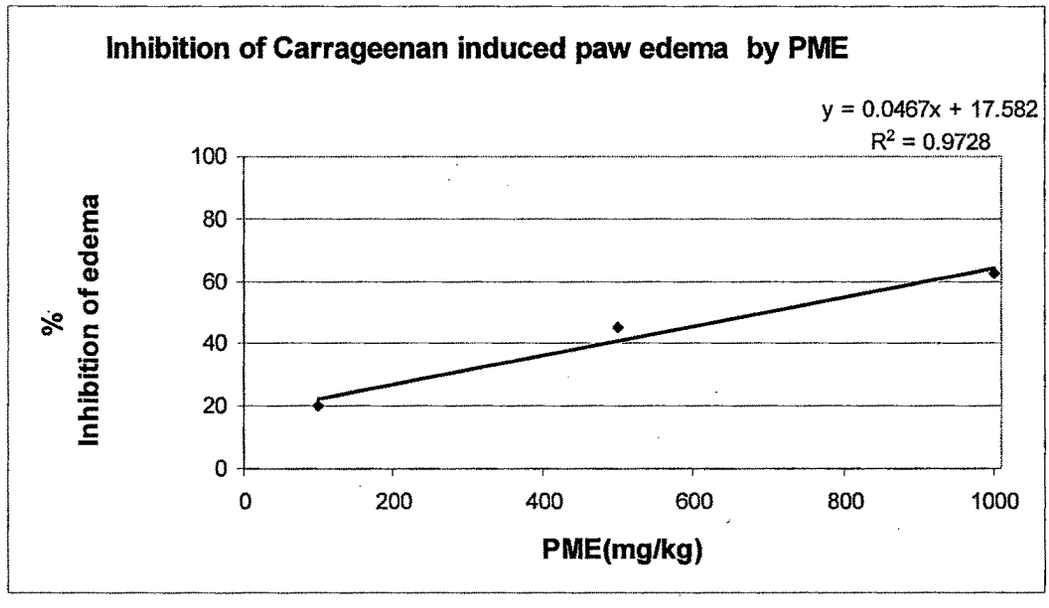


Figure 31. Effects of on PME rat paw edema induced by carrageenan. PME was dosed p.o. 1 h before the intraplantar injection of carrageenan at three doses of PME (100-1000 mg/kg/p.o.).). Following plot obtained from paw edema inhibition measured for the animals pre-treated three different doses 100, 500, 1000 mg/kg/p.o. of PME. ED_{50} value was calculated based on the % inhibition of edema from three different doses. ED_{50} 694±27mg/kg/p.o..



Chronic model of inflammation- Adjuvant-induced arthritis in rats

Table 14. Effect of test drugs on paw edema in adjuvant-induced arthritis model, Adjuvant *mycobacterium tuberculosis* was injected s.c. at day zero, Test drugs , Indomethacin, MCR-363, BCOV, BNB, and PME were administered orally 0-21 days after injection of adjuvant. n=5 for each dose. The Data showed as MEAN±SEM. **P<0.05 or as compared to adjuvant induced control group.

Sr.No	Test Substance	Dose mg/kg/p.o.	Adjuvant Injection at day 0	MEAN Increase in Paw(right hind) volume (ml) (0-21 days)	Rat adjuvant Arthritis % inhibition
1	Normal Control	Vehicle (1% CMC)	--	0.33±0.07	---
2	Adjuvant Control	- Vehicle (1% CMC)	√	2.23±0.33	0
3	Indomethacin	6	√	0.60±0.15**	73.25
4	MCR 363	100	√	0.81±0.17**	63.74
5	BCOV	100	√	0.72±0.14**	67.85
6	BNB	500	√	0.99±0.21**	55.34
7	PME	500	√	1.24±0.33**	43.91

Figure. 32. Effect of test drugs on body weight of rat in adjuvant-induced arthritis. Effect of adjuvant administration at day 0 and followed by 21 days treatment of indomethacin (5mg/kd/day/p.o.), MCR 363(100mg/kg/p.o.), BCOV(100mg/kg/p.o.) BNB(1000mg/kg/p.o.), PME(1000mg/kg/p.o.) on rat body weight. Results are expressed as the mean body weight grams. At day 21, Treatment groups animals showed significant attenuation of weight loss indomethacin (5mg/kd/day/p.o.), MCR 363(100mg/kg/p.o.), BCOV(100mg/kg/p.o.) as compared to the vehicle treated control adjuvant arthritic rats(**P<0.05 as compared to arthritis control group, n=5)

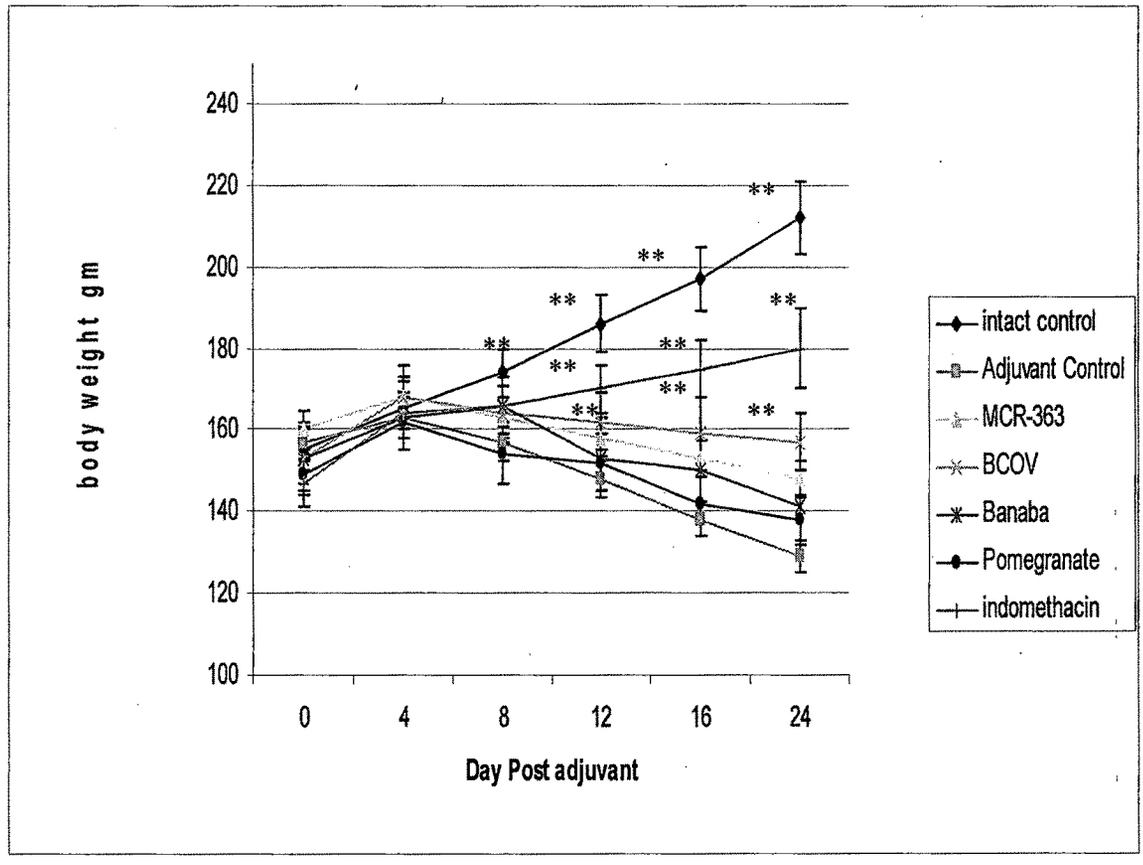


Figure 33. Effect of test drugs on the footpad volume in rat adjuvant induced arthritis. Effect of adjuvant administration and drug treatment on paw swelling. Results are expressed as the mean paw volume±SEM. Hind limb swelling in treatment groups indomethacin (5mg/kd/day/p.o.), MCR 363(100mg/kg/p.o.), BCOV(100mg/kg/p.o.) animals had significant attenuation of paw inflammation compared with that seen in the vehicle -treated control arthritic rats(**P<0.05, n=5)

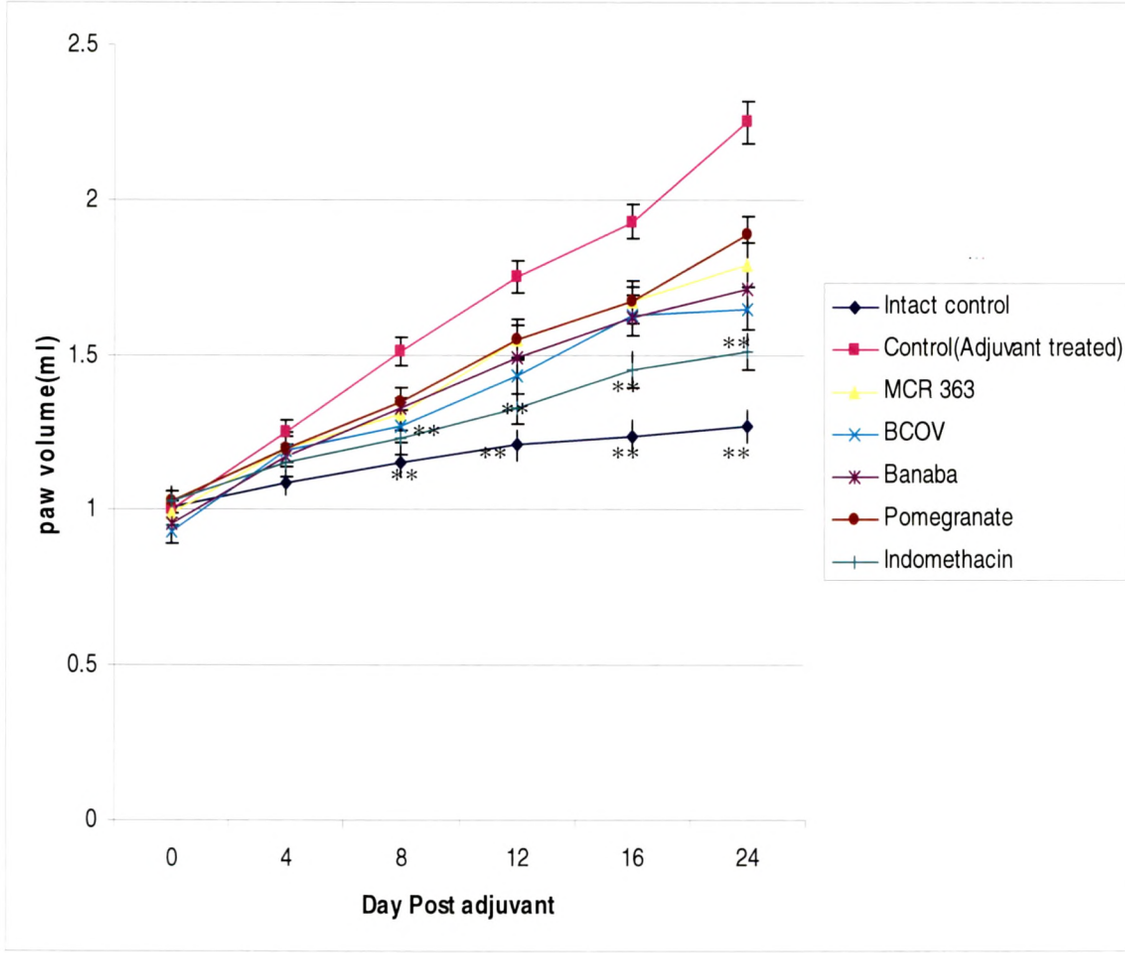
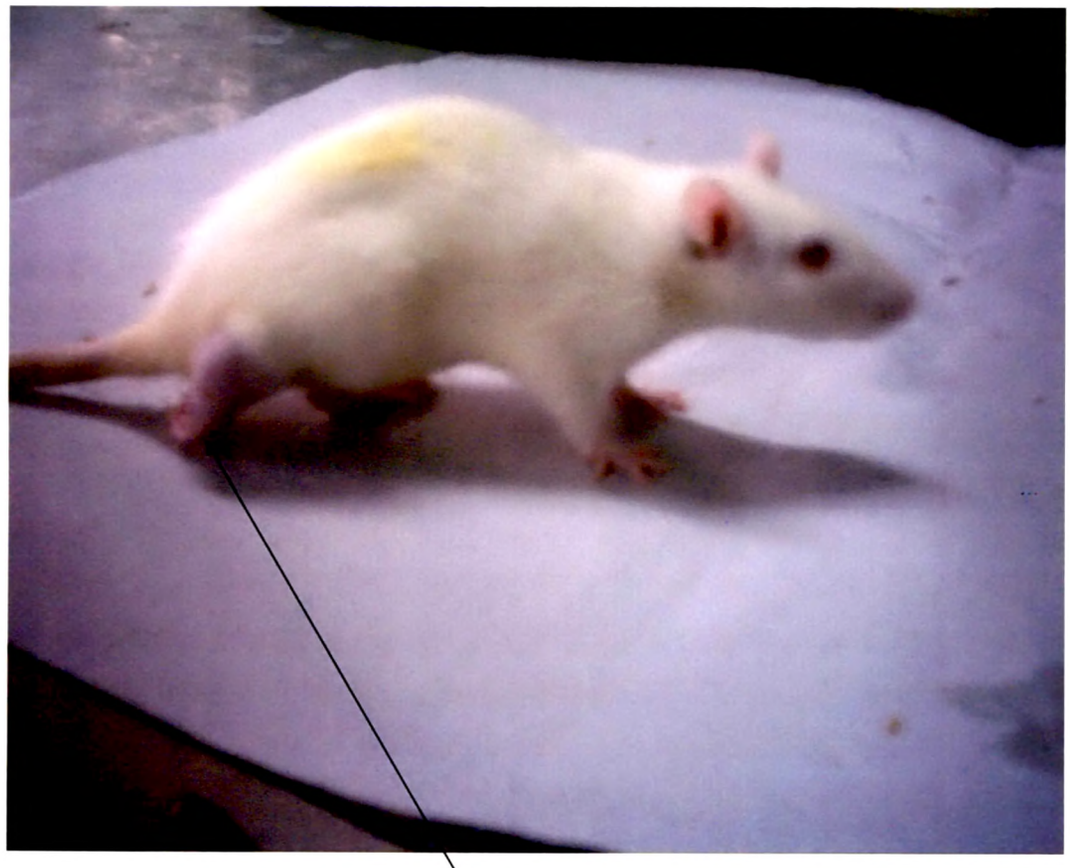


Table 15. Data shows mean organ weights in mg. Evaluated as an organ weight per 100 g of the body weight of each rat. Data are expressed as the mean ± SEM (n=5). Organ weight in MCR-363, BCOV, BNB, PME treated arthritic animals was non significant compared with vehicle treated AA control group. Indomethacin treatment 5mg/kg/p.o./po showed significant tissue weight difference compared to AA control (**P <0.05, as compared to arthritic control rat).

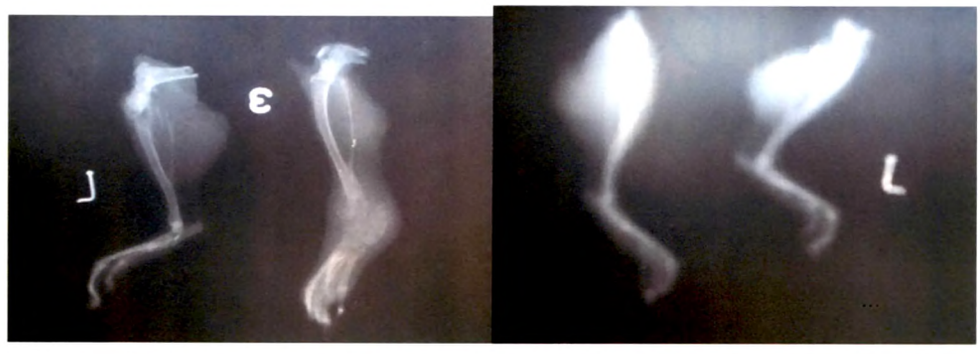
		Tissue weight in milligrams		
Groups	**Dose**	**Thymus**	**Spleen**	**Adrenals**
Normal Control	--	170.3 ± 5.4	227.8 ± 10	22.0 ± 1.0
AA control	--	297.0 ± 13.3	330.9 ± 7.8	26.6 ± 0.6
MCR-363	100mg/kg/p.o./po	274.0 ± 4.2	327.4 ± 16.4	26.7 ± 0.5
BCOV	100mg/kg/p.o./po	270.1 ± 21.1	353.8 ± 4.3	26.1 ± 1.6
BNB	1000mg/kg/p.o./po	291.8 ± 14.4	350.5 ± 24.8	25.4 ± 1.3
PME	1000mg/kg/p.o./po	291.8 ± 14.4	350.5 ± 24.8	25.4 ± 1.3
Indomethacin	5mg/kg/p.o./po	167.3 ± 5.4**	226.8 ± 10.7**	21.0 ± 1.0**

Figure 34. Picture shows development of arthritis in control rat at 16th day post adjuvant injection in vehicle treated control group, inflammation in hind paw clearly seen. Hind limb swelling in indomethacin, MCR-363, BCOV, BNB, and PME treated arthritic animals showed inhibitied as compared vehicle(1% CMC) treated arthritic control rats.



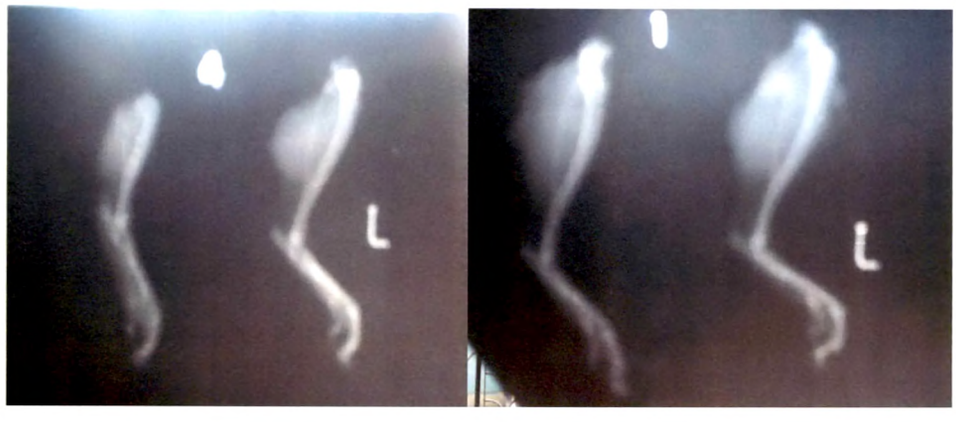
Paw inflammation in adjuvant induced-arthritis control animal

Figure 35. Radio graph of control, MCR 363, BCOV, BNB, PME, Indomethacin treated group in the rat adjuvant arthritis model. There was marked periarticular soft tissue swelling. The tarsocrural joint is collapsed and the intertarsal articulations are narrow in AA control group . There were multiple sites of cortical and trabecular osteolysis. Relatively less damage can bee seen in x-rays of groups PME(1000mg/kg/p.o./day.), MCR363(100mg/kg/p.o./day), BCOV(100mg/kg/p.o./day) and BNB(1000mg/kg/p.o./day) as compared to adjuvant arthritic control group.



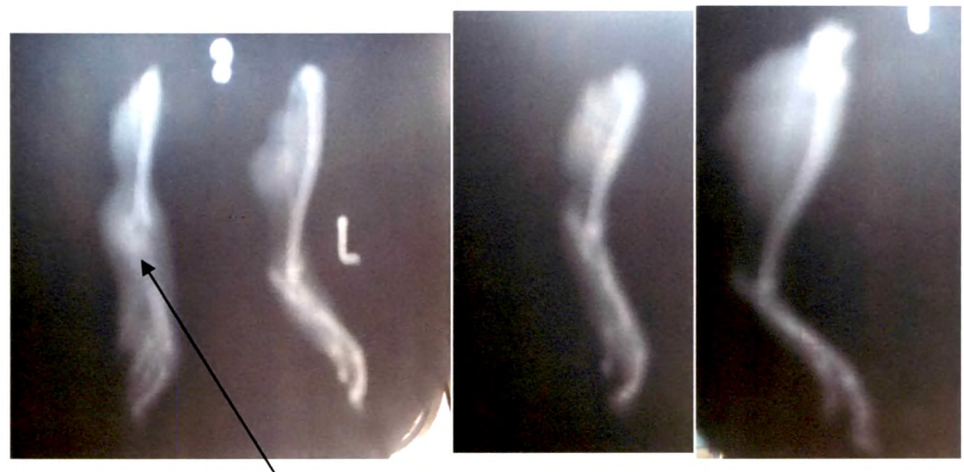
a. PME

b. BNB



c. BNB

d. MCR 363



e. AA control f. Indomethacin

Joint inflammation in paw of Adjuvant induced arthritic rat.

Chronic model of Inflammation- TNBS induced colitis

Table 16. Mean Change in body weight in gram after 0-18 days treatment with MCR 363, BCOV, BNB and PME. There was significant weight loss in TNBS control animals as compared to normal control. (n=5,** $p < 0.05$)

No.	GROUPS	DOSE (0-18days)	Change in Body Weight (grams)		
			Day 7	Day 14	Day 18
1	-VE CONTROL	--	-2.00	6.83	3.17
2	TNBS (at day 0)	--	-1.00	-17.33**	-21.67**
3	TNBS (at day 0) + **MCR-363**	MCR-363 100mg/kg/p.o./day	4.33	5.33	-1.33
4	TNBS (at day 0) + **BCOV**	BCOV 100mg/kg/p.o./day	2.00	7.50	3.33
5	TNBS (at day 0) + **BNB**	BNB 500mg/kg/p.o./day	2.83	4.33	-2.00
6	TNBS (at day 0) + **PME**	PME 500mg/kg/p.o./day	1.95	4.6	1
7	TNBS (at day 0) + **Sulphasalazine**	Sulphasalazine 20mg/kg/p.o./day	-2	5.92	3.3

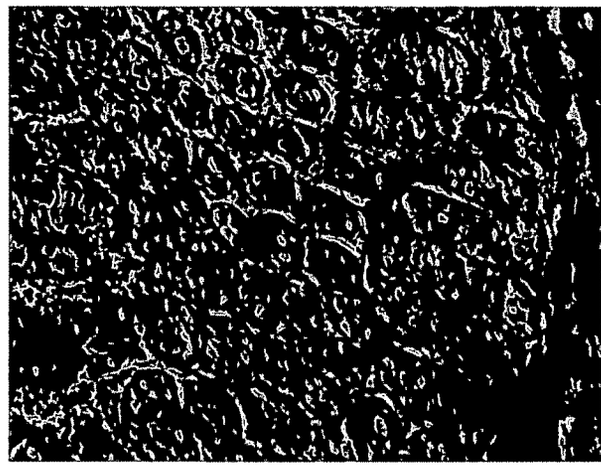
Table 17. Mean Change in colon weight in gram after 0-18 days treatment with MCR 363, BCOV, BNB and PME. There was significant colon weight difference in MCR 363, BCOV, BNB and PME treated group as compared to TNBS control animals (n=5,** $P < 0.05$)

No.	GROUPS	DOSE (0-18days)	Relative weight of colon in grams
1	-VE CONTROL	--	0.32+0.02
2	TNBS (at day 0)	--	0.51+0.05
3	TNBS (at day 0) + **MCR-363**	MCR-363 100mg/kg/p.o./day	0.30+0.06**
4	TNBS (at day 0) + **BCOV**	BCOV 100mg/kg/p.o./day	0.36+0.04**
5	TNBS (at day 0) + **BNB**	BNB 500mg/kg/p.o./day	0.27+0.04**
6	TNBS (at day 0) + **PME**	PME 500mg/kg/p.o./day	0.39+0.05**
7	TNBS (at day 0) + **Sulphasalazine**	Sulphasalazine 20mg/kg/p.o./day	0.25+0.04**

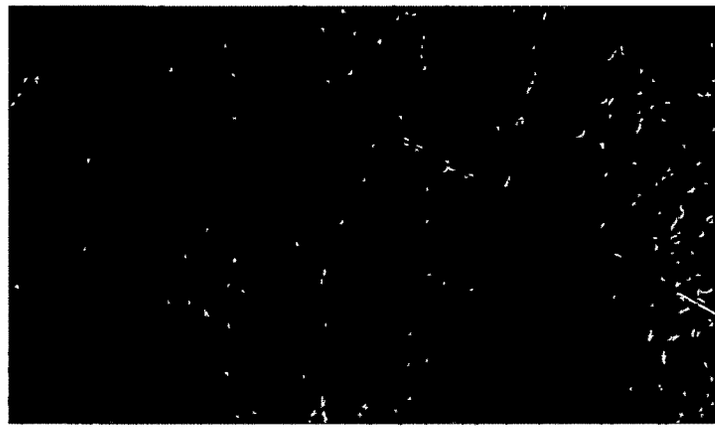
Table 18. Effect of test drugs on MCR 363, BCOV, BNB and PME. On MPO level of colon tissue. The data express as mean± IU/mg of tissue. There was significant difference between MCR 363, BCOV, BNB, PME and Sulphasalazine as compared to TNBS control group. (**P<0.05, n=5)

No.	GROUP	DOSE (0-18days)	MPO IU/mg
1	-VE CONTROL	--	0.53±0.05
2	TNBS (at day 0)	--	3.53±0.23
3	TNBS (at day 0) + **MCR-363**	MCR-363 100mg/kg/p.o./day	0.57±0.03**
4	TNBS (at day 0) + **BCOV**	BCOV 100mg/kg/p.o./day	0.45±0.04**
5	TNBS (at day 0) + **BNB**	BNB 500mg/kg/p.o./day	0.53±0.04**
6	TNBS (at day 0) + **PME**	PME 500mg/kg/p.o./day	0.43±0.03**
7	TNBS (at day 0) + **Sulphasalazine**	Sulphasalazine 20mg/kg/p.o./day	0.40±0.04**

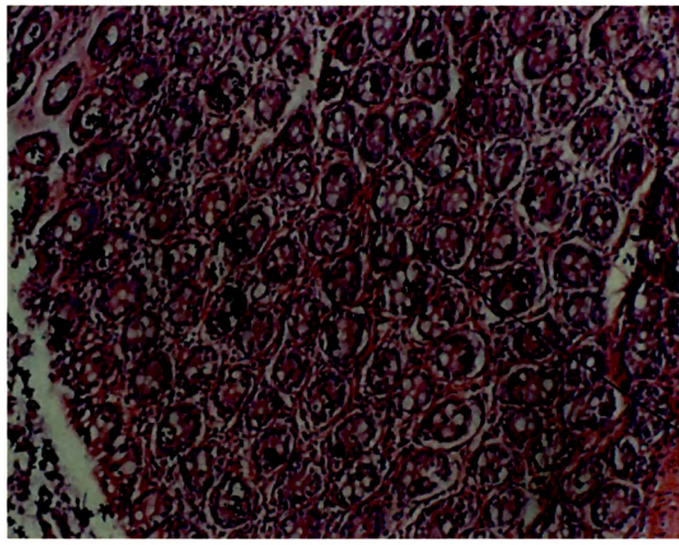
Figure 36. Histological sections of colonic mucosa from colitis rats 4 weeks after trinitrobenzenesulphonic acid (TNBS) instillation. (A) Normal control (B) TNBS control group showing the persistence of wide areas of ulceration, inflammation and fibrosis with associated regenerative changes in the adjacent mucosa (C) MCR 363-treated group (100mg/kg/p.o.). (D)BCOV -100/mg/kg/p.o. (E) BNB-500mg/kg/p.o. (F) PME- 500mg/kg/p.o. (G) Sulphasalazine- showing the healing intestinal wall with residual patchy inflammatory infiltrate and submucosa brosis (original magnification,x 40).



A. Normal Control- no damage seen in colonic mucosa

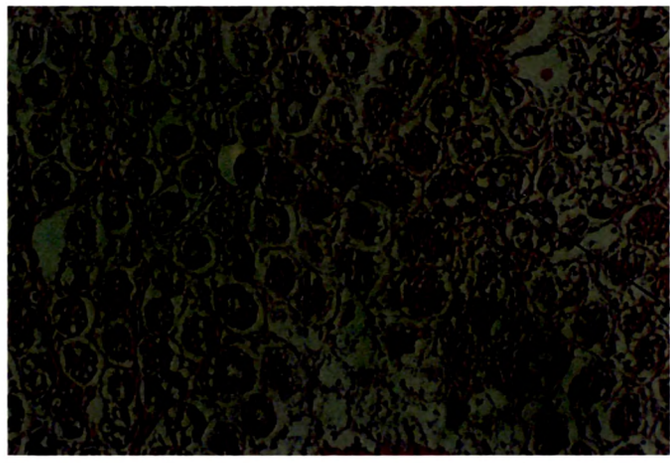


B. TNBS –control- damage seen in colonic mucosa



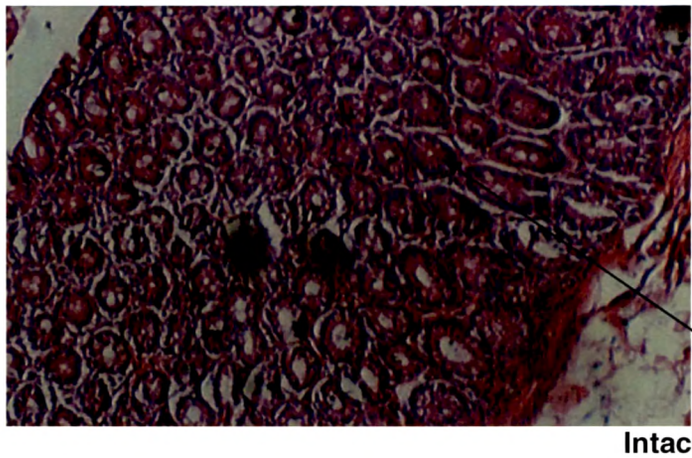
Intact mucosa

C. MCR- 363- no damage in colonic mucosa

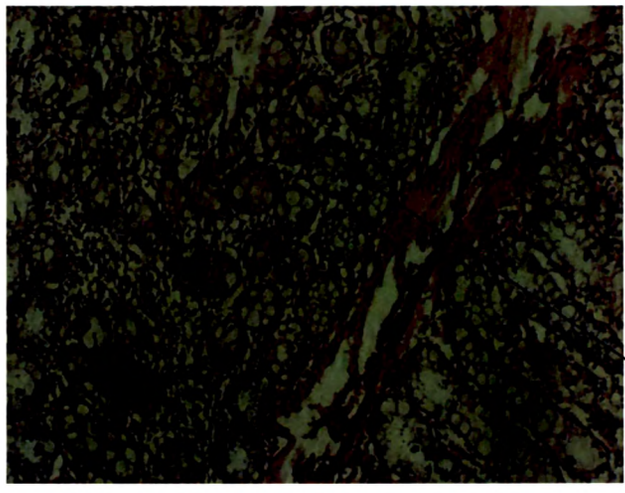


Intact mucosa,

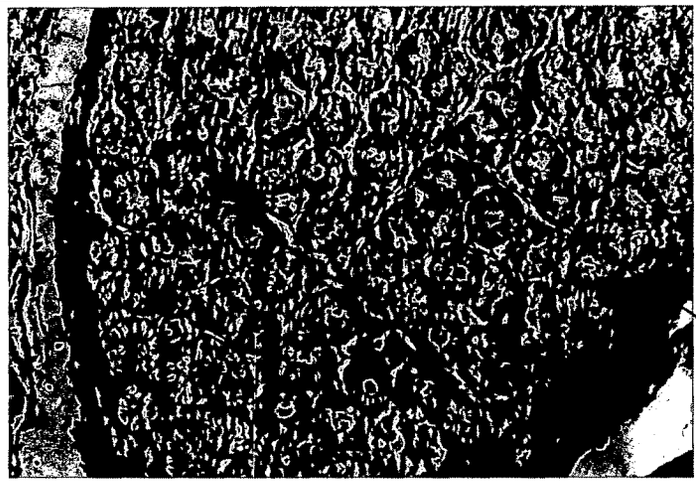
D. BCOV- less damage in colonic mucosa



E. BNB- very less damage in colonic mucosa



F. PME- less damage in colonic mucosa



G. Sulphasalazine - no damage in colonic mucosa

Table 19:

Morphological grades for colon for control and test drugs on MCR 363, BCOV, BNB and PME. Data shows mean score(min-0, max-4) n=5

No.	GROUP	DOSE	Morphological grades for colon (Mean)
1	Normal Control	--	0.00
2	TNBS Control	--	4.00
3	MCR-363	100mg/kg/p.o./day	1.00
4	BCOV	100mg/kg/p.o./day	0.50
5	BNB	500mg/kg/p.o.	0.5
6	PME	500mg/kg/p.o.	0
7	Sulphasalazine	20mg/kg/p.o.	0.3

Analgesic activity in mice

Table 20. Analgesic activity of compounds on acetic acid induced abdominal constriction test method in mice. Data shows total counts of writhes/ 10 mins after acetic acid i.p. administration. Mean ± SEM ,n=5. Significant difference in writing counts have seen in treatment group Diclofenac sodium, MCR 363, BCOV, BNB, PME as compared to vehicle treated acetic acid control group. (n=5, **P<0.05)

			No. of writhes/ 10 mins	**Acetic Acid induced writhing**
Sr.No	**Test**	**Dose**		**% Inhibition**
	Substance	**mg/kg/p.o.**	Mean± SEM	Mean
1.	Normal control	--	0	--
2.	Acetic acid Control	--	45±7	--
3	Diclofenac sodium	40	18±3**	72.5
4	MCR 363	25	26±3**	35.78
5	BCOV	25	28± 7**	33.45
6	BNB	500	29± 10**	31.23
7.	PME	500	37± 7**	17.23

Table 21. Analgesic activity of test drugs on tail immersion method in mice. Analgesic activity of test in hot water (55°C) tail immersion method in mice. Data shows time taken to flick the tail immersed in hot water(55°C) in sec. Mean ± SEM ,n=5. Only Tramadol has shown some significant inhibition as compared to vehicle treated control group. (n=5, **P<0.05)

			Time in seconds	**Tail Flick**
Sr.No	**Test**	**Dose**	**Tail withdrawal time in sec.**	**% Inhibition**
	Substance	**mg/kg/p.o.**		Mean± SEM
1	Control	--	5.5±2	
2	Tramadol	5	15±3**	75.27
2	MCR 363	25	6.7±3	10.23
3	BCOV	25	5.9±3	7.2
4	BNB	500	6.2±3	4.90
5	PME	500	6±3	2.66

Endotoxin-induced pyresis in rat

Table 22. Lipopolysaccharide (0.36 mg/kg/i.p.) was injected i.p. at time zero; Test drugs MCR-363, BCOV, BNB, PME, Paracetamol were administered orally 5hr after injection of LPS. MCR 363, BNB, BCOV and standard drug paracetamol treatment group showed significant reversal of body temperature as compared to control. (n=5, **P<0.05)

Sr.No	Test	Dose	Change in Temperature- °C	% Inhibition
	Substance	**mg/kg/p.o.**		Mean± SEM
1	Normal Control	--	0.0	--
2	LPS Control	--	2.35±0.25	
3	MCR-363	25	1.25±0.21**	54.2
		12.5	1.77±0.3**	25.1
		6.25	2.05±0.2	10.5
4	BNB	500	1.90±0.26**	23.05
5	PME	500	1.29±0.23**	52.30
6	BCOV	100	1.11±0.27**	61.90
7	Paracetamol	25	0.93±0.22**	63.8
		10	1.67±.25**	39.9

4.3 Toxicological evaluations including safety pharmacological studies.

4.31 (Toxicological studies)-SAFETY PHARMACOLOGICAL STUDIES:

4.31.1 Gastrointestinal Tract

- *In vivo* assay for Ulcerogenicity[209]

It has been shown unequivocally and consistently in experimental models that COX-2 inhibitors do not cause gastric lesions even at above effective anti-inflammatory doses, [176,177] in stark contrast to NSAIDs, which induce gastrointestinal lesions after a single acute dose. The major side effect of conventional NSAIDs is gastropathy manifested as gastric bleeding, ulceration, and alterations in gut motility, emesis and diarrhea, thought to be caused by their inhibition of COX-1 in the gastrointestinal tract. Indomethacin, at 20 induced visible, hemorrhagic gastric lesions in all rats 4 hr after dosing (figure 36). In comparison MCR 363, BCOV, BNB, PME were without any gastrointestinal damaged at doses up to 100mg/kg/p.o. for synthetic compounds and 1000mg/kg/p.o. for herbal drugs (table- 23). This contrasts strongly with current NSAIDs, in which case the major cause of treatment withdrawal is because of gastrointestinal complications.

5.22. Cardiovascular safety study

5.22.1 *In vivo* CVS safety studies[210].

In the present study, the resting mean arterial blood pressure of urethane anaesthetized g. pigs was found to be 123±5 mmHg which is similar to the value reported for barbiturate anaesthetized g. pigs Gardiner *et* al., 1980. Test substance suspended in 1% CMC was administered to the guinea pig in a single oral dose by gavages using a feeding needle before half hour of the blood pressure monitor Administration of 100 mg/kg/p.o. of the MCR 363 no significant difference found up to 3hrs. BCOV treatment with 500mg/kg/p.o. dose gives mean blood pressure 125±5 mmHg which is similar to control group (figures 37, 38). Neither BNB at 1g/kg dose nor PME at 1g/kg have any significant effect on mean blood pressure compared to control group.

In vehicle treated control animals the heart rate was 350±21 and the QT interval 88±1ms given in table 24. Test compounds MCR 363, BCOV, BNB and PME exhibit no significant effect on heart rate and QT interval in g. pigs. The results of this study demonstrate the safety of test substances MCR 363, BCOV, BNB and PME on CVS system. (Figures 37, 38, table 24)

The figure and table shows that there is non significant effect of test drugs MCR 363, BCOV, BNB and PME on Heart rate, Mean blood pressure and ECG in guinea pigs.(figure 37, 38, table 24)

5.22.2 Isolation, mounting and stabilization of isolated aortic rings[211]

PE induced contraction of all the groups is shown in Fig. 39. There was no significant change in PE induced contraction of any of the groups except MCR 363. MCR 363 30μM shown vasodilating effect (fig. 39, 40) and shown relaxation of PE induced contraction of rat aorta. This was expected because of the NO release from MCR 363 gave local vasorelaxation effect. No significant effect of either presence of BCOV found on the PE induced contraction. Herbal drugs BNB and PME have no effect on aortal contraction induced by PE.

5.23 Central nervous system safety studies:

5.23.1 Locomotor activity in mice[212]

Acute treatment of MCR 363, BCOV, BNB, PME have no effect on locomotor activity in mice (table 25). While acute treatment of the diazepam on locomotor was depressant and the total counts was significantly reduced. Whereas the effect of test drugs MCR 363, BCOV, BNB, and PME was non significant compared to vehicle treated control group.

5.23.2 Observational assessment

Irwin's test is well accepted protocol for assessment of any behavioral effect of test drug. There is no significant effect found in awareness, grooming, motor activity, motor incoordination, muscle tone and posture was seen in animals treated MCR 363, BCOV, BNB and PME. Table 26 shows assessment of animal behavioral response after administration of test drugs MCR 363, BCOV, BNB, and PME. Each number is a median (plus interquartile range) of behavioral scores in 5 separate mice.

5.23.3 Pentobarbital sleeping time in mice[214]

The absolute values of sleep latency and sleeping time showed table 27 demonstrate that animals treated MCR 363, BCOV at 100mg/kg/p.o. dose and BNB and PME at 1000mg/kg/p.o. dose, diazepam (1 mg/kg/p.o.), 1hr before injection of pentobarbital, presented a no significant effect found on sleeping time of MCR363, BCOV treated mice as compared to control group (table 27). Same result observed with herbal drug BNB and PME, no significant changes found as compared to pentobarbital treated control group. Diazepam treated group showed significant prolongation of pentobarbital-induced sleeping time as compared to vehicle treated pentobarbital control group. ($P < 0.05$).

5.23.4 Test for motor co-ordination (rota-rod test) in mice[212]

No alteration was observed on the rota rod test after treatment with test drugs MCR 363, BCOV, BNB, PME as compared to control (table 28), while diazepam (2 mg/kg/p.o.), as expected, significantly decreased this parameter as compared to vehicle treated control group.(P <0.05).

5.3 TOXICITY STUDIES- Acute and sub acute

5.3.1 Acute toxicity studies

Changes in general behaviors, body weight and internal organ weight are critical for the objective evaluation of the effect of a compound on test animals, since such changes are often the first signs of toxicity. In acute toxicity study, after the single administration of oral doses 0.3,1, 2, 4, 5g/kg test drugs MCR 363, BCOV, BNB, and PME. Even at a single dose of 5,000 mg/kg/p.o. in all groups, neither sign of toxicity nor death of mice was observed during the 14 days of the experimental period except at very high dose 4, 5 g/kg 1 mouse in each group found dead at 2nd day of experiment. The LD_{50} values of compounds MCR 363, BCOV, BNB, PME is >5 g/kg (table 29). The no-toxic effect level (NOEL) found in a single dose up to 5g/kg dose of all test drugs BCOV, BNB, and PME. In group MCR 363 doses 4, 5 g/kg found to be slightly lethal LD_{10} (table 29). Toxicity evaluation was further carried out by gross pathological examinations of the internal organs revealed no pathological abnormality as compared with the control. There was no significant difference found in either body weight or any organs weight (kidney, lung, liver, heart, brain, spleen). These results suggest that the test drugs MCR 363, BCOV, BNB, and PME are practically non toxic after an acute exposure.

5.3.2 Sub Acute toxicity(Pilot study)

Maximal therapeutic dose from previous *in vivo* study was selected for all test compounds in this study. Rats were divided in the group of 10(5 males + 5 females) and fed oral dose of compound MCR-363-100mg/kg/p.o., BCOV-100mg/kg/p.o., BNB 1000mg/kg/p.o., PME-1000mg/kg/p.o. daily. It was observed that the animals fed with MCR 363, BCOV, BNB, PME were healthy through out the study of 28 days. No symptomatic ocular toxicity found in all group up to 28 days of studies. No unusual changes in behavior or in locomotor activity, no ataxia, and no signs of intoxication were observed during the 28-day period. No differences were found in growth found between the control group and the animals fed with different levels MCR 363, BCOV, BNB, and PME. The difference between the food consumption and body weight of control and experimental groups animals were non significant, indicating that the feed intake and utilization was not affected (Tables 30, 31). At the end of study animal were sacrificed, there was no observable toxic effect seen in tissue weights and in gross pathological observation of all major organs including lung, liver, kidney, heart, brain spleen, adrenal, testis and ovary.(Table 33). All hematological parameters found to be non significantly affected in all treatment groups as compared to control group (Table 32). No significant changes found in histological observations of kidney and liver in treatment groups and vehicle treated control group. The no-toxic effect level (NOEL) found in a repeated oral administration of 100mg/kg/p.o. for MCR 363 and BCOV, and 1000mg/kg/p.o. for BNB and PME. (Figure 41, 42)

***In vivo* assay for Ulcerogenicity**

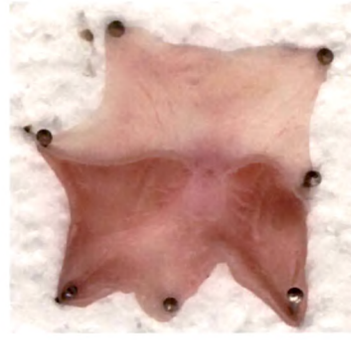
Table 23. Effect of test drugs MCR 363, BCOV, BNB and PME on rat gastric mucosa. The data expressed as Mean± SEM and % of animals having ulcers. Test compounds were administered orally 4hr before the rats were euthanized. Visible gastric lesion was scored and the sum determined. Indomethacin treatment 20mg/kg/p.o. showed significant ulceration as compared to control group (n=5, ***P<0.01)

Sr.No	Test	Dose	Mean score	Percentage of animals having ulcer
	Substance	**mg/kg/p.o.**		
1	Control	--	0	0
2	Indomethacin	20	23±2***	100
3	MCR 363	100	0	0
4	BCOV	100	0	0
5	BNB	1000	0	0
4	PME	1000	0	0

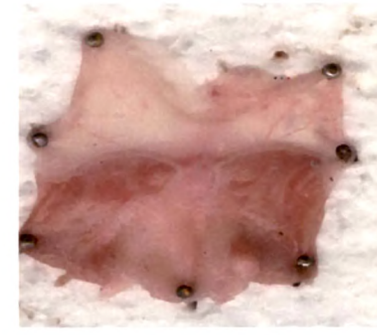
Figure 36. Ulcerogenic effect in rat, figure shows opened stomach after 4 hrs treatment of test compounds and standard drug Indomethacin. Test compounds were administered orally 4hr before the rats were euthanized. Visible gastric lesion was scored and the sum determined.

A. MCR 363 -100mg/kg/p.o.- no ulcer detected

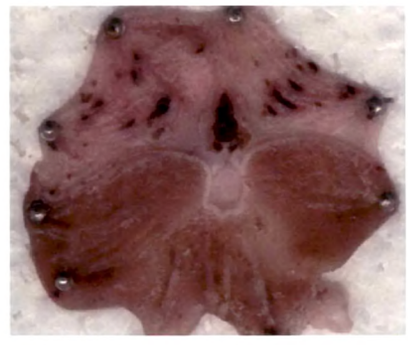
B. BCOV -100mg/kg/p.o. no ulcer detected



C. BNB- 1g/kg- no ulcer detected.



D. PME -1g/kg- no ulcer detected



E. Indomethacin 20mg/kg/p.o.- sever ulcers can seen

Cardiovascular safety study

Figure 37.

The mean arterial blood pressure (mmHg) in the animals treated with vehicle control, MCR-363(100mg/kg/p.o.), BCOV(100mg/kg/p.o.), BNB(banaba-1000mg/kg/p.o.), and PME (Pomegranate 1000mg/kg/p.o.) using BIOPAC Data acquisition system(BIOPAC MP30 SYSTEM, There were no significant difference found as compared to vehicle treated control group and other experimental groups.

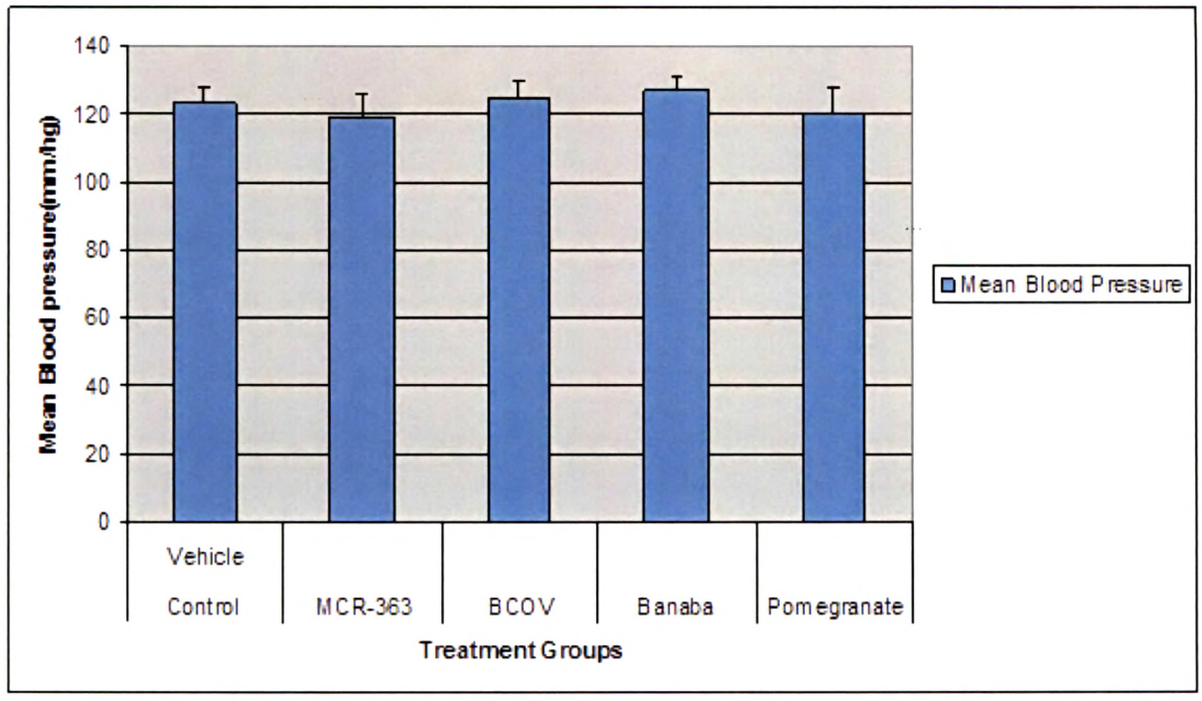
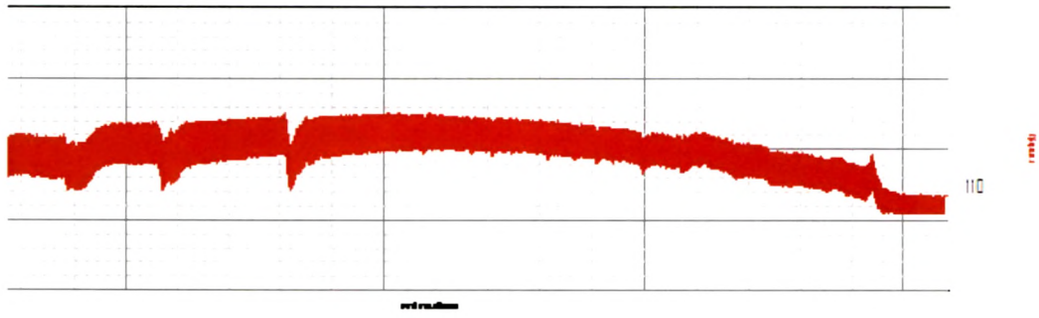
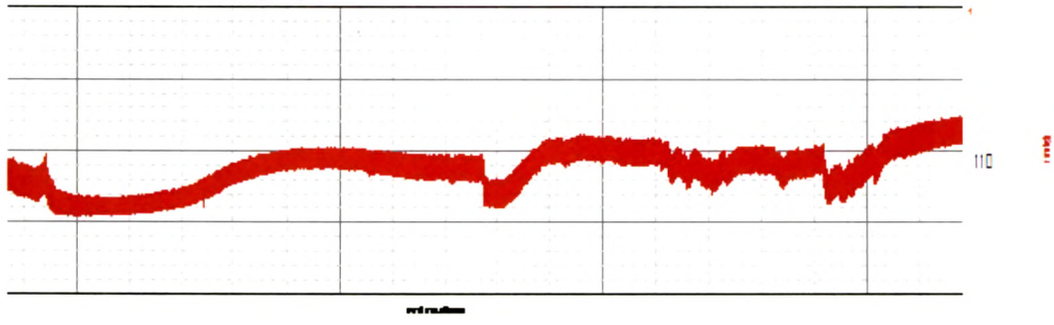


Figure 38. Tracing of mean arterial blood pressure (mmHg) on animal treated with vehicle control, MCR-363(100mg/kg/p.o.), BCOV(100mg/kg/p.o.), BNB(1000mg/kg/p.o.), PME(1000mg/kg/p.o.) in g. pig using BIOPAC Data acquisition system(BIOPAC MP30 SYSTEM, USA). There were no significant difference found as compared to vehicle treated control group and other experimental groups.(n=6)

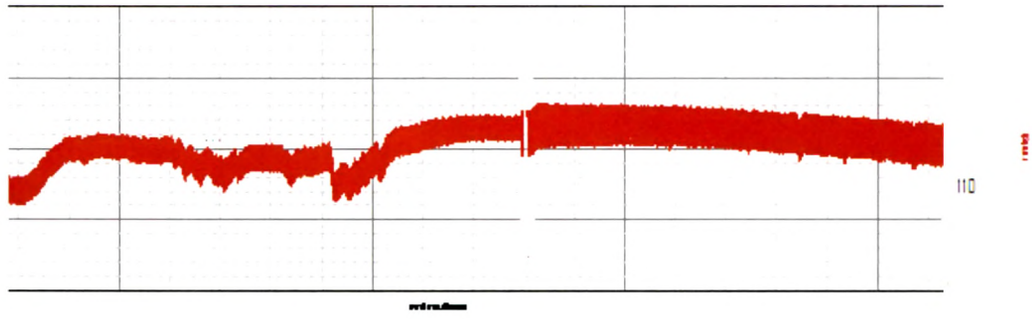
1. Control group- vehicle(1% CMC) treated.



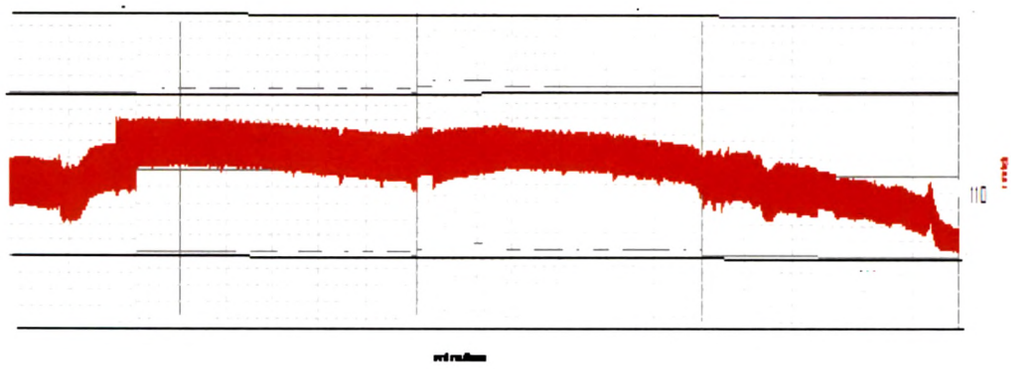
2. MCR 363- 100mg/kg/p.o.



3. BCOV- 100mg/kg/p.o.



4. BNB – 1000mg/kg/p.o.



5. PME- 1000mg/kg/p.o.

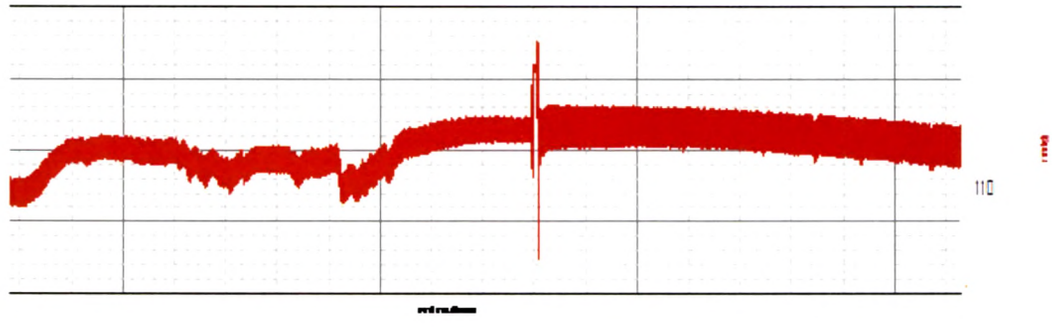
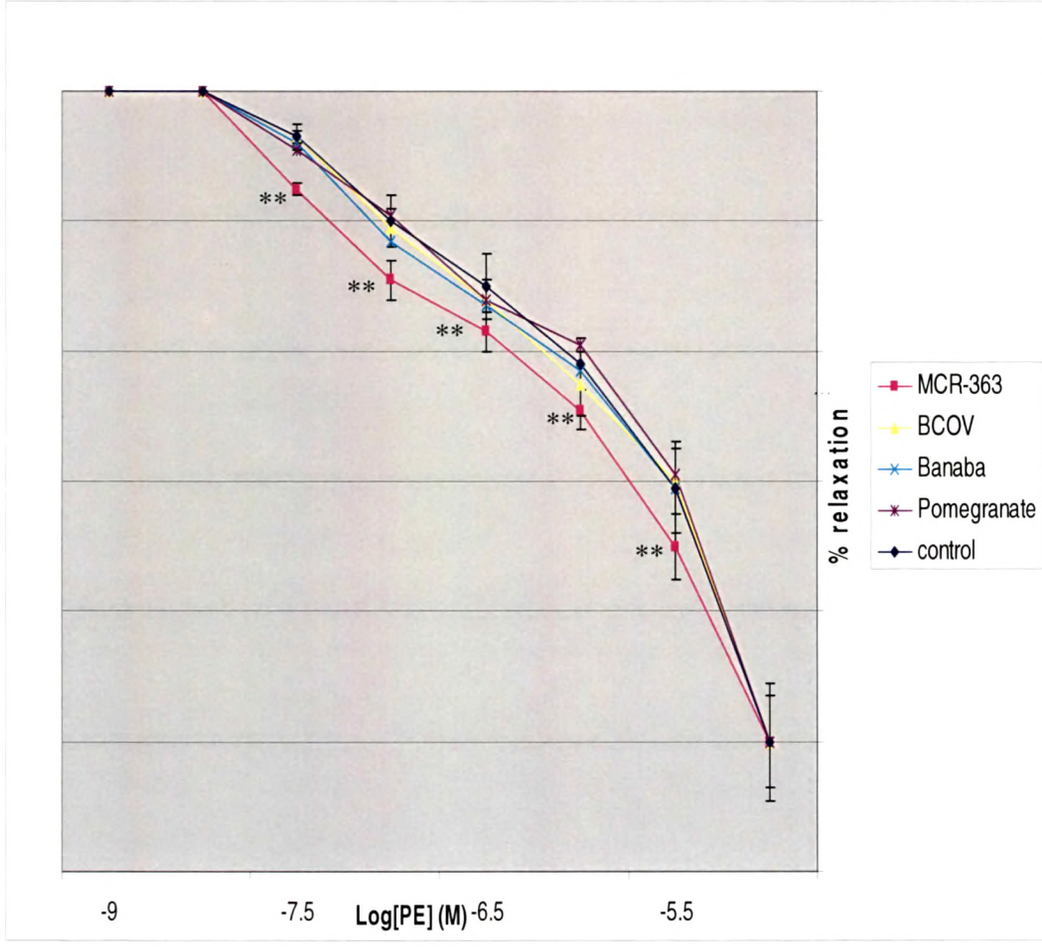


Table 24– Heart beats and QT interval(millisecond) on animal treated with vehicle control, MCR-363(100mg/kg/p.o.), BCOV(100mg/kg/p.o.), BNB(banaba-1000mg/kg/p.o.), PME(Pomegranate 1000mg/kg/p.o.) determined in g. pig using BIOPAC Data acquisition system(BIOPAC MP30 SYSTEM, USA). There were no significant differences in either heart rate or QT interval found as compared to vehicle treated control group and other experimental groups.(The data expressed as mean ±SEM, *n* = 6 in each group)

Groups	Dose	Heart beats	QT interval in millisecond
Control	Vehicle (1% CMC)	351±21	88±1
MCR-363	100mg/kg/p.o.	348±19	89±3
BCOV	100mg/kg/p.o.	355±23	85±4
BNB	500mg/kg/p.o.	347±27	87±3
PME	500mg/kg/p.o.	365±29	86±4

Figure 40. Effect of test drugs on PE induced contraction on isolated aorta graph shows concentration response curves for PE of isolated rat aorta in presence of MCR-363, BCOV, BNB, and PME. There was significant difference in % relaxation of aorta found between the MCR 363 and vehicle control group (n=5, **P<0.05)



Locomotor activity in mice

Table 25. Effect of test drugs MCR 363, BCOV, BNB, and PME on locomotor activity of mice using actophotometer. Data expressed as Mean±SEM There were no statistically significant differences between locomotor activity for control groups and the test MCR 363, BCOV, BNB, PME groups. There was significant difference between diazepam 1mg/kg/p.o. as compared to vehicle treated control group (**p<0.05, n=5)

Groups	Dose	Mean Counts
Control	Vehicle	175±11
MCR-363	100mg/kg/p.o.	185±12
BCOV	100mg/kg/p.o.	167±10
BNB	500mg/kg/p.o.	180±13
PME	500mg/kg/p.o.	172±10
Diazepam	1mg/kg/p.o.	24±5**

Observational assessment test in mice

Table 26– Irwin's behavioral assessment in mice showed response after 1hr of administration of test drugs MCR 363, BCOV, BNB, and PME. Each number is a median (plus interquartile range) of behavioral scores in 5 separate mice. There was no significant difference between the vehicle treated group and test drugs MCR 363(100mg/kg/p.o.), BCOV(100mg/kg/p.o.), BNB(1000mg/kg/p.o.), PME(1000mg/kg/p.o.).n=5

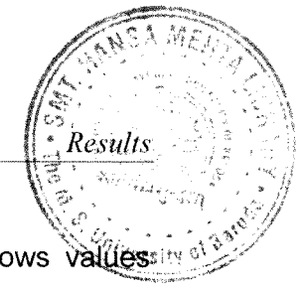
Treatments		Control	MCR 363	BCOV	BNB	PME
Awareness	Awareness	4.5(4-5)	4(4-5)	4.5(4-5)	4.5(4-5)	4.5(4-5)
Visual Placing	4(4-4)	4(4-4)	4(4-4)	4(4-4)	4(4-4)	4(4-4)
Passivity	0(0-0)	0(0-0)	0(0-0)	0(0-0)	0(0-0)	0(0-0)
Stereotypy	0(0-0)	0(0-0)	0(0-0)	0(0-0)	0(0-0)	0(0-0)
Mood	Grooming	4(4-4)	3.5(3-4)	4(4-4)	4(4-4)	4(4-4)
Vocalization	0(0-0)	0.5(0-1)	0(0-1)	0.5(0-1)	0.5(0-1)	0.5(0-1)
Restlessness	0(0-0)	0(0-0)	0(0-0)	0(0-0)	0(0-0)	0(0-0)
Irritability (aggression)	0(0-0)	0(0-0)	0(0-0)	0(0-0)	0(0-0)	0(0-0)
Fearfulness	0(0-0)	0(0-0)	0(0-0)	0(0-0)	0(0-0)	0(0-0)
Motor Activity	Reactivity (envir.)	4(4-4)	4(3-5)	4(4-4)	4(4-4)	4(4-4)
Spontaneous Activity	4(4-5)	4(3-4)	4(2-5)	4(3-4)	4(3-4)	4(3-4)
Touch Response	4(4-4)	4(3.5-4)	4(3-4)	4(3.5-4)	4(3.5-4)	4(3.5-4)
Pain Response	4(4-4)	4(4-4)	4(4-4)	4(4-4)	4(4-4)	4(4-4)
CNS Excitation	Startle Response	0(0-0)	0(0-0)	0(0-0)	0(0-0)	0(0-0)
Straub Tail	0(0-0.5)	0(0-0)	0(0-0)	0(0-0)	0(0-0)	0(0-0)
Tremors	0(0-0)	0(0-0)	0(0-0)	0(0-0)	0(0-0)	0(0-0)
Twitches	0(0-0)	0(0-0)	0(0-0)	0(0-0)	0(0-0)	0(0-0)
Convulsions	0(0-0)	0(0-0)	0(0-0)	0(0-0)	0(0-0)	0(0-0)
Posture	Body Posture	4(4-4)	4(4-4)	4(4-4)	4(4-4)	4(4-4)
Limb Position	4(4-4)	4(4-4)	3(3-4)	3(4-4)	3(4-4)	4(4-4)
Motor Incoord	Staggering Gait	0(0-0)	0(0-0)	0(0-0)	0(0-0)	0(0-0)
Gait Incapacity	0(0-0)	0(0-0)	0(0-0)	0(0-0)	0(0-0)	0(0-0)
Righting Reflex	0(0-0)	0(0-0)	0(0-0)	0(0-0)	0(0-0)	0(0-0)
Muscle Tone	Limb Tone	4(4-4)	4(4-4)	4(4-4)	4(4-4)	4(4-4)
Grip Strength	4(4-4)	4(3.5-4)	4(4-4)	4(3.5-4)	4(3.5-4)	4(3.5-4)
Body Sag	0(0-0)	0(0-0)	0(0-0)	0(0-0)	0(0-0)	0(0-0)

Abdominal Tone	4(4-4)	4(4-4)	4(4-4)	4(4-4)	4(4-4)	4(4-4)
Reflex	Pinna	1(1-1)	1(1-1)	1(1-1)	1(1-1)	1(1-1)
Corneal	1(1-1)	1(1-1)	1(1-1)	1(1-1)	1(1-1)	1(1-1)
IFR (Toe Pich)	4(4-4)	4(4-4)	4(4-4)	4(4-4)	4(4-4)	4(4-4)
Autonomic	Salivation	0(0-0)	0(0-0)	0(0-0)	0(0-0)	0(0-0)
Writhing	0(0-0)	0(0-0)	0(0-0)	0(0-0)	0(0-0)	0(0-0)
Palpebral opening	4(4-4)	4(3-4)	4(3-4)	4(3-4)	4(3-4)	4(3-4)
Exopthalmos	0(0-0)	0(0-0)	0(0-0)	0(0-0)	0(0-0)	0(0-0)
Urination	1(0-2)	1(0-1)	1(0-1)	1(0-1)	1(0-1)	1(0-1)
Piloerection	0(0-0)	0(0-0)	0(0-0)	0(0-0)	0(0-0)	0(0-0)
Skin colour	1(1-1)	1(4-4)	1(1-1)	1(4-4)	1(4-4)	1(4-4)
Respir. Rate	6(6-6)	5(5-6)	5(5-5)	5(5-6)	5(5-6)	5(5-6)
Miscellaneous	Lacrimation	0(0-0)	0(0-0)	0(0-0)	0(0-0)	0(0-0)
Diarrhea	0(0-0)	0(0-0)	0(0-0)	0(0-0)	0(0-0)	0(0-0)
Dead	No. Acute	0	0	0	0	0

Pentobarbital-induced sleeping time in mice

Table-27. Effect of test drugs on pentobarbital induced sleeping time in mice. Table shows values mean± SEM sleeping time in minutes for the treatment groups (vehicle control, MCR-363(100mg/kg/p.o.), BCOV(100mg/kg/p.o.), BNB(1000mg/kg/p.o.), PME(1000mg/kg/p.o.), and Diazepam (2mg/kg/p.o.)) All groups remained non significant except diazepam group which showed significantly higher sleeping time as compared to control group(**p<0.05) n=5.

Groups	**Dose, p.o.**	**Sleeping time(min)**
Pentobarbital Control	Vehicle	45±6
MCR-363	100mg/kg/p.o.	49±5
BCOV	100mg/kg/p.o.	45±6
BNB	500mg/kg/p.o.	51±7
PME	500mg/kg/p.o.	43±5
Diazepam	2mg/kg/p.o.	75±7**



Test for motor co-ordination (rota-rod test) in mice

Table 28. Effect of test drugs on rota rod test in mice. Table shows values mean± SEM time in seconds for the treatment groups (vehicle control, MCR-363(100mg/kg/p.o.), BCOV(100mg/kg/p.o.), BNB(1000mg/kg/p.o.), PME(1000mg/kg/p.o.), and Diazepam 2mg/kg/p.o.)) All groups remained non significant except diazepam which showed significantly lower in time performed by mice rota rod as compared to vehicle treated control(**p<0.05) n=5.

Groups	Dose	Time of permanence (s).
Control	Vehicle	125±11
MCR-363	100mg/kg/p.o.	126±13
BCOV	100mg/kg/p.o.	120±10
BNB	1000mg/kg/p.o.	130±12
PME	1000mg/kg/p.o.	121±9
Diazepam	2mg/kg/p.o.	10±3**

Acute toxicity study

Table 29: Single dose acute toxicity studies. Data expressed as no. of mice dead after 14 days observation of single dose ranges form 0.3-5 g/kg. The test performed in accordance to OECD-420 guideline. (n=12/dose).

		Treatment				
No. of mice	**Dose g/kg**	**Control (vehicle)**	**MCR 363**	**BCOV**	**BNB**	**PME**
		Number of mice dead				
12	0.3	0	0	0	0	0
12	1.0	0	0	0	0	0
12	2.0	0	0	0	0	0
12	4.0	0	1	0	0	0
12	5.00	0	1	0	0	0
LD_{50}		--	>5	>5	>5	>5

Data expressed as no. of mice dead. n=12.

Sub acute toxicity

Table 30. Food consumption of rats in the subacute toxicity study. Data expressed as mean food consumption in group. Mean food consumption of the Animals during the study period (g/animal) with treatment group

Groups	Dose	Day 0	Day 7	Day 14	Day 21	Day 28
Control	Vehicle	10.4±3	12.6±4	14.8±4	16.1±4	17.8±5
MCR-363	100mg/kg/p.o.	10.7±3	13.1±3	15.2±5	15.9±4	18.1±4
BCOV	100mg/kg/p.o.	10.0±2	11.9±4	14.2±4	15.1±5	17.0±5
BNB	500mg/kg/p.o.	10.6±3	12.2±3	14.5±3	15.5±3	17.6±4
PME	500mg/kg/p.o.	10.7±4	12.3±4	14.7±4	15.3±4	18.1±5

Values are expressed as mean ± SEM, n = 10.

There were no significant differences between vehicle treated control group and other experimental groups.

Table 31. Body weights of rats in the subacute toxicity study. Data expressed as mean food consumption per animal in group. Mean body weights animals during the study period in grams.

		Mean body weights of rats in grams				
Groups	**Dose**	**Day 0**	**Day 7**	**Day 14**	**Day 21**	**Day 28**
Control	Vehicle	201±10	212±12	235±13	245±12	257±13
MCR-363	100mg/kg/p.o.	207±11	219±11	238±13	248±13	262±15
BCOV	100mg/kg/p.o.	204±12	213±13	227±12	243±14	267±13
BNB	500mg/kg/p.o.	205±11	218±12	223±14	249±13	264±13
PME	500mg/kg/p.o.	203±12	219±13	235±12	245±15	259±14

Values are expressed as mean ± SEM, n = 10.

There were no significant differences between vehicle treated control group and other experimental groups

Table 32. Effect of 28 days multiple dose oral administration MCR 363, BCOV, BNB, PME on hematological parameters of rats in the subacute toxicity study. Values are expressed as mean ± SEM, n = 12, All treated groups were ns(non significant) as compared to vehicle treated control group.

Groups	Dose	Hb (g%)	RBC(10^6/mm3	Rt(%)	HCT(%)
Control	Vehicle	14.5+05	7.55+0.6	1.34+0.4	44.06+1.7
MCR-363	100mg/kg/p.o.	14.46+0.3	7.51+0.3	1.5+0.4	43.9+0.9
BCOV	100mg/kg/p.o.	14.44+0.4	7.57+0.45	1.5+0.3	43.62+0.8
BNB	500mg/kg/p.o.	14.45+0.3	7.57+0.3	1.52+0.3	44.02+1.0
PME	500mg/kg/p.o.	14.40+0.3	7.44+0.3	1.42+0.3	43.68+0.8

Groups	Dose	MCV(um3)	MCH(pg)	MCHC(%)	Platelets(105 /mm3)
Control	Vehicle	58.46+2.4	19.22+0.85	32.92+0.2	3.4+0.2
MCR-363	100mg/kg/p.o.	58.52+1.3	19.23+0.4	32.94+0.2	3.42+0.5
BCOV	100mg/kg/p.o.	59.08+2.3	19.18+0.37	33.06+0.2	3.5+0.3
BNB	500mg/kg/p.o.	58.18+1.6	19.14+0.5	32.99+0.3	3.37+0.25
PME	500mg/kg/p.o.	58.34+1.5	19.22+0.37	32.94+0.2	3.38+0.3

Groups	Dose	Total Leucocytes	N%	L%	E%	M%
Control	Vehicle	9.07+1.2	21.8	74.8	1	2
MCR-363	100mg/kg/p.o.	9.2+1.9	21.2	75.6	1.2	2
BCOV	100mg/kg/p.o.	9.9+2.1	22.6	74.6	0.6	2
BNB	500mg/kg/p.o.	10.0+1.9	21.8	75.2	1.2	1
PME	500mg/kg/p.o.	9.32+1.8	21.2	75.4	1	2

Table 33. Effect of 28 days multiple dose oral administration MCR 363, BCOV, BNB, PME on different organs weight lung, heart, liver, kidney, spleen, adrenal, ovary, testis and brain in the subacute toxicity study.

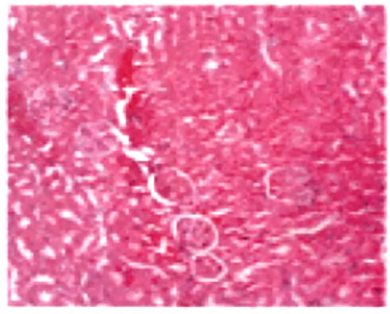
Values are expressed as mean ± SEM, n = 12.

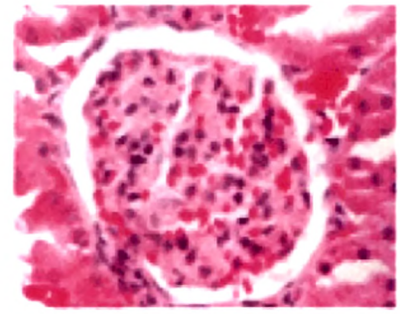
There were no significant differences found between vehicle treated control group and other experimental groups

	Organ weight in grams				
Organ	**Control**	**MCR 363 100mg/kg/p.o./day**	**BCOV 100mg/kg/p.o./day**	**BNB 1g/kg/day**	**PME 1g/kg/day**
Lung	1.09±0.06	1.10±0.05	1.14±0.04	1.18±0.04	1.11±0.06
Heart	0.88±0.02	0.93±0.05	0.85±0.03	0.94±0.06	0.87±0.03
Liver	8.23±0.38	8.48±0.54	8.18±0.57	8.30±0.46	8.35±0.38
Spleen	0.53±0.01	0.79±0.04	0.58±0.01	0.66±0.01	0.53±0.01
Adrenal	0.04±0.00	0.04±0.01	0.04±0.00	0.03±0.01	0.04±0.00
Kidney	1.29±0.1	1.29±0.2	1.27±0.2	1.31±0.2	1.33±0.05
Brain	1.02± 0.04	0.99± 0.04	1.06± 0.03	1.04± 0.5	1.09± 0.06
Testis (in male)	1.36±0.04	1.37±0.03	1.33±0.04	1.38±0.03	1.35±0.03
Ovary (in female)	0.06±0.00	0.06±0.00	0.07±0.00	0.07±0.00	0.06±0.00

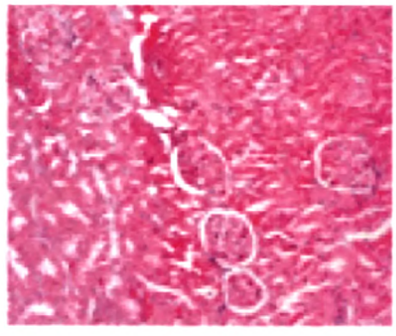
Figure 41: Histology of kidney of rat from sub acute toxicity. The histology of rat kidney from the control and treated groups (the 10x and 40x magnifications). No visible damage detected in any treatment group.

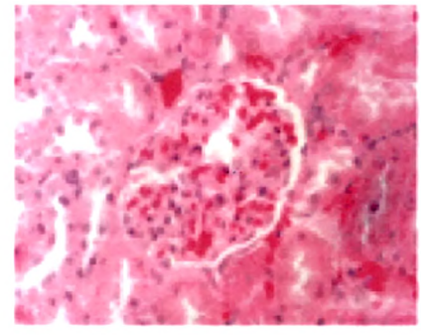
10x 40x



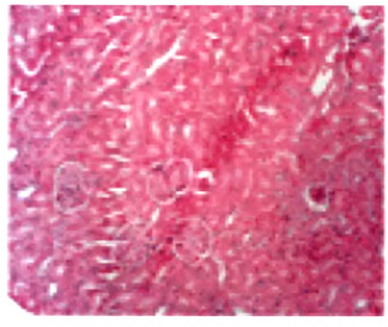


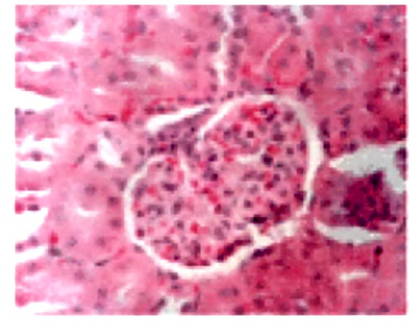
1. Control group



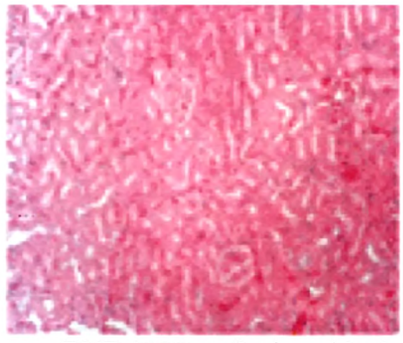


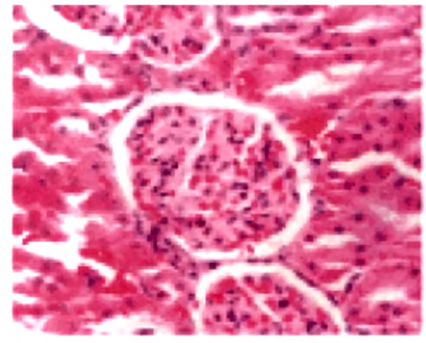
2. MCR 363(100mg/kg/p.o./day)



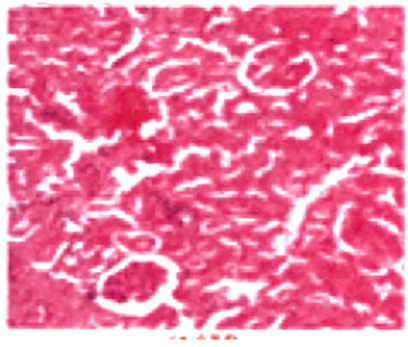


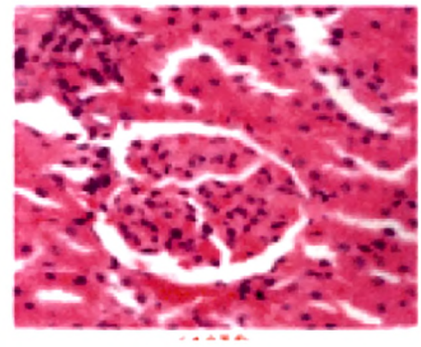
3. BCOV (100mg/kg/p.o./day)





4. BNB (500mg/kg/p.o./day)

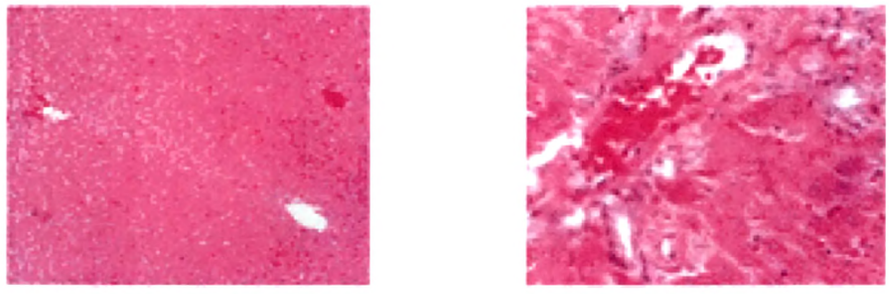




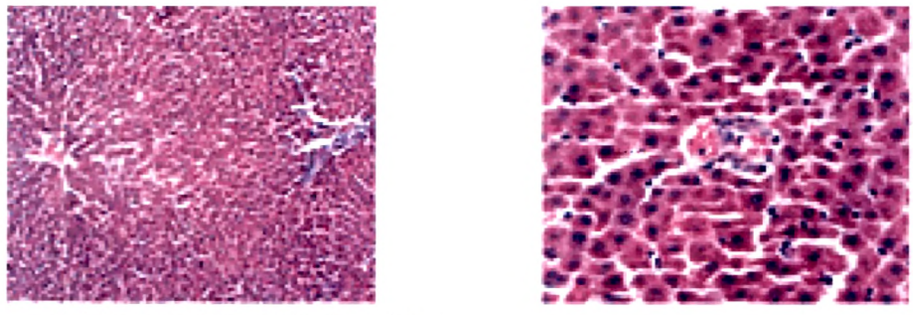
5. PME (500mg/kg/p.o./day)

Figure 42 : Histology of rat liver from sub acute toxicity. The histology of rat kidney from the control and treated groups (the 10x and 40x magnifications). No visible damage detected in any treatment group.

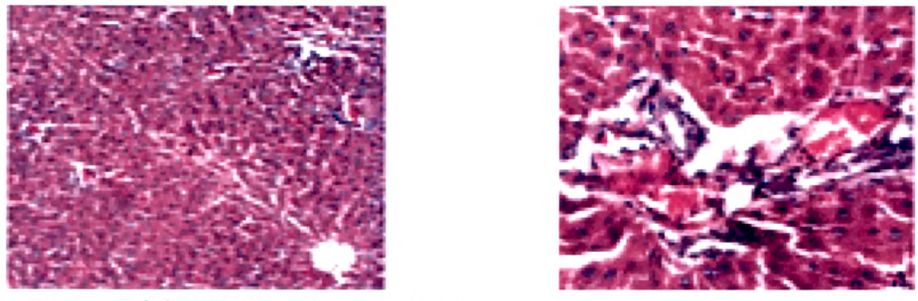
10x 40x



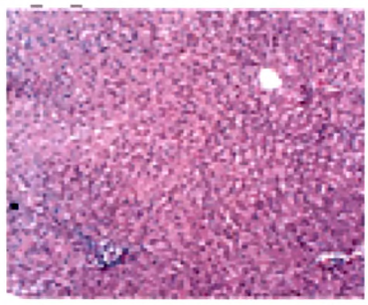
1. Control group

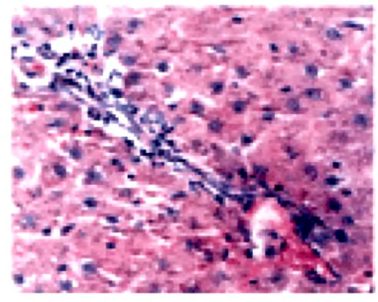


2. MCR 363 (100mg/kg/p.o./day)

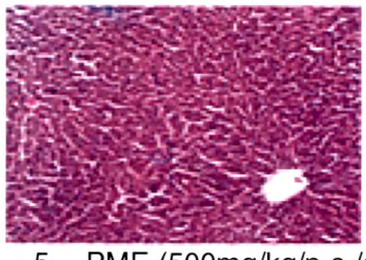


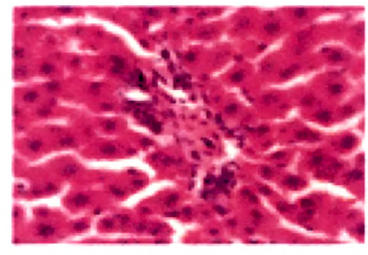
3. BCOV (100mg/kg/p.o./day)





4. BNB (500mg/kg/p.o./day)





5 PME (500mg/kg/p.o./day)