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Pharmacological Activities of Diclofenac Sodium-Loaded Microspheres (DSMSs) in Rats

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ABSTRACT

Diclofenac sodium (DS), a non-steroidal anti-inflammatory drug (NSAID) has been used as a novel anti-inflammatory, analgesic and antipyretic agent for many years both in human and animal pharmacotherapy. It is however, commonly associated with side effects including a high incidence of gastric and duodenal ulceration. Peptic ulceration, perforation, and gastrointestinal bleeding, sometimes severe and occasionally fatal, have been reported during the drug therapy. The present study has been designed to evaluate and compare the ulcerogenic effect of various doses of DS-loaded microspheres of polymethylmethacrylate (DSMSs), a sustained release dosage form, with DS in 150-200 g adult male albino Wistar rats and to make a comparative study of anti-inflammatory activities of DSMS with DS. Results indicate that ulcerogenicity decreases significantly with DSMS in comparison to the DS, while retaining the anti-inflammatory property intact.

Keywords: anti-inflammatory, duodenal ulceration, perforation, sustained release, ulcerogenicity

INTRODUCTION

Diclofenac sodium (DS) is a non-steroidal anti-inflammatory drug (NSAID) that has been used for more than 30 years to treat pain and inflammation. The pharmacological efficacy of DS is through potential inhibition of prostaglandin synthesis at relatively specific inhibition of cyclo-oxygenase 2 enzyme (COX-2) isoform (Fitzgerald *et al.* 2001). It is one of the most useful anti-inflammatory, analgesic and antipyretic agents in the treatment of rheumatoid arthritis, osteoarthritis (Galer 2010) and others joint pains (Schwartz *et al.* 1964). In case of rheumatoid arthritis and osteoarthritis, patients generally remember morning and evening medication but tend to forget doses in between (Krogh 1983). One or two daily doses improves therapy by maintaining steady state plasma concentration of the drug in the blood and avoids the peaks of high plasma concentration as well as the troughs of low plasma concentration (Nagasankar *et al.* 2000). Use of this drug by patients has become restricted because of its adverse complication, particularly to the gastrointestinal (GI) system (Zhang 2009). DS-loaded microspheres of polymethylmethacrylate (DSMSs) DSMS has been formulated to reduce GI tract irritation and to release the drug over a prolong period (12-14 hrs) (Tao *et al.* 2003). The mechanism of drug release from the microsphere is due to erosion of polymer matrices and by diffusion through the matrices (Gohel *et al.* 2010). The present experiment has been designed to know whether the formulation reduces the ulcerogenic property and shows better anti-inflammatory activity (Ray *et al.* 2010).

MATERIALS AND METHODS

Chemicals

Calcium chloride, dichloromethane, polyoxyethylene sorbitan monooleate [Tween 80] and sorbitan monooleat [Span-80] HLB value-4.3 were purchased from Merck Pvt. Ltd., Mumbai. Sodium alginate was obtained from SDFine-Chem Ltd., Mumbai and Diclofenac Sodium (Indian Pharmacopoeia) was a gift by M/S Dey's Medical Stores (Mfg) Ltd.

Animals

Adult male albino wistar rats (8 weeks), weighing 150–200 g were used. The ethical committee Jadavpur University approved all animal experiments (approval number: CPCSEA Regn no: (0367/01/C/CPCSEA) India). The animals were fed a normal laboratory pellet diet and water *ad libitum*. Animals were maintained under a constant 12-h light and dark cycle at an environmental temperature of 21-23°C for at least 1 week prior to the experiment.

Experimental design

The drug and the dosage forms were suspended in normal saline with sodium-carboxy methyl cellulose (Na-CMC) and gum acacia as suspending agent. A required volume was administered orally by gavages with an Fr 8 × 23-inch feeding tube. Fasted rats that were deprived of food but not water for 24 hrs prior to experiment were used to assess the effect of DS and DSMS on gastric mucosa.

Comparative ulcer study of DS with DSMS in rats

1. Formulation of DSMS

The microspheres were prepared by the water in oil in water (w/o/w) method (Viswanathan *et al*. 1999). An adequate amount of DS was uniformly dispersed in 1.5 ml of double distilled water (DDW) and emulsified with 2-4% of polymethylmethacrylate (PMMA) previously dissolved in dichloromethane with span 80 as the emulsifier. This w/o emulsion was again emulsified with 75 ml DDW with Tween 80 as the stabilizer. The microspheres formed by this normal multi-emulsion method were washed thoroughly, collected, dried and preserved for the analysis of pharmacological activity in rodent.

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Original Research Paper

2. In-vivo ulcerogenicity study

Ulcerogenicity studies were conducted according to the procedure reported by Cioli *et al.* (1980), Dalal and Narurkar (1991) and Tammara *et al.* (1993).

Adult male Wistar albino rats fasted for 24 hrs but provided water *ad libitum* were randomly divided into four groups, each containing 6 animals. The first group A received a suspension of CMC and gum acacia in distilled water, while group B, group C and group D were administered with suspension of DS (Suspension of pure drug), DSMS (Diclofenac sodium loaded microsphere) and MWDS (microsphere without diclofenac sodium), respectively. After 6 hrs of administration of suspensions the animals were sacrificed. The stomachs of sacrificed animals were removed and opened through their greater curvature. Mucosal damages (ulcer formation) were examined under the microscope (Magnus MLX Microscope magnification 40X10). The severity of mucosal damages was assessed by modification of a rating scale reported by Tammara *et al.* (1993). The rating scale used was as follows:

Observation score

No lesions: 0.0
Punctiform lesions (< 1 mm): 1.0
Five or more punctiform lesions: 2.0
One to five small ulcers (1-2 mm): 3.0
More than five small ulcers or one large ulcer: 4.0
More than one large ulcer (greater than 4 mm): 5.0

Based on the severity of mucosal damage, each specimen was assigned a score. The scores were averaged and the mean score was tabulated as the severity indexes for the drug and for the formulated drug suspension (Tammara *et al.* 1993). Statistical significance ($P < 0.01$) were performed by Dunnett's test (GraphPad prism3 software) to test the significance of difference in the severity index between DS and DSMS.

3. Preparation of stomach tissue homogenate

After collection, the stomach tissues were rinsed with ice-cooled normal saline and the surface water was removed with tissue paper. The stomach tissues of the different groups of animals were kept in So-Low Ultra Low freezer (-80°C) for 12 hrs (So-Low Ultra Low Freeze, Model No. C85-5, Serial No. 0708756, Environmental Equipment, Cincinnati, Ohio USA). On the following day, a small portion of stomach tissue was homogenized using a homogenizer (Remi Motor, Type-RQ 127A) with specific buffer solution for different biochemical testing for assay of ulceration.

4. Biochemical investigations

Assay of reduced glutathione (GSH): Reduced glutathione (GSH) in stomach was assayed by the method of Ellman (1959). At first the stomach tissue was homogenized in phosphate buffer (0.2 M, pH-8.0). 0.5 ml of the homogenized mixture was treated with 2.0 ml 5% trichloroacetic acid (TCA). The mixture was kept on an ice bath ice for 10-20 min and then centrifuged at 3000 rpm for 15 min. 2.0 ml of the supernatant was treated with 1.0 ml of Ellman's reagent and 4.0 ml of 0.3 M disodium hydrogen phosphate solution. The absorbance of yellow colour developed was measured in an UV spectrophotometer (JASCO-V-650) at 412 nm within 2-3 min. The amount of glutathione was expressed as Units/min/mg-protein.

Assay of Catalase (CAT): The stomach tissue was homogenized with phosphate buffer (0.01 M, pH-7.0) solution and was centrifuged at 5000 rpm for 10 min. In 0.1 ml of the supernatant, 0.9 ml phosphate buffer solution (pH 8.0), and 0.4 ml of 2 mM H_2O_2 were added. The reaction was arrested after 15, 30, 45 and 60 sec by adding 2.0 ml of dichromate acetic acid mixture (5% potassium dichromate and glacial acetic acid 1: 3 ratio) and kept in a boiling water bath for 10 min. After 10 min the mixtures were taken out of the water bath and allowed to cool. The absorbance of the resultant mixture was measured at 620 nm as per method reported by Sinha (1972).

Assay of Thiobarbituric Acid Reactive Substances (TBARS): Lipid peroxidation was determined by measuring the concentration of malanodialdehyde in the gastric mucosa following the modified method of Ohkawa *et al*. (1979). 0.5 ml of tissue homogenate (0.025 M Tris-HCl buffer, pH-7.8), 0.5 ml saline and 1.5 ml of 20% TCA were added, mixed well and then centrifuged at 3000 rpm for 20 min. In 1.0 ml of the protein raw supernatant, 1.5 ml of 0.8%TBA (thiobarbituric acid) reagent was added, mixed well and boiled for 1 hr at 95°C. The tubes were then cooled under tap water. The absorbance of clear supernatant was measured against reference blank at 532 nm (Ohkawa *et al.* 1979).

Estimation of total protein

Total protein was estimated by the method of Lowry *et al*. (1951) using bovine serum albumin (BSA) as standard at 660 nm with a Jasco V650 spectrophotometer.

Comparative study of anti-inflammatory activity of DS with the DSMS in animals

1. Carrageenan-induced paw edema in rats

This model was based on the principle of release of various inflammatory mediators by carrageenan (Winter *et al.* 1962). The Wistar albino rats (n = 6) were divided into four groups. The first group received a simple suspension of CMC and gum acacia in distilled water. The second, third and fourth groups were fed with suspensions containing DS, DSMS and MWDS, respectively through a feeding needle. The right hind paw was marked with a marker at the level of lateral melleolus. The basal paw volume was measured by volume displacement method using mercury Plethysmometer (PL048/08 supplied by CHEMICO Pvt. Ltd., India) by immersing the paw up to the mark at the level of lateral malleolus. One hr after dosing, 0.1 ml of 1.0% carrageenan was injected into the right hind paw of each rat. The paw volume was measured again at 1, 2, 3, 4, and 5 hrs after challenge. The increase in paw volume was calculated as percentage compared with the basal volume. The percentage of inhibition was calculated using the following formula of Lanher *et al.* (1992):

Percentage inhibition = $(V_t - V_o)$ control – $(V_t - V_o)$ treated / $(V_t - V_o)$ control X 100

where V_t is the final average volume (after carrageenan treatment) and V_o is the initial average volume (before carrageenan treatment).

2. Histamine-induced paw edema in rats

Histamine-induced paw edema was studied using same method that was used to determine the carrageenan induced paw edema. 0.1 ml of a 1.0% solution of histamine was administered into the sub-plantar side of the right hind paws. The paw volume was measured time to time. The percentage of inhibition of the inflammation was calculated using the same formula that has been given above and compared with the normal group (Amann *et al.* 1995).

3. Acetic acid-induced vascular permeability

The inhibitory activity of DSMS against increased vascular permeability which was induced by acetic acid was evaluated following the method of Whittle (1964). Male mice were randomly divided into three groups (n = 6). The first group received blank suspension whereas the second group received suspension of pure DS (50 mg/kg.bw). Third group was given with suspension of DSMS (50, 100 mg/kg.bw) orally. After thirty minutes, 0.50 ml of 1.0% acetic acid was injected intraperitoneally (i.p.) to the each animal. After 6 hrs, the viscera were exposed by abdominal incisions. The abdomen was then irrigated with normal saline containing 0.9% NaCl. The total protein content of each sample was determined using the Lowry method (Lowry *et al.* 1951).

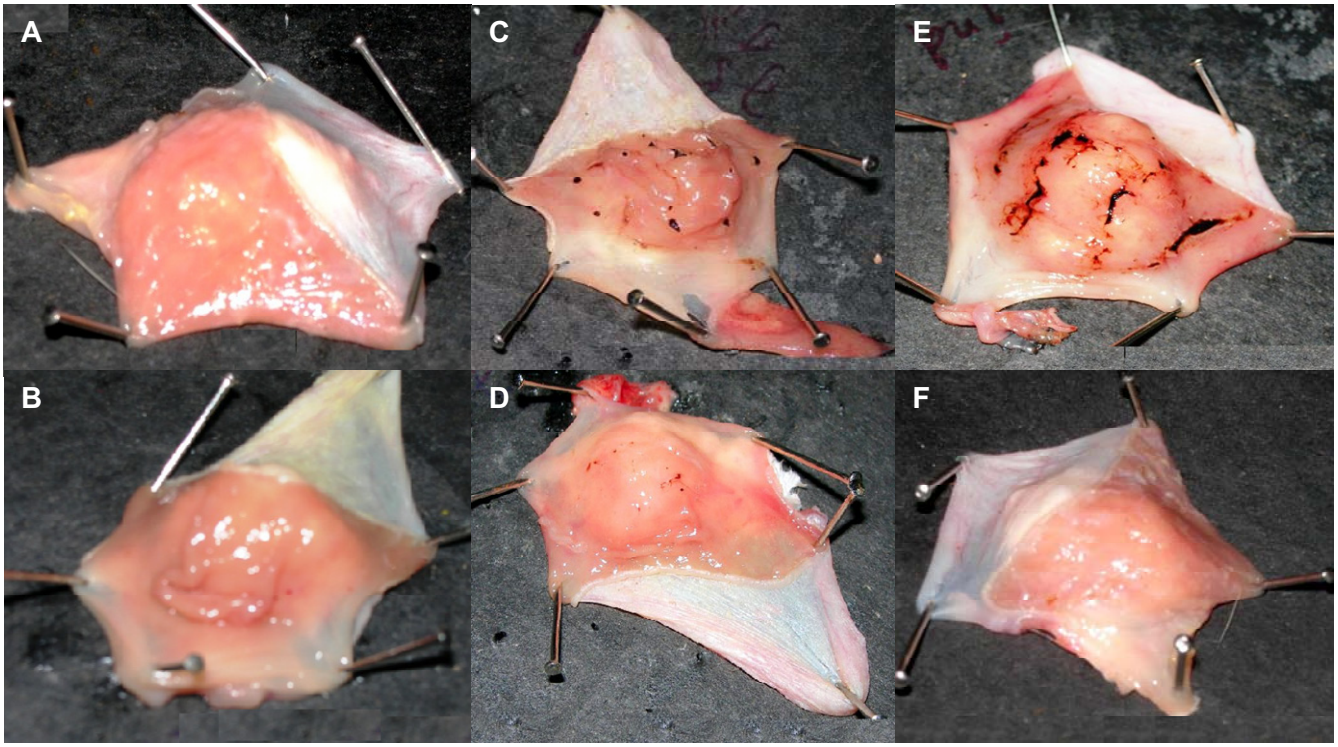

Fig. 1 Effect of DS, DSLM and MWDS on the rat stomach. (**A**) = Normal rat stomach; (**B**) = DSMS (50 mg/kg.bw) treated rat stomach; (**C**) = DS (75 mg/kg.bw) treated rat stomach; (**D**) = DSMS (75 mg/kg.bw) treated rat stomach; (**E**) = DSMS (100 mg/kg.bw) treated rat stomach; (**F**) = MWDS (based on 75 mg/kg.bw) treated rat stomach.

4. Peritoneal capillary permeability test (dye leakage method)

Peritoneal capillary permeability test was conducted following the method of Filderman and Kovacs (Filderman *et al.* 1969). The test formulation with suspension of gum acacia and CMC were administered orally to each group (n = 6). After 1 hr, 0.25 ml of 0.6% solution of acetic acid was administered to each animal i.p. Immediately after administration, 0.1 ml of 4% pontamine blue was injected intravenously as quickly as possible. After 5 hrs, the animals were sacrificed by cervical dislocation. The peritoneal cavity was cut and drained. The collected exudates were centrifuged for 10 min at 2000 rpm and 0.5 ml of the supernatant was diluted with 4.5 ml of physiological saline. Dye concentrations were measured at 625 nm using a spectrophotometer (JASCO-V-650), using saline as blank.

Statistical analysis

The experimental results were expressed as mean ± SEM. Data were assessed by the method of analysis of one way ANOVA followed by Dunnett's *t*-test. $P < 0.05$ (by GraphPad Prism 3 software) was considered as statistically significant.

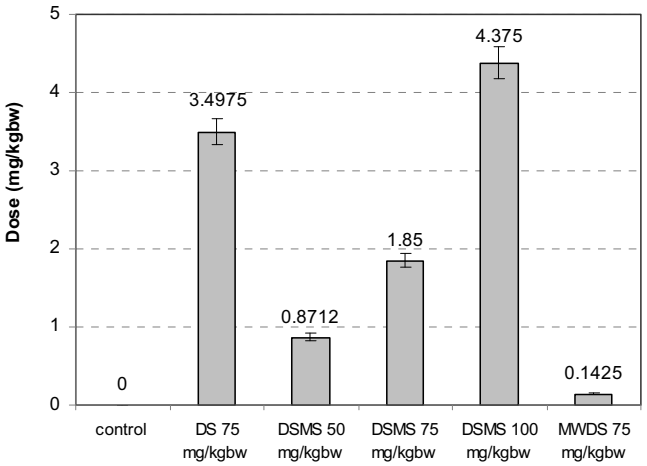

Fig. 2 Comparative study of DSMS with DS for gastric ulcer as a side effect in rats.

RESULTS AND DISCUSSION

Damage to the gastric mucosa by NSAIDs, including a high incidence of gastric and duodenal ulceration (Sugimori *et al.* 2007), can be arrested to a great extent by embedding the drug in a polymer matrix. There has been an increase in the design of a drug delivery system containing NSAIDs-loaded micro-particles or microsphere (Ray *et al.* 2010). The toxic effect of DS on gastric mucosa of adult albino Wistar rats was investigated by Devi *et al.* (1991). Formulations of DSMS with various biopolymeric hydrophilic matrices were found to give prolonged release and avoid adverse GI effects (Bravo *et al.* 2004). Gonçalves *et al.* (2005) investigated on the development of DS-loaded microspheres in chitosan cross-linked with gluteraldehyde and found sustained release at pH 1.2, 6.8 and 9.0. Gurusamy *et al.* (2006) compared analgesics, anti-inflammatory and ulcerogenic effects of solid dispersion of Meloxicam in polyethylene glycol 6000 and polyvinylpyrrolidone, their physical mixture and with Meloxicam alone. Results indicated that both the physical mixture and solid dispersion possesses better effect with less ulcerogenic activity as compared with raw Meloxicam. Solid dispersion and co-precipitate of indomethacin were compared with raw indomethacin. The ulceration was assessed four hrs after single dose as also 24 hrs following the last dose of a chronic four day dosing regimen. It was found that the co-precipitate formulation produced less ulceration than solid dispersion and pure drug. This suggests that severity of ulceration could be related with formulation design and drug release kinetics (Karnachi *et al.* 1997).

In the present study gastric mucosal injuries were evaluated by examining the stomach tissue of DS- and DSMS-treated rats under a microscope. **Fig. 1A** shows the photograph of the stomach mucosa lining of the rats of Group A i.e., the group fed with suspension of CMC and gum acacia in distilled water, while **Fig. 1C** and **1F** show the photograph of stomach mucosal lining for DS and MWDS treated rats, respectively. **Fig. 1B**, **1D** and **1E** represents the photo-

Table 1 Comparative study of DSMS with DS for gastric ulcer as side effect in rats.

Groups	Dose (mg/kg.bw)	Ulcer index
Control	75	0.00
DS	50	3.5 ± 0.05
	75	0.87 ± 0.04
	100	1.85 ± 0.04
DSMS	Based on 75 mg/kg.bw	4.37 ± 0.07
MWDS		0.14 ± 0.02*

Values expressed as mean ± SEM for six independent observations (n=6).
Statistical differences were determined by ANOVA followed by Dunnett's test.
*$P < 0.01$ when all treated groups are compared with control group.

graph of stomach mucosa linings of rats treated with three different doses of DSMS viz., 50, 75 and 100 mg/kg.bw, respectively. It was observed that the DS treated group produced severe gastric damage in the stomach mucosa lining (**Fig. 1C**), while in the group treated with CMC and gum acacia no hemorrhaging surface was noted. The stomach tissue of rats treated with MWDS show slight hemorrhage, almost to a negligible level, indicating that the polymer might be causing slight irritation on the gastric mucosa. However, the gastric mucosal damage of the rats treated with DSMS was much less in comparison to the stomach tissue lining of DS treated rats. It was further revealed that the gastric damage was related with the administered dose. The mucosal injury was increased when the dose was raised from 50 to 100 mg/kg.bw (**Fig. 2**). The ulcer index of different groups of animals has been presented in **Table 1**. The ulcer index of rats treated with 75 mg/kg.bw DSMS was found to be 1.85 ± 0.43 whereas the ulcer index for the rat stomach treated with same dose of DS was 3.5 ± 0.054. The ulcer index of DSMS treated rat reduced further to 0.87 ± 0.047 when the dose was brought down to 50 mg/kg.bw. The results shown in **Table 1** are in line with the observation made by earlier research workers cited above and therefore substantiate the observation that NSAID-loaded polymer microsphere has the potential to reduce its ulcerogenic effect.

The effect of DS, DSMS and MWDS on antioxidant and lipid peroxide levels of different groups animals have been presented in **Table 2**. From the enzymatic study of stomach tissue, it can be inferred that the formulated dosage forms are more beneficial than DS. Total protein, catalase and reduced glutathione level of DSMS treated rats are higher than that of DS treated animals. The enzymatic condition in stomach tissue of DSMS dosages were close to normal saline treated rats. TBARS level was found to be low in normal group and high in DS treated animals. The TBARS level of DSMS-treated rats is near the normal level but differs significantly with the DS-induced animals. The group treated with MWDS showed results similar to animals treated with normal saline. The group treated with MWDS showed a TBARS level almost equal to the TBARS level of animals treated with normal saline. These results confirmed that DSMS (50, 75, 100 mg/kg.bw) decreases the antioxidant level when compared with DS (75 mg/kg.bw) (**Table 2**), although it increases the TBARS level marginally. From this experiment, it can be concluded that DSMS (50 mg/kg.bw) is more acceptable.

The anti-inflammatory effect of DS, DSMS and MWDS were compared by measuring the percentage inhibition of paw edema induced by carrageenan and histamine.

Table 3 reveals that the anti-inflammatory effect of DS, as evident by the percentage inhibition of carrageenan-induced paw edema, was higher at the first hour in comparison to that produced by DSMS. However the anti-inflammatory effect produced by DS reached a maximum at third hour and then decreased. On the other hand, although the initial anti-inflammatory effect produced by DSMS was less at the initial hour, the effect was more profound at later hour and sustained over a longer period of time (**Fig. 3**). The increase in the amount of DS in the microsphere increased the anti-inflammatory effect at different hours. Similar observation was found against the histamine-induced inflammation (**Fig. 4**).

In acetic acid-induced vascular permeability study, the anti-inflammatory activity was evaluated in three different groups of mice treated with normal saline, or DS or DSMS. **Table 4** represents the total amount of protein count present in exudates collected after 6 hrs induction in the rats treated with normal saline or DS or DSMS. While the protein count for DS treated animal was 68.13 ± 0.055 which was near to the value shown by group treated with normal saline (72.47 ± 0.046). On the other hand, the group treated with DSMS the value came down to 45.89 ± 0.044. The reduction in protein count in DS and DSMS compared to the normal group is attributed to decrease in permeability in the blood vessels of DS and DSMS treated animals. Lower value of DSMS in comparison with DS suggests better therapeutic efficacy of DSMS formulation. The similar results were observed in dye leakage method that has been shown in the **Table 5**.

However, vascular permeability was found to be the dosage related. DS inhibits the vascular permeability but to a lesser extent, whereas the inhibitory effect produced by DSMS was found over a longer period of time.

Table 2 Effect of DSMS and DS on anti oxidant and lipid peroxidation of the same group of animal in stomach tissue.

Parameter	Control	Inducer (DS)	Formulation (DSMS)			MWDS
		75 mg/kg.bw	50 mg/kg.bw	75 mg/kg.bw	100 mg/kg.bw	
TP (mg/ml)	24.58 ± 0.05	14.84 ± 0.03	18.77 ± 0.05 a	17.86 ± 0.03 a	17.43 ± 0.05 a	23.2 ± 0.09 a
TBARS (nmol/mg protein)	1.02 ± 0.01	2.66 ± 0.07	1.62 ± 0.05 a	1.81 ± 0.01 a	2.09 ± 0.04 a	1.1 ± 0.04 b
CAT (U/min/mg protein)	2.32 ± 0.11	1.78 ± 0.07	2.03 ± 0.03 c	1.98 ± 0.03 a	1.92 ± 0.02 a	2.07± 0.05 b
GSH (U/min/mg protein)	2.22 ± 0.08	1.61± 0.11	2.08 ± 0.05 b	2.05 ± 0.04 b	1.93 ± 0.04 b	2.11± 0.04 b

$P^a < 0.01$, $P^b > 0.05$, $P^c < 0.05$ compared with control value. The results were analyzed by ANOVA followed by Dunnett's test. Each value represents the mean ± SEM of 6 animals in each group.

Table 3 Effect of DSMS and DS on carrageenan- and histamine-induced edema in rats.

Groups	Dose	Inducer	Percentage of edema inhibition in successive hrs					
	mg/kg	(Carrageenan/Histamine)	0	1	2	3	4	5
DS	50	Carrageenan	0.00	36.36 ± 0.16	52.00 ± 0.03	61.11 ± 0.13	41.37 ± 0.15	40.00 ± 0.06
		Histamine	0.00	35.71 ± 0.09	36.00 ± 0.11	66.66 ± 0.12	83.33 ± 0.12	70.83 ± 0.27
DSMS	50	Carrageenan	0.00	9.09 ± 0.06***	20.00 ± 0.16***	55.56 ± 0.08***	65.52 ± 0.08***	60.00 ± 0.15***
		Histamine	0.00	27.27 ± 0.14***	32.00 ± 0.14***	50.00 ± 0.22***	83.34 ± 0.17***	79.16 ± 0.18***
	75	Carrageenan	0.00	36.36 ± 0.15***	58.33 ± 0.09***	65.51 ± 0.26***	73.33 ± 0.11***	66.66 ± 0.19***
		Histamine	0.00	21.43 ± 0.21***	24.00 ± 0.06***	36.66 ± 0.16***	76.19 ± 0.29***	75.00 ± 0.21***
	100	Carrageenan	0.00	27.27 ± 0.06***	64.00 ± 0.09***	66.66 ± 0.19***	75.86 ± 0.04***	70.85 ± 0.23***
		Histamine	0.00	28.57 ± 0.11***	36.00 ± 0.16***	73.33 ± 0.20***	85.71 ± 0.15***	83.33 ± 0.18***

Values expressed as mean±SEM for six independent observations (n=6)
Statistical differences were determined by ANOVA followed by Dunnett's test *P*** < 0.0001, compared with DS value.

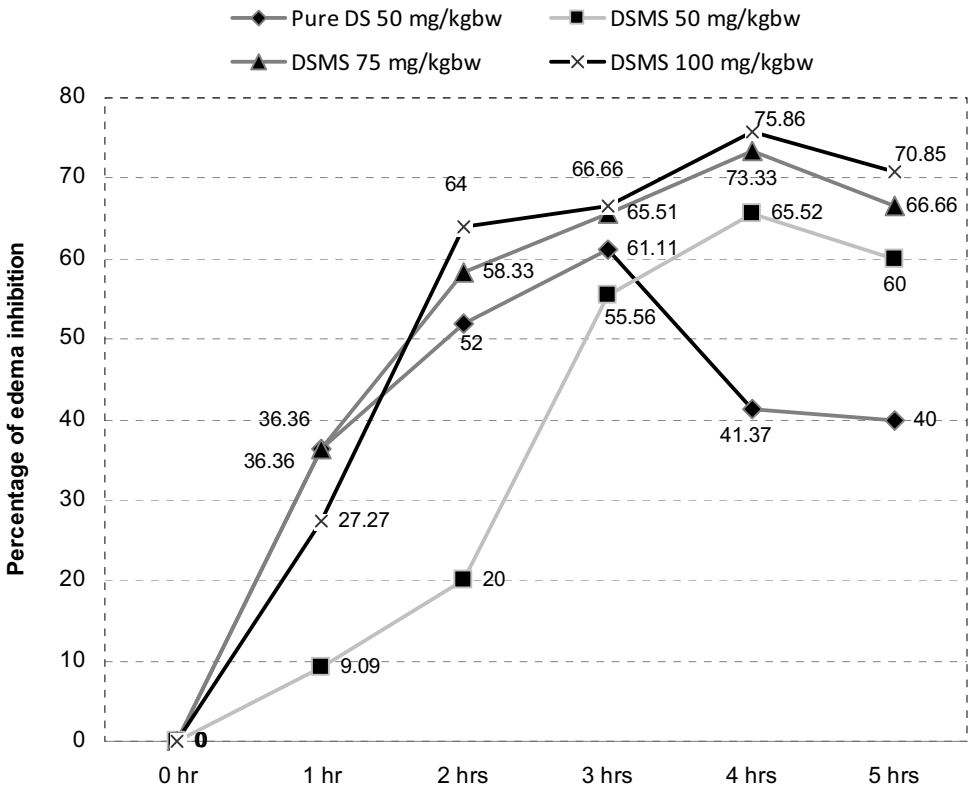

Fig. 3 Percentage of inhibition of carrageenan-induced paw edema over 5 hrs.

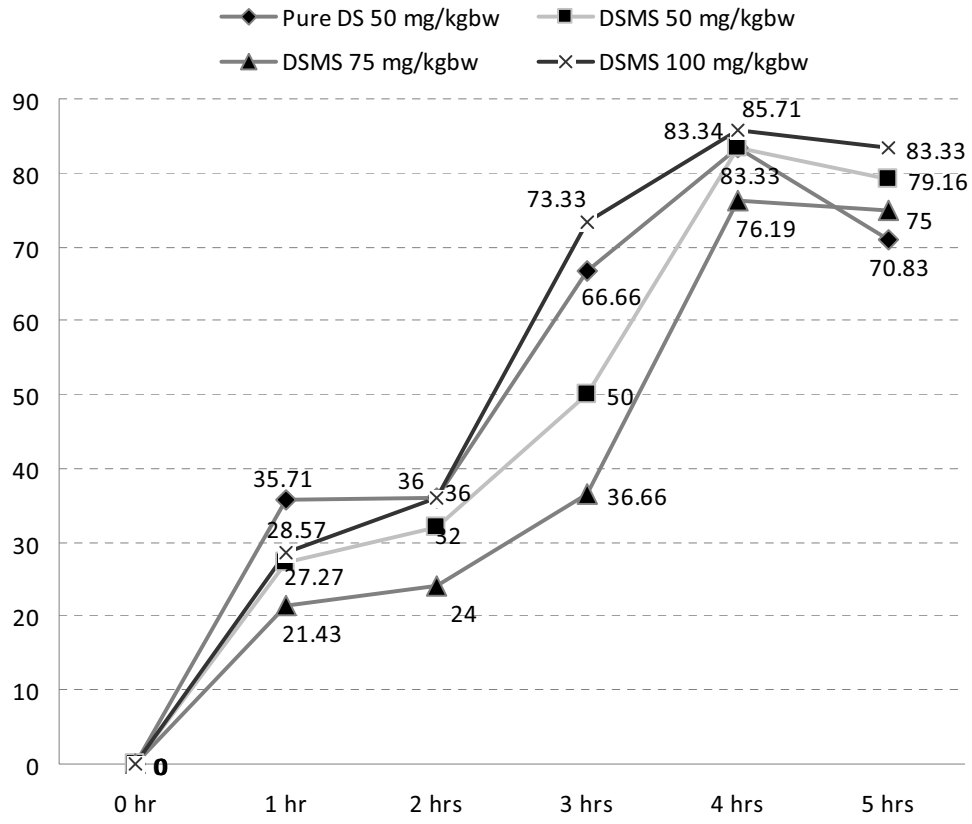

Fig. 4 Percentage of inhibition of histamine-induced paw edema over 5 hrs.

CONCLUSION

In conclusion, all the discussed results suggest that DSMS offers more beneficial effects in the management of inflammation with low ulcerogenicity property. From the several animals' studies, it is concluded that Diclofenac Sodium loaded microsphere is a better choice for the patients suffering from rheumatoid arthritis, osteoarthritis and other joint pains because of its sustained action and low toxicity.

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Table 4 Acetic acid induced inflammatory exudation.

Groups	Dose mg/kg.bw	Volume of exudates (ml)	Total amount of protein (mg) after 6 hrs
Normal Saline		4	72.47 ± 0.04
DS	50	4	54.48 ± 0.14***
	100	4	68.13 ± 0.05***
DSMS	50	4	45.18 ± 0.09***
	100	4	45.89 ± 0.04***

Each value represents the mean ± SEM of 6 animals in each group. Statistical differences were determined by ANOVA followed by Dunnett's test. $P^{***} < 0.0001$ compared with normal group.

Table 5 Acetic acid induced vascular permeability (dye leakage method).

Groups	Dose (mg/kg.bw)	Volume of exudates (ml)	Absorbance
Normal saline		4	0.18 ± 0.010
DS	50	4	0.14 ± 0.005[a]
	100	4	0.11 ± 0.149[a]
DSMS	50	4	0.045 ± 0.010[a]
	100	4	0.019 ± 0.005[a]

Statistical differences were determined by ANOVA followed by Dunnett's test $P^a > 0.05$ compared with normal. Each value represents the mean ± SEM of 6 animals.

REFERENCES

Amann R, Schuligoi R, Lanz I, Donnerer J (1995) Histamine induced edema in the rat paw-effect of capsaicin denervation and a CGRP receptor antagonist. *European Journal of Pharmacology* **279**, 227-231

Batycky RP, Hanes J, Langer R, Edwards DA (1997) A theoretical model of erosion and macromolecular drug release from biodegrading microspheres. *Journal of Pharmaceutical Sciences* **86 (12)**, 1464-1477

Galer BS (2010) A comparative subjective assessment study of PENNSAID® and Voltaren Gel®, two topical formulations of diclofenac sodium. *Pain Practice* in press

Bravo SA, Lamas MC, Salomon CJ (2004) Swellable matrices for the controlled-release of diclofenac sodium: formulation and *in vitro* studies. *Pharmaceutical Development and Technology* **9 (1)**, 75-83

Carmen K (1983) *Compendium of Pharmaceuticals and Specialties*, Canadian Pharmacists Association, Ottawa, Canada, 676 pp

Cioli V, Putzolu S, Rossi V, Corradino C (1980) A toxicological and pharmacological study of ibuprofen guaiacol ester (AF 2259) in the rat. *Toxicology and Applied Pharmacology* **54**, 332-339

Dalal PS, Narurkar MM (1991) *In vitro* and *in vivo* evaluation of sustained release suspensions of ibuprofen. *International Journal of Pharmaceutics* **73**, 157-162

Delporte C, Backhouse N, Erazo S, Negrete R, Vidal P, Silva X, Lopez-Perez JL, **San Feliciano A, Munoz O** (2005) Analgesic-antiinflammatory properties of *Proustia pyrifolia*. *Journal of Ethnopharmacology* **99**, 119-124

Devi RS, Narayan S, Vani G, Devi CSS (2007) Gastroprotective *effect of Terminalia arjuna* bark on Diclofenac sodium induced gastric ulcer. *Chemico-Biological Interactions* **167**, 71-83

Ellman GC (1959) Tissue sulfhydryl groups. *Archives of Biochemistry and Biophysics* **82**, 70-77

Filderman RB, Kovacs BA (1969) Anti-inflammatory activity of the steroid alkaloid glycoside, tomatine. *British Journal of Pharmacol*ogy **37 (3)**, 748-755

FitzGerald GA, Patrono C (2001) The coxibs, selective inhibitors of cyclooxygenase-2. *The New England Journal Medicine* **345**, 433-442

Gohel MC, Parikh RK, Nagori SA, Jethwa BA (2010) Fabrication and evaluation of bi-layer tablet containing conventional paracetamol and modified release diclofenac sodium. *Indian Journal of Pharmaceutical Sciences* **72 (2)**, 191-6

Gonçalves VL, Laranjeira MCM, Fávere VT, Pedrosa RC (2005) Effect of crosslinking agent on chitosan microspheres in controlled release of diclofenac sodium. *Polímeros: Ciência e Tecnologia* **15 (1)**, 6-12

Gurusamy S, Kumar B, Mishra D (2006) Analgesic anti-inflammatory and ulcerogenic studies of Meloxicam solid dispersion in rodent. *Iranian Journal of Pharmacology and Therapeutics* **5**, 77-79

Karnachi AA, Reddy IK, Degennaro MD, Khan MA (1997) Comparative evaluation of the severity of gastric ulceration by a solid dispersion and coprecipitates of indomethacin. *Journal of Drug Targeting* **4 (5)**, 297-301

Kurahara K, Matsumoto T, Iida M (2007) Clinical features of nonsteroidal anti-inflammatory drug-induced colopathy. *Nippon Rinsho* **65 (10)**, 1875-1878 (in Japanese)

Lanhers MC (1992) Activite anti-inflammatoire d'un extrait de *Peumus boldus*. *Phytotherapy* **38-39**, 12-13

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951) Protein measurement with the Folin phenol reagent. *Journal of Biological Chemistry* **193**, 265-275

Nagasankar MS, Meshram RN, Ramprakash G (2000) Solid dispersion of hydroxy propyl beta cylodextrin and ketorolac, enhancement of in vitro dissolution rates, improvement in anti-inflammatory activity and reduction in ulcerogenicity in rats. *Journal of Pharmacy and Pharmacology* **(52)**, 949-956

Nikkola L, Viitanen P, Ashammakhi N (2009) Temporal control of drug release from biodegradable polymer: Multicomponent Diclofenac sodium releasing PLGA 80/20 rod. *Journal of Biomedical Material Research. Part B: Applied Biomaterials* **89B (2)**, 518-526

Ohkawa H, Ohishi N, Yaki K (1979) Assay for lipid peroxide for animal tissue by thiobarbituric acid reaction. *Analytical Biochemistry* **95**, 351-358

Rao YM, Veni JK, Jayasagar G (2001) Formulation and evaluation of Diclofenac sodium using hydrophilic matrices. *Drug Development and Industrial Pharmacy* **27**, 759-766

Ray S, Banerjee S, Maiti S, Laha B, Barik S, Sa B, Bhattacharyya UK (2010) Novel interpenetrating network microspheres of xanthan gum-poly (vinyl alcohol) for the delivery of diclofenac sodium to the intestine – *in vitro* and *in vivo* evaluation. *Drug Delivery* **17 (7)**, 508-519

Savaşer A, Ozkan Y, Işimer A (2005) Preparation and *in vitro* evaluation of sustained release tablet formulations of diclofenac sodium. *Farmaco* **60 (2)**, 171-177

Schwartz GR (1999) *Principles and Practice of Emergency Medicine* (Vol 2), Lippincott Williams & Wilkins, Baltimore, MD, 1964 pp

Sinha KA (1972) Colorimetric assay of catalase. *Analytical Biochemistry* **47**, 389-394

Tammara VK, Narurkar MM, Crider MA, Khan MA (1993) Synthesis and evaluation of morpholinoalkyl ester prodrugs of indomethacin and naproxen. *Pharmaceutical Research* **10 (8)**, 1191-1199

Sugimori S, Watanabe T, Shiba M, Higuchi K, Arakawa T (2007) Clinical features of NSAID-induced small intestinal damage. *Nippon Rinsho* **65 (10)**, 1862-1865 (in Japanese)

Tao SL, Desai TA (2003) Microfabricated drug delivery system from particles to pores. *Advance Drug Delivery Reviews* **55**, 315-328

Thirunavukkarasu P, Ramkumar L, Ramanathan T (2009) Anti-ulcer activity of *Excoecaria agallocha* bark on NSAID-induced gastric ulcer in albino rats. *Global Journal of Pharmacology* **3 (3)**, 123-126

Viswanathan NB, Thomas PA, Pandit JK, Kulkarni MG, Mashelkar RA (1999) preparation of non porous microsphere with high entrapment efficiency of protein by a (water in oil)-in oil emulsion technique. *Journal of Controlled Release* **58**, 9-20

Whittle BA (1964) The use of changes in capillary permeability in mice to distinguish between narcotic and non-narcotic analgesic. *British Journal of Pharmacology and Chemotherapy* **22**, 24-253

Winter CA, Risley E, Nuss G (1962) Carrageenan-induced edema in hind paw of the rat as an assay for anti-inflammatory drugs. *The Society for Experimental Biology and Medicine* **111**, 544-547

Zhang S, Lü B, Si JM, Chen FM, Meng LN, Wu WF, Yu LM (2009) Effect of non-steroidal anti-inflammatory drugs on small intestinal barrier function in rats. *Zhonghua Nei Ke Za Zhi* **48 (1)**, 44-47 (in Chinese)