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The Comparison of Anti-inflammatory and Analgesic Effect of Diclofenac Alone with Combined Treatment with Diclofenac and Serratiopeptidase in Patients having Osteoarthritis of Knee Joint

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ABSTRACT

Oral proteolytic enzymes like serratiopeptidase are very commonly used by clinicians either alone or in combination with non-steroidal anti-inflammatory drugs for analgesia and anti-inflammatory purpose. As the activity of these drugs is not proved in trials, and they are not listed in any country's official pharmacopoeia, it was planned to study their effect in osteoarthritis patients. Two groups (n= 30 each) of diagnosed knee osteoarthritis patients, were treated with diclofenac 50 mg twice a day (BID) and serratiopeptidase 10mg three times a day (TID) + Diclofenac 50 mg BID for two weeks. The pain and difficulty in daily activities were assessed by Visual Analogue Scale (VAS) and Western Ontario and Mc Masters Universities Osteoarthritis (WOMAC OA) index scale before and after the treatment. Highly significant improvement in both scales was seen in both groups. There was no statistically significant difference in the improvements found in both groups. Addition of serratiopeptidase has not potentiated analgesic and anti-inflammatory effects of diclofenac. Thus, the analgesic and anti-inflammatory efficacy of serratiopeptidase are not proved.

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INTRODUCTION

Inflammation is the local response of the living tissues to injury caused by any agent. It is the natural defence reaction to eliminate the injury-causing agent. Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) and glucocorticoids are used in therapy for their potent anti-inflammatory effect for many decades. Because of the potential adverse effects of glucocorticoids, NSAIDs are preferred for controlling inflammation and suppress associated symptoms in cases of joint pains, sprains, dental pain. NSAIDs are available without prescription in chemist shops and maybe overused by patients as self-medication also.

Besides, there is a group of drugs called proteolytic enzymes (chymotrypsin, serratiopeptidase, hyaluronidase) which are widely used alone or in combination with NSAIDs. Oral preparations of serratiopeptidase are prescribed by many clinicians for its proposed anti-inflammatory action, for trauma, arthritis, carpal tunnel syndrome, respiratory tract congestion, parotitis etc.

Serratiopeptidase is a proteolytic enzyme having a high molecular weight (Nakahama *et al.*, 1986; Menon and Nirale, 2010). In the gastrointestinal tract, complex proteins are degenerated to simple proteins and then only they can be absorbed. There-

fore, it is doubtful that serratiopeptidase reaches the blood circulation intact after oral administration. Therefore, it is also doubtful that it produces an anti-inflammatory effect at the site of action when it is given orally. Serratiopeptidase fixed-dose combinations have very poor rationality score also (Vandana Roy and Vandana Tayal, 2017). Besides, serratiopeptidase is neither listed in any official pharmacopoeia nor any essential drug list. Considering all these facts, the present study was planned to evaluate whether the anti-inflammatory effect of diclofenac is potentiated by its combination with serratiopeptidase given orally, in patients having osteoarthritis of the knee joint.

MATERIALS AND METHODS

It was an open-labelled comparative, prospective, interventional study involving 60 patients of either sex having age more than 30 years attending orthopaedic Outpatient Department (OPD) suffering from mild to moderate osteoarthritis of the knee joint. These patients were enrolled after confirmation of diagnosis by an orthopaedic specialist and after taking their written informed consent. The study was conducted in a tertiary care hospital of Krishna Institute of Medical Sciences, Karad, Maharashtra. The Institutional Ethics Committee of Krishna Institute of Medical Sciences, Karad approved the study protocol.

Patients fulfilling inclusion criteria (Table 1) were randomly assigned in 1:1 fashion to either of two treatment groups. Each group consisted of 30 patients. Group A received tablet diclofenac sodium 50 mg twice a day (BID) for two week. Group B received tablet diclofenac sodium 50 mg BID and tablet serratiopeptidase 10 mg three times a day (TID) for two weeks. Efficacy of treatment was assessed by measuring the severity of pain in the affected joint by using Visual Analogue Scale (VAS) (0-10 scale) (Collins *et al.*, 1997) before starting treatment and at the end of 2 weeks treatment. Pain, stiffness and level of difficulty during different daily activities was assessed before starting treatment and at the end of 2 weeks of treatment, by using Western Ontario and Mc Masters Universities Osteoarthritis (WOMAC OA) index (Wolfe, 1999).

Each sub-dimension in WOMAC OA index was graded according to the Likert scale (0-4 points). Total pain score, total stiffness score, the total level of difficulty score was calculated by adding scores of individual sub-dimensions of each patient. Means of total scores of pain, stiffness and level of difficulty were calculated for all 60 patients at day 0 and end of 2 weeks. Safety of treatments was assessed by the recording of adverse effects complained by patients after telephonic calls every week and by questions asked at the end of the two weeks.

Statistical Analysis

Student's paired t-test was used to compare results obtained in group A and group B at day 0 and the end of 2 weeks of treatment. The difference in efficacy was assessed by comparing the mean difference in symptom scores between baseline and 2-week score in both groups, in both VAS and WOMAC scale tests. The statistical analysis of the difference in the efficacy of two treatments was done by using student's unpaired t-test. P-value <0.05 was considered significant, and <0.01 was considered highly significant.

RESULTS

All 60 enrolled patients of senile osteoarthritis of knee joint completed the study. Out of the total, 60 patients, 32 were female, and 28 were males. Maximum patients were having age between 41 to 70 years (Table 2).

In control group A treated with tablet diclofenac 50 mg BID, it was observed that individual symptom scores for each parameter like pain in the knee joint, stiffness and level of difficulty during various daily activities were very highly significantly reduced at the end of 2 weeks of treatment as compared to day 0 scores, as assessed by WOMAC OA Index ($p< 0.0001$). The intensity of pain in the arthritic knee joint was also found to be very highly significantly reduced at the end of 2 weeks treatment with diclofenac in this group ($p< 0.0001$) (Table 3).

The group B received diclofenac 50 mg BID and serratiopeptidase 10 mg TID for two weeks. In this group also there was a great reduction($p<0.0001$) in the mean individual scores for pain, stiffness and difficulty during daily activities at the end of 2 weeks of treatment as assessed by WOMAC OA scale. The overall intensity of pain in this group was also very highly significantly decreased ($p< 0.0001$) at the end of 2 weeks of treatment, as assessed by using the VAS scale (Table 4).

The differences in all scores between 0 week and 2 weeks, for all parameters of WOMAC and VAS, were calculated and means of group A and B were compared using student's unpaired t-test. There was no significant statistical difference seen in the improvements in group A and B ($p> 0.05$). Thus, both treatments were equally effective, and no treatment was superior as compared to the other treatment. (Table 5)

During the entire treatment course in both the

Table 1: Inclusion and exclusion criteria

Inclusion criteria	Exclusion criteria
1. Either sex	1. Rheumatoid/ psoriatic arthritis
2. Mild/moderate senile osteoarthritis: knee pain and/or knee stiffness	2. Pregnant/ lactating mothers
3. Age more than 30 years	3. Patients with cardiovascular, hepatic, central nervous system disorders
	4. Known hypersensitivity to serratiopeptidase or diclofenac
	5. Patients receiving any other drug by oral/ injectable or topical route for osteoarthritis
	6. Patients participated in any clinical trial in the last 30 days

Table 2: Age-wise distribution of osteoarthritis patients

Age Group	Number of patients
30- 40 years	3
41-50 years	12
51-60 years	18
61-70 years	22
71-80 years	5

Table 3: Symptom scores as per WOMAC OA and VAS in group A (Diclofenac) n=30

Scale	Symptom	Score at 0 week Mean ±SD	Score at 2 weeks Mean ± SD	P-value
WOMAC	Pain in knee joint	8.33± 2.74	4.46± 2.25	< 0.0001
	Stiffness of joint	3.73 ± 1.59	1.63 ± 1.47	< 0.0001
	Level of difficulty in daily activities	17.86± 3.83	10.96± 3.57	< 0.0001
	Total WOMAC OA score	29.93± 6.56	17.06± 6.08	< 0.0001
VAS	Overall pain intensity	5.57± 1.65	3.033 ± 1.45	< 0.0001

Table 4: Symptom scores as per WOMAC OA and VAS in group B (Diclofenac + Serratiopeptidase) n=30

Scale	Symptom	Score at 0 week Mean ± SD	Score at 2 weeks Mean ± SD	P-value
WOMAC	Pain in knee joint	8.56 ± 2.90	4.13± 2.82	< 0.0001
	Stiffness of joint	5.03± 2.34	2.46 ± 1.97	< 0.0001
	Level of difficulty in daily activities	21±5.58	12 ± 6.27	< 0.0001
	Total WOMAC score	34.66 ± 9.31	18.8 ± 10.29	< 0.0001
VAS	Overall pain intensity	6.50 ± 1.757	3.93 ± 1.721	< 0.0001

Table 5: The comparison of improvement in scores as per WOMAC and VAS in Groups A and B

Scale	Symptom	Improvement in Group A Mean ± SD	Improvement in Group B Mean ± SD	P-value
WOMAC	Pain in knee joint	3.86± 2.25	4.43± 2.82	0.39
	Stiffness of joint	2.1± 1.34	2.56± 1.81	0.263
	Level of difficulty in daily activities	6.9± 3.9	8.93± 5.17	0.108
	Total WOMAC score	12.86± 5.65	15.86 ± 9.04	0.17
VAS	Overall pain intensity	2.53 ± 1.38	2.57± 1.56	0.93

p > 0.05 is considered not significant

groups, no serious adverse effects were observed/ reported. All the patients completed the treatments, and no treatment was stopped due to any adverse effect. Only 2 patients in the diclofenac group, while one patient in diclofenac + serratiopeptidase group has reported nausea and epigastric pain. The difference was not statistically significant.

DISCUSSION

The present study was aimed to evaluate the efficacy of serratiopeptidase, a proteolytic enzyme obtained from a nonpathogenic bacterium Serratia E15 (Nakahama *et al.*, 1986), in controlling inflammation and pain associated with osteoarthritis of knee joint in human. In this study, a comparison was done between anti-inflammatory and analgesic effects of diclofenac 100 mg/day and combination treatment of diclofenac 100 mg/day with serratiopeptidase 30 mg/day, administered orally for two weeks. The efficacy of treatments was assessed by measuring the improvement in symptom scores in pain, stiffness and level of difficulty in various daily activities by using WOMAC OA index (Wolfe, 1999). The overall intensity of pain was assessed by using a visual analogue scale.

Our study results show that there was a very highly significant reduction in inflammation and pain at the end of 2 weeks of both treatments. Further, it was also observed that there was no significant difference in the efficacy of both treatments in producing relief in parameters like joint pain, joint stiffness and difficulty in performing daily activities. Thus, it can be concluded from these observations that the addition of oral serratiopeptidase to diclofenac did not potentiate the anti-inflammatory and analgesic effect of diclofenac in osteoarthritis. This result creates doubt about the anti-inflammatory effect of oral serratiopeptidase. This result also raises a question about the bioavailability of oral serratiopeptidase. Serratiopeptidase is a polypeptide having large molecular weight.

Polypeptides given orally are usually degraded by intestinal proteases and then absorbed. There is a meagre number of animal and human studies showing the presence of serratiopeptidase in blood after oral administration (Dallas *et al.*, 1989; Moriya *et al.*, 1994). For supporting adequate oral bioavailability, more authentic pharmacokinetic data is required. Few studies have shown the anti-inflammatory activity of serratiopeptidase in animal models (Jadav *et al.*, 2010; Mundhava *et al.*, 2016). Antiinflammatory effect of serratiopeptidase in inflammatory venous disease was shown in a clinical study (Bracale and Selvetella, 1996). Other two clinical studies have also documented anti-inflammatory effect of serratiopeptidase (Panagariya and Sharma, 1999; Klein and Kullich, 2000). In 6 clinical triasls, the efficacy of oral proteolytic enzymes in osteoarthritis is found to be comparable to diclofenac and tolerability was found better than diclofenac (Ueberall *et al.*, 2016). In one study, bromelain, another proteolytic enzyme, was found to produce an equal anti-inflammatory effect as diclofenac in osteoarthritis patients (Kasemsuk *et al.*, 2016). Two other studies have suggested that oral proteolytic enzyme therapy with bromelain, rutosid and wobenzyme gives either comparable or superior analgesic effect in osteoarthritis patients when compared to oral diclofenac (Akhtar *et al.*, 2004; Bolten *et al.*, 2015). Our results are not matching with all these studies. A study in which the relative efficacy of various analgesic and anti-inflammatory drugs used in practice was compared, failed to show the significant activity of serratiopeptidase (Chopra *et al.*, 2009). Nirale and Menon used gel formulations of serratiopeptidase and diclofenac for testing local anti-inflammatory effect in rodents. They claim that both had an anti-inflammatory effect, but there was no statistical difference in their effects (Menon and Nirale, 2010).

One study concludes that the cost of treatment increases more than five times if serratiopeptidase is combined with NSAIDs. This study also con-

cludes that the trend of prescribing oral proteolytic enzymes along with NSAIDs is very common and is mostly influenced by recommendations of medical representatives (Shah and Nerurkar, 2013). It is also a worrying fact that this drug is not listed in any official pharmacopoeia, national formulary, and it is not marketed in countries like the USA and UK (Mathew and Sivasubramanian, 2004).

In a review article, it is suggested that the existing scientific evidence is insufficient to support analgesic anti-inflammatory actions of serratiopeptidase (Bhagat *et al.*, 2013).

Therefore, we suggest that more authentic larger clinical trials and pharmacokinetic studies will be required to prove or disprove the efficacy of serratiopeptidase and other proteolytic enzymes as an analgesic and anti-inflammatory drugs. Though few preparations containing serratiopeptidase were banned in India in 2016, many formulations having combinations of serratiopeptidase and NSAIDs and isolated serratiopeptidase are still available in the market. These are being used by clinicians very commonly. Therefore, awareness should be created among clinicians about the lack of proven efficacy of this drug and its absence in any official drug formulary or pharmacopoeia and also essential drug lists. Its use should be discouraged by controlling authorities. This will increase cost-effectiveness and rationality in drug treatment of common ailments like arthritis and other painful and inflammatory conditions.

CONCLUSION

Addition of serratiopeptidase does not potentiate analgesic and anti-inflammatory effect of diclofenac in osteoarthritis patients. Therefore the combined use of these drugs or use of fixed-dose combination having these drugs as analgesic or anti-inflammatory can not be recommended.

Conflict of interest

The authors declare that they have no conflict of interest for this study.

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REFERENCES

Akhtar, N. M., Naseer, R., Farooqi, A. Z., Aziz, W., Nazir, M. 2004. Oral enzyme combination versus diclofenac in the treatment of osteoarthritis of the knee ? a double-blind prospective randomized study. *Clinical Rheumatology*, 23(5):410–415.

Bhagat, S., Agarwal, M., Vandana Roy 2013. Serratiopeptidase: A systematic review of the existing evidence. *International Journal of Surgery*, 11(3):209–217.

Bolten, W. W., Glade, M. J., Raum, S., Ritz, B. W. 2015. The Safety and Efficacy of an Enzyme Combination in Managing Knee Osteoarthritis Pain in Adults: A Randomized, Double-Blind, Placebo-Controlled Trial. *Arthritis*, 2015:1–7.

Bracale, G., Selvetella, L. 1996. Clinical study of the efficacy of and tolerance to see prose S in inflammatory venous disease. Controlled study versus serratiopeptidase. *Minerva cardioangiologica*, 44(10):515–524.

Chopra, D., Rehan, H. S., Mehra, P., Kakkar, A. K. 2009. A randomized, double-blind, placebo-controlled study comparing the efficacy and safety of paracetamol, serratiopeptidase, ibuprofen and betamethasone using the dental impaction pain model. *International Journal of Oral and Maxillofacial Surgery*, 38(4):350–355.

Collins, S. L., Moore, A. R., McQuay, H. J. 1997. The visual analogue pain intensity scale: what is moderate pain in millimetres? *Pain*, 72(1):95–97.

Dallas, P., Rekkas, D., Choulis, N. H. 1989. HPLC determination of serratiopeptidase in biological fluids. *Pharmazie*, (4):44.

Jadav, S. P., Patel, N. H., Shah, T. G., Gajera, M. V., Trivedi, H. R., Shah, B. K. 2010. Comparison of anti-inflammatory activity of serratiopeptidase and diclofenac in albino rats. *Journal of Pharmacology and Pharmacotherapeutics*, 1(2):116.

Kasemsuk, T., Saengpetch, N., Sibmooh, N., Unchern, S. 2016. Improved WOMAC score following 16-week treatment with bromelain for knee osteoarthritis. *Clinical Rheumatology*, 35(10):2531–2540.

Klein, G., Kullich, W. 2000. Short-Term Treatment of Painful Osteoarthritis of the Knee with Oral Enzymes. *Clinical Drug Investigation*, 19(1):15–23.

Mathew, J. R., Sivasubramanian, K. 2004. Letter to Editor-Use of oral enzyme preparations: Is there any evidence? *Indian Journal of Plastic Surgery*, 37(1):80.

Menon, M. D., Nirale, N. M. 2010. Topical formulations of serratiopeptidase: development and pharmacodynamic evaluation. *Indian Journal of Pharmaceutical Sciences*, 72(1):65.

Moriya, N., Nakata, M., Nakamura, M., Takaoka, M., Iwasa, S., Kato, K., Kakinuma, A. 1994. Intestinal absorption of serrapeptase (TSP) in rats. *Biotechnology and applied biochemistry*, 201:101–109.

Mundhava, S. G., Mehta, D. S., Thaker, S. J. 2016. A comparative study to evaluate anti-inflammatory and analgesic activity of commonly used proteolytic enzymes and their combination with diclofenac in rats. *International Journal of Pharmaceutical Sciences and Research*, 7(6).

Nakahama, K., Yoshimura, K., Marumoto, R., Kikuchi, M., Lee, I. S., Hase, T., Matsubara, H. 1986. Cloning and sequencing ofSerratiaprotease gene. *Nucleic Acids Research*, 14(14):5843–5855.

Panagariya, A., Sharma, A. K. 1999. A preliminary trial of serratiopeptidase in patients with carpal tunnel syndrome. *The Journal of the Association of Physicians of India*, 47(12):1170–1172.

Shah, S. A., Nerurkar, R. P. 2013. Evaluation of prescribing trends and rationality of use of oral proteolytic enzymes. *Indian Journal of Pharmacology*, 45(3):309.

Ueberall, M., Mueller-Schwefe, G., Wigand, R., Essner, U. 2016. Efficacy, tolerability, and safety of an oral enzyme combination vs diclofenac in osteoarthritis of the knee: results of an individual patient-level pooled reanalysis of data from six randomized controlled trials. *Journal of Pain Research*, Volume 9:941–961.

Vandana Roy, Vandana Tayal 2017. An Assessment of Availability, Cost and Rationality of Serratiopeptidase Preparations in India. *MAMC Journal of Medical Sciences*, 3(3):152.

Wolfe, F. 1999. Determinants of WOMAC function, pain and stiffness scores: evidence for the role of low back pain, symptom counts, fatigue and depression in osteoarthritis, rheumatoid arthritis and fibromyalgia. *Rheumatology*, 38(4):355–361.