ABSTRACT

Objective: Knee osteoarthritis is a common disabling chronic disease globally. Many pharmacological agents have been used efficiently in treatment of knee osteoarthritis. This study aims to evaluate metformin and serratiopeptidase together in treatment and stop of osteoarthritis progression by different mechanisms.

Methods: Present study was a randomized clinical trial study conducted in Al-Kindi teaching hospital through the period from 1st January to 30th of May, 2017 on two groups of 80 osteoarthritis patients (group I; treated with metformin 850 mg oral tablets and group II; treated with metformin 850 mg oral tablets and serratiopeptidase 20 mg oral tablets). Parameters of two groups were compared with those of 40 normal healthy controls.

Results: Patients in group II showed a highly significant reduction in pain scores post-treatment (p<0.001). Tumor necrosis factor alpha (TNF-α), interleukin 1 beta (IL-1β) and interleukin 8 (IL-8) levels were significantly lowered among patients in group II treatment (p<0.001). Lower inflammatory parameters levels were observed among healthy controls and the parameters levels of group II patients were lower than those of group I patients.

Conclusion: Metformin and serratiopeptidase regimen was efficient and safe in the treatment of knee osteoarthritis.

Keywords: Knee osteoarthritis, Inflammatory parameters, Metformin, Serratiopeptidase

INTRODUCTION

Knee osteoarthritis (OA) is a common disabling chronic disease globally [1]. OA is a disorder of synovial joints characterized pathologically by damaging of articular cartilage, increased load, thickening of the capsule, subchondral bone changes and osteophytosis [2]. The obesity is a major epidemic all over the world and it is greatly linked to OA [3] and its effect on OA is multi-factorial [4]. The obesity is responsible on initiating the mechanical effects which lead to knee joint damage by high load, muscular weakness and biomechanical changes [5], in addition to the effect of metabolic factors [6] like lipids and humoral mediators [7]. The symptoms of knee OA are commonly pain, stiffness, tenderness and swelling [8].

Management

The goal of knee OA management is the eliminating of pain, movement maintenance, stopping joint cartilage destruction and enhancing the quality of life [8]. The management is based on three ways; non-pharmacological, pharmacological and surgical. Non-pharmacological ways involved common lifestyle changes like weight reduction, physical activity and programmed dieting [9]. Reduction of weight loss was proved to improve both pain and movement in obese patients with knee OA [10]. Many pharmacological agents were used efficiently in treatment of knee OA like systemic non-steroidal anti-inflammatory drugs (NSAIDs) [11], topical creams [12], glucosamine [13], diacerein [14], platelet-rich-plasma [15], metformin [16] and serratiopeptidase [17].

Metformin

The Metformin (biguanide) is the widely used treatment of type II diabetes mellitus. Among diabetics, metformin alters glucose production and may have an effect on insulin production. It also had an effect in weight loss [18]. In addition to anti-diabetic treatment effect, metformin is shown to relieve the pain and inflammation intensity of OA with no reported side effects that make it as a treatment choice in patients with knee OA and serve as a potential drug for inflammation-related disorders [19]. Anti-inflammatory mechanism of metformin unknown till now, however, many researchers documented that Metformin had an effect in lowering inflammatory markers levels and oxidative stress [20].

Serratiopeptidase

Serratiopeptidase which is also known as serralysin/serratia-protease/serrapeptase is a proteolytic enzyme has anti-inflammatory benefits [21]. Drugs involved this enzyme are regarded as modern medicines due to their selectivity and efficiency [22]. These enzymes are proteins including the tremendous catalytic capacity and offer robust implications in modern healthcare [23]. Nowadays, serratiopeptidase enzymes are highly used in Japan and Europe as the anti-inflammatory and pain treatment of choice [24].

In Iraq, the prevalence of overweight and obesity was increased in last two decades leading to steadily increase of knee OA cases especially among elderly age population causing disabled function, poor life quality and a big burden on health system [25]. Many pharmaceutical prescriptions for knee OA treatment were established with no obvious efficiency and numerous adverse effects. For that, our study aimed to evaluate metformin and serratiopeptidase together in treatment and stop of OA progression by different mechanisms.

MATERIALS AND METHODS

Study design and settings

This study is a randomized clinical trial study conducted in Al-Kindi teaching hospital through the period from 1st January to 30th of May, 2017. The patients with knee osteoarthritis presented to consultancy clinic of Al-Kindi teaching hospital were the study population. Inclusion criteria were adulthood, overweight and obesity and knee osteoarthritis. Exclusion criteria were pregnancy, bleeding and coagulation disorders, systemic diseases like hypertension and diabetes mellitus and current treatment of knee osteoarthritis with other drug regimens.
Study participants
A total of 80 patients with knee OA were selected non-randomly and were randomized digitally into two groups (group I; treated with metformin 850 mg oral tablets) and (group II; treated with metformin 850 mg oral tablets and serratiopeptidase 20 mg oral tablets). The patients with knee OA were diagnosed clinically and radiologically by Rheumatologist in Al-Kindi teaching hospital. A group of 40 healthy controls was selected from relatives of patients. The weight and height of each all study participants (n=120) was measured using a calibrated scale to calculate the body mass index (BMI) before selection in the study. All the study participants were matched for BMI.

Ethical considerations
A written informed consent was taken from each study participant and the research work team was responsible for the treatment of knee OA patients and any complications of the drug regimens according to Helsinki Declaration. The patients were advised to stop the drug and call the researchers if there is any sign of bleeding or bruising.

Clinical trial
After labelling of selected OA patients, pain scores (1-10) were taken from patients and recorded in a prepared questionnaire for each study participant. A sample of 5 ml blood was taken from each study participants in the laboratory of the hospital to measure the inflammatory markers (serum levels of IL-1ß, IL-8, TNF-α, resistin and adiponectin).

After taking patients parameters, the two drug regimens were prescribed by rheumatologists for two study groups and the patients were followed up after 12 w. In the second visit, the BMI and pain scores were recorded in the questionnaire of each patient and the investigations of anti-inflammatory parameters for three groups of study participants were done in the laboratory of the hospital. The outcome of drugs was assessed by measuring pain scores, BMI and level of inflammatory markers of knee joint OA patients and then compare it with the healthy group. The adverse effects for both study groups through a period of 12 w were recorded.

Statistical analysis
All the data were entered analyzed by using a statistical package of social sciences software program. The results were categorized in contingency table. Paired t-test was used to compare between two means before and after treatment. One way ANOVA analysis was used to compare between means of three study groups while fishers exact test was used to compare between categorical variables of adverse effects. Level of significance was set as ≤0.05.

RESULTS
Demographic characteristics
This study included 80 knee OA patients, 42.5% of patients in group I were in age group ≥60 y while 47.5% of patients in group II were in age group ≥60 y. Female OA patients in two studied groups were more than females. No significant differences were observed between study groups patients regarding age and gender (table 1).

| Variable | Knee OA patients | P |
|----------|------------------|---|
| Age      |                  |   |
| <40 y    | 4 (10.0)         | 4 (10.0) | 0.8* NS |
| 40-49 y  | 10 (25.0)        | 11 (27.5) |   |
| 50-59 y  | 9 (22.5)         | 6 (15.0) |   |
| ≥60 y    | 17 (42.5)        | 19 (47.5) |   |
| Gender   |                  |   |
| Male     | 12 (30.0)        | 11 (27.5) | 0.6** NS |
| Female   | 28 (70.0)        | 29 (72.5) |   |

Total number of patients (n) =80, No. =40 patients, NS=Not significant, * Fishers exact test, ** Chi-square test.

The BMI of OA patients in both study groups were slightly decreased post treatment but with no significant difference (p=0.7, p=0.1). OA patients in group I (treated with metformin) showed no significant changes in pain scores post-treatment (p=0.07), while patients in group II (treated with metformin and serratiopeptidase) showed a highly significant reduction in pain scores post-treatment (p<0.001). Regarding inflammatory parameters, TNF-α was significantly lowered among OA patients in both study groups post-treatment (p<0.001). There was a highly significant lowering in serum levels of IL-1ß and IL-8 post-treatment (p<0.001), on another hand, no significant changes were observed post-treatment for IL-1ß and IL-8 levels among patients of group I. There were no significant changes in serum levels of resistin and adiponectin post-treatment for OA patients of both study groups (table 2).

| Parameters | Group I | Group II | P* |
|------------|---------|----------|----|
|            | Pre     | Post     |    |
|            | mean±SD | mean±SD  |    |
| Group I    |         |          |    |
| BMI        | 34.6±5.1| 34.2±4.6 | 0.7 NS |
| Pain scores| 7.9±2.1 | 6.9±2.8  | 0.07 NS |
| IL-1ß (pg/ml) | 425.2±22.1 | 419.8±20.9 | 0.2 NS |
| IL-8 (pg/ml)  | 370±25.4 | 366.7±28.5 | 0.5 NS |
| TNF-α (pg/ml) | 65.9±15.5 | 60.3±7.0  | <0.001 5 |
| Resistin (µg/ml) | 0.018±0.009 | 0.016±0.007 | 0.2 NS |
| Adiponectin (µg/ml) | 31.3±4.2 | 29.8±4.5  | 0.1 NS |

| Group II   |         |          |    |
|            | Pre     | Post     |    |
|            | mean±SD | mean±SD  |    |
| BMI        | 33.7±5.8| 32.1±4.2 | 0.1 **NS |
| Pain scores| 8.0±2  | 4.5±2.5  | <0.001 5 |
| IL-1ß (pg/ml) | 427±20.3 | 412±17.5  | 0.001 5 |
| IL-8 (pg/ml)  | 368.1±30.3 | 228.2±21.4 | <0.001 5 |
| TNF-α (pg/ml) | 70.3±17 | 58.4±7.0  | <0.001 5 |
| Resistin (µg/ml) | 0.02±0.001 | 0.02±0.009 | 0.3 NS |
| Adiponectin (µg/ml) | 30.4±5.5 | 29.4±4.9  | 0.3 NS |

Total number of patients (n) =80, NS=Not significant, S= Significant, *Paired t-test, SD=Standard deviation, BMI=Body mass index, IL-1ß= Interleukin-1 Beta, IL-8=Interleukin-8, TNF-α=Tumor necrosis factor-alpha.
After comparing inflammatory parameters of OA patients of both study groups with that of healthy controls, there were highly significant differences in serum levels of IL-1ß, IL-8, TNF-α and adiponectin between study groups and healthy controls (p<0.001). Lower levels were observed among healthy controls and the parameters levels of group II patients were lower than those of group I patients. No significant differences were observed between study groups and healthy controls regarding resistin level (p=0.2) (table 3).

### Table 3: Distribution of inflammatory parameters according to study groups and healthy controls

| Parameters        | Group I mean±SD | Group II mean±SD | Healthy mean±SD | P*    |
|-------------------|-----------------|------------------|-----------------|-------|
| IL-1ß (pg/ml)     | 419.8±20.9      | 412.6±17.5       | 3.2±0.8         | <0.001* |
| IL-8 (pg/ml)      | 366.7±28.5      | 228.2±21.4       | 33.8±12.4       | <0.001* |
| TNF-α (pg/ml)     | 60.3±0.7        | 58.4±0.7         | 38.5±2.9        | <0.001* |
| Resistin (µg/ml)  | 0.016±0.007     | 0.022±0.009      | 0.021±0.001     | 0.2 NS |
| Adiponectin (µg/ml)| 29.8±4.5       | 29.4±4.9         | 17.3            | <0.001* |

Total number of study participants (n) =80, NS=Not significant, *One-way ANOVA analysis, SD=Standard deviation, IL-1ß=Interleukin-1 Beta, IL-8=Interleukin-8, TNF-α=Tumor necrosis factor-alpha.

As shown in table 4, no significant differences were observed between two study groups regarding the adverse effects 3 mo post-treatment. The main side effects of group I regimen were nausea and vomiting (10%), headache (5%), dizziness (5%), diarrhea (2.5%) and weakness (2.5%). The main side effects reported after use of group II regimen were nausea and vomiting (2.5%) and dizziness (2.5%).

### Table 4: Distribution of adverse effects for two treated knee OA patients groups' post-treatment

| Adverse effects               | Study groups | P*    |
|-------------------------------|--------------|-------|
|                               | Group I No. (%) | Group II No. (%) |       |
| Nausea and vomiting          | 4 (10.0)      | 1 (2.5) | 0.3 NS |
| Diarrhea                      | 1 (2.5)       | 0      | 1.0 NS |
| Headache                      | 2 (5.0)       | 0      | 0.2 NS |
| Dizziness                     | 2 (5.0)       | 1 (2.5) | 0.6 NS |
| Muscle weakness               | 1 (2.5)       | 0      | 1.0 NS |
| Bleeding/Bruising             | 0             | 0      | -     |

Total number of patients (n) =80, No. of adverse effects for group I=10 effects, No. of adverse effects for group II=2 effects, NS=Not significant, *Fishers exact test.

### DISCUSSION

Many drugs formulations were used in the treatment of knee OA such as NSAIDs and drugs have the ability to interrupt the extracellular matrix metabolism, especially proteoglycans synthesis [11, 26]. However, these pharmacological agents had several adverse effects like gastrointestinal ulcers. Therefore, new drug formulations are needed to relieve symptoms with safe and long duration effects [26].

Current study revealed that after 3 mo of treatment, knee OA patients group treated with metformin only had only significant decline in TNF-α inflammatory marker (p<0.001), while knee OA patients group II (treated with metformin and serratiopeptidase) had a significant reduction in pain scores, IL-1ß, IL-8 and TNF-α (p<0.001). These findings indicated the great symptomatic efficacy and laboratory efficacy of metformin and serratiopeptidase regimen for treatment of knee OA. The synergistic effect of metformin with many drugs and in different diseases was observed [27]. Mohammed et al. [28] study in Iraq documented that metformin when used with other anti-inflammatory agents like NSAIDs for treatment of knee OA, resulted in improvement of Osteoarthritis Outcome Score. The metformin is first-line drug of type II diabetes mellitus through suppression of glucose production by liver [29]. It was found that metformin reduced the inflammation markers and participated in oxidative stress reduction with unknown mechanism till now [30]. These inflammatory markers like cytokines and chemokines are elevated after knee joint trauma with the great role of oxidative in the pathophysiology of knee OA [8]. Some authors confirmed in the vitro osteogenic effect of metformin [31]. Chen et al. [32] study in China showed that metformin had a therapeutic activity for intervertebral disc degeneration. Although no significant relationship, the BMI of patients in both study groups was reduced after three months of treatment. This finding is similar to results of Levri et al. [33] study in USA which found no sufficient evidence to use metformin for treatment of overweight and obesity.

Serratiopeptidase enzymes had a great role in biological life by its function as biocatalysts [34]. They are proteins that widely used as anti-inflammatory agents in addition to their analgesic effect [35]. Ingle et al. [36] study in India used serratiopeptidase in both study groups of patients with knee OA in combination with different drugs and revealed higher efficacy with mild adverse effects of both modalities including serratiopeptidase in the treatment of knee OA. The molecular mechanism of serratiopeptidase enzyme action in knee OA is not well organized completely, but it was proved that it dissolves the dead and damaged tissues without harming living tissues [37]. Previous Indian study proved that serratiopeptidase provides anti-inflammation effect postoperatively [38].

In comparing inflammatory parameters of both study groups with those of healthy controls, serum levels of IL-1ß, IL-8, TNF-α and adiponectin of patients treated with metformin and serratiopeptidase were significantly lower than those of patients treated with metformin only. This finding confirmed the synergistic anti-inflammatory effect of metformin and serratiopeptidase.

Additionally, these findings coincide with results of Kim et al. [39] in South Korea and Bhagat et al. [17] et al. study in India which clarified the anti-inflammatory effect of metformin and serratiopeptidase enzyme.

The adverse effects of both study groups were minimal especially for group II regimen (metformin and Serratiopeptidase) with no significant differences between the study groups. These findings referred to the safety of this drugs regimen that is confirmed by several studies conducted previously [28, 37].

The main limitations of the present study were lost to follow up, single centre study and short period of time for assessing the adverse effects, so further long duration follow up studies are needed to check the long effect of drug regimen of metformin and serratiopeptidase.
In conclusion, application of drug regimen of metformin and serratopептидаза in the treatment of knee osteoarthritis is efficient and safe. This regimen is efficient in reducing the pain and inflammatory markers but with no effect on body mass index of patients with knee osteoarthritis.

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AUTHORS CONTRIBUTIONS
This work was the result of the author's works in Al-Kindy hospital according to their specialities.

Yasir Abbas Ateia: Principle investigator (Selection of the drugs, drug doses and reporting adverse effects). Implementing and monitoring the trial. Writing and supervising the methods, results and discussion. Editing of the manuscript.

Dr. Mohammed Sh. Al-Edanne: Co-investigator helps in confirming knee OA diagnosis and selection of patients. This author is responsible also for assessing the outcome drugs among patients with regular patients monitoring in addition to managing the adverse effects accordingly.

Dr. Mohammed Ismael Al-Qurtas: Co-investigator helps in technical works and logistics in addition to data collection, statistical analysis and writing the introduction.

CONFLICT OF INTERESTS
Declared none.

REFERENCES
1. Cross M, Smith E, Hoy D, Nolte S, Akerman I, Fransen M, et al. The global burden of hip and knee osteoarthritis: estimates from the global burden of disease 2010 study. Annals Rheumatic Diseases; 2014. Available from: https://www.google.iq/url?sa=t&rct=j&q=&esrc=s&source=web&cd=&ved=2ahUKEwjOxJq5ho9RAhXJmEwHHWpocP4Ch0wH6AEoAA&usg=AOvVawj2UFXcetume0f-EfQ91mFe.
2. Pritzker K. Pathology of osteoarthritis. In: Osteoarthritis. 2nd edition. Brandt K, Doherty M, Lohmander LS, eds. Oxford University Press: Oxford; 2003. p. 248-58.
3. Anandacoomarasamy A, Caterson I, Sambrook P, Fransen M, March L. The impact of obesity on the musculoskeletal system. Int J Obes (Lond); 2008;32:211–22.
4. Szoeke C, Dennerlein L, Guthrie J, Clark M, Ciccitini F. The relationship between prospectively assessed body weight and physical activity and prevalence of radiological knee osteoarthritis in postmenopausal women. J Rheumatol; 2006;33:1835–40.
5. Runhaar J, Koes BW, Cnockaerts S, Bierma-Zeinstra SM. A systematic review on the changed biomechanics of lower extremities in obese individuals: a possible role in the development of osteoarthritis. Obes Rev; 2011;12:1071–82.
6. Brooks PM. Impact of osteoarthritis on individuals and society: how much disability? Social consequences and health economic implications. Curr Opin Rheumatol; 2002;14:573–7.
7. Velasquez MT, Katz JD. Osteoarthritis: another component of metabolic syndrome? Metab Syndr Relat Disord; 2010;8:295–305.
8. Ayra RK, Jain V. Osteoarthritis of the knee: an overview. J IACM; 2013;14:54–62.
9. Richette PJ, Pointou C, Garnier P. Beneficial effects of massive weight loss on symptoms, joint biomarkers and systemic inflammation in obese patients with knee OA. Ann Rheum Dis; 2011;70:139-44.
10. Gadbergens H, Boesen M, Lohmander LS, Christensen R, Henriksen M, Bartels EM, et al. Weight loss is effective for symptomatic relief in obese subjects with knee osteoarthritis independently of joint damage severity assessed by high-field MRI and radiography. Osteoarthritis Cartilage; 2012;20:495-502.
11. Srinivasan A, Venkatachalam T, Thamothanaran G, Sekar G. Assessment of the efficacy of acetyllicenar versus tramadol in osteoarthritis patients. Int J Appl Pharm Biol Res; 2016;1:40-6.
12. Mason L, Moore RA, Edwards J. Topical NSAIDs for chronic musculoskeletal pain: systematic review and meta-analysis. BMC Musculoskelet Disord; 2004;5:28.
13. Gegg D, Reda DJ, Harris CL, Klein MA, O’Dell JR, Hooper MM, et al. Glucosamine, chondroitin sulfate, and the two in combination for painful knee osteoarthritis. N Engl J Med; 2006;354:795-8.
14. Bartels EM, Bliédal H, Schondorf PK, Altman RD, Zhang W, Christensen R. Symptomatic efficacy and safety of diacerein in the treatment of osteoarthritis: a meta-analysis of randomized placebo-controlled trials. Osteoarthritis Cartilage; 2010;18:289-96.
15. Kon E, Mandelbaum B, Bida R, Filardo G, Delcoqilo M, Timoncini A, et al. Platelet-rich plasma intraarticular injection versus hyaluronic acid vissosupplementation as treatments for cartilage pathology: from early degeneration to osteoarthritis. Arthroscopy; 2011;27:1490-501.
16. Barnett LA, Jordan KP, Edwards JJ, van der Windt DA. Does metformin protect against osteoarthritis? An electronic health record cohort study. Prim Health Care Res Dev; 2017;18:623-8.
17. Bhagat S, Agarwal M, Roy V. Serratopептидаза: a systematic review of the existing evidence. Int J Surg; 2013;11:209-17.
18. Seifarth C, Schehler B, Schneider H. Effectiveness of metformin on weight loss in non-obese individuals with obesity. Exp Clin Endocrinol Diabetes; 2013;121:27-31.
19. Yuan H, Li L, Zheng W, Wan J, Ge P, Li H, et al. Antidiabetic drug metformin alleviates endotoxin-induced liminal liver injury in mice. Int Immunopharmacol; 2012;12:682-8.
20. Berenbaum F. Osteoarthritis as an inflammatory disease (osteoarthritis is not osteoarthrosis). Osteoarthritis Cartilage; 2013;21:16-21.
21. Klein G, Kullich W. Short-term treatment of painful osteoarthritis of the knee with oral enzymes. A randomized, double-blind study versus diclofenac. Clin Drug Invest; 2000;19:15-23.
22. Vakayanopoulos V, Brassier A, Chabli A, Caillaud L, Lemoine M, Odent T, et al. Enzyme replacement therapy for lysosomal storage disorders. Arch Pediatr; 2011;18:1119-23.
23. Verma MK, Pulcherla KR. Enzyme promiscuity in Earthworm serine protease-Substrate versatility and therapeutic potential. Amino Acids; 2016;48:941-8.
24. Malehi PC. Orally administered serratopептидаза: can it work? J Assoc Physicians India; 1998;46:492.
25. Iraqi ministry of health, Directorate of public health and primary health care and Iraqi ministry of planning and development cooperation, central organization for statistics and information in collaboration with WHO. Chronic non-communicable diseases risk factors survey; 2006. Available from: http://www.fineprint.com. [Last accessed on 02 Jul 2017]
26. Leong DJ, Choudhury M, Hirsh DM, Hardin JA, Cobelli NJ, Sun HB. Nutraceuticals: potential for chondroprotection and molecular targeting of osteoarthritis. Int J Mol Sci; 2013;14:23063-85.
27. Ortiz ML. Synergistic interaction between meftormín and sulfonliureas in diclofenac-induced antinocepcion measured using the formalin test in rats. Pain Research and Management. Pain Res Manag; 2013;18:2536-7.
28. Mohammed MM, Al-Shamma KJ, Jassim NA. Evaluation of the clinical use of meftormín or pioglitazone in combination with meloxican in patients with knee osteoarthritis; using knee injury and osteoarthritis outcome score. Iraqi J Pharm Sci; 2014;23:13-23.
29. Cheng AY, Fantus IG. Oral antihyperglycemic therapy for type 2 diabetes mellitus. CMAJ; 2005;172:213-26.
30. Andrews M, Soto N, Arrendondo M. Effect of metformin on the expression of tumor necrosis factor-α, Toll-like receptors 2/4 and C reactive protein in obese type-2 diabetic patients. Rev Med Chile; 2012;14:1377-82.
31. Cortizo AM, Sedlinsky C, McCarthy AD, Blanco A, Schurman L. Osteogenic actions of the anti-diabetic drug meftormín on osteoblasts in culture. Eur J Pharmacol; 2006;536(1-2):38-46.
32. Chen D, Xia D, Pan Z, Xu D, Zhou Y, Wu Y, et al. Meftormín protects against apoptosis and senescence in nucleus pulposus cells and ameliorates disc degeneration in vivo. Cell Death Dis; 2016;7:e2441.
33. Levri KM, Slaymaker E, Last A, Ference J, D’Amico F, Wilson SA. Metformin as a treatment for overweight and obese adults: a systematic review. Ann Fam Med 2005;3:457-61.
34. Imbimbo BP. The potential role of non-steroidal anti-inflammatory drugs in treating Alzheimer’s disease. Expert Opin Investig Drugs 2004;13:1469–81.
35. Jadav SP, Patel NH, Shah TG, Gajeri MV, Trivedi HR, Shah BK. Comparison of antiinflammatory activity of serratiopeptidase and diclofenac in albino rats. J Pharmacol Pharmacother 2010;1:116-7.
36. Ingle P, Lathi V, Patil P, Surana S, Shirath N. Evaluation of symptomatic efficacy and safety of diclofenac versus etrocoxib in combination with serratiopeptidase in knee osteoarthritis. Pharmagene 2014;1:10-4.
37. Tiwari M. The role of serratiopeptidase in the resolution of inflammation. Asian J Pharm Sci 2017;12:209–15.
38. Chappi DM, Suresh KV, Patil MR, Desai R, Tauro DP, Bharani KNSS, et al. Comparison of clinical efficacy of methylprednisolone and serratiopeptidase for reduction of postoperative sequelae after lower third molar surgery. J Clin Exp Dent 2015;7:e197-202.
39. Aiswarya S, Parvathy S, Aneesh TP, Viswanad V. Design and in vitro characterization of metformin loaded resealed erythrocytes. Asian J Pharm Clin Res 2017;10:231-8.