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Effects of single or combined administration of salmon calcitonin and omega-3 fatty acids vs. diclofenac sodium in sodium monoiodoacetate-induced knee osteoarthritis in male Wistar rats

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Abstract

Background: There is a continuous search for a better therapy in osteoarthritis (OA) management. Therefore, this study investigated the effects of salmon calcitonin (Sct) and/or omega-3 fatty acids (N-3) relative to diclofenac sodium (DF) in induced knee osteoarthritic male Wistar rats.

Methods: The 40 rats that were used in this study were divided into 8 groups (n = 5 rats), viz: Normal control; OA control; OA + N-3; OA + Low dose of Sct (Sct.Lw); OA + High dose of Sct (Sct.Hi); OA + N-3 + Sct.Lw; OA + N-3 + Sct.Hi; and, OA + DF. OA was induced with 4 mg of sodium monoiodoacetate in 40 μL of saline. The solution was injected into the left knee joint space of anaesthetised rats. Sct was administered at 2.5 and 5.0 IU/kg b.w. (im), whereas N-3 and DF were administered at 200 and 1 mg/kg b.w. (p.o.), respectively. Treatments commenced 9 days after the induction of OA, and they lasted for 28 days.

Results: Sct and/or N-3 significantly reduced c-telopeptide of type 1 collagen (CTX-1), collagen type 2 α-1 (C2M), malondialdehyde (MDA), uric acid (UA), and interleukin-6 (IL-6), but, significantly increased superoxide dismutase (SOD) after OA induction. Both therapies had additive effects on C2M, MDA, SOD, and catalase (CAT), but, non-additive actions on UA, IL-6, and CTX-1. Like the Sct and N-3, DF significantly reduced CTX-1, C2M, UA, and IL-6. However, it had no significant effect on SOD and MDA, even though it significantly reduced CAT activity. None of the therapies had significant effect on total alkaline phosphatase activity, except N-3 + Sct.Lw.

Conclusions: The combined, and sometimes the single administration of Sct and N-3 proved to be better therapies in OA management than DF.

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Introduction

Osteoarthritis (OA) is the most prevalent disorder of the musculoskeletal system, and the greatest cause of disability in both advanced and the so-called emerging countries [1]. More than 10% of the world population is affected by this disease [2]. Although OA affects the hand, spine, hip, wrist, and ankle [3], knee OA represents the most widespread form of this disease [4].

Even though several animal models have been used for studying the pathogenesis of OA [5], the initiation of this disease by intra-articular injection of sodium monoiodoacetate (MIA) is one of the most widely used methods [6]. MIA triggers a pro-inflammatory response, and attenuates the glyceraldehyde-3-phosphate dehydrogenase activity in chondrocytes, resulting in the alteration of glycolytic process and cell death [7].

Joint inflammation features elevate free radical activities, which are linked with macrophages phagocytosis, neutrophils activation, and the uncoupling of varieties of cellular redox systems [8]. These events result to an increased peroxidation of membrane lipids, and the derangement of the endogenous antioxidant system. The provocation of lipid peroxidation by free radicals has been considered to be the primary mechanism of cell membrane damage, and cell death [9]. Apart from joint inflammation, OA is also accompanied by the gradual degradation of joint ligaments, menisci, and cartilage, increased subchondral bone formation [10, 11], and decreased bone quality [12], among others.

However, it has been opined that the optimal therapy for OA should include a combination of pharmacological and non-pharmacological (e.g. regular nursing, regular physical exercises and aerobics, dietary supplements, etc.) methods of treatment [13]. Therefore, the present study investigated the effects of the single or combined administration of salmon calcitonin (Sct) and omega-3 fatty acids (N-3) (eicosapentaenoic acid and docosahexaenoic acid, ratio – 3/2), relative to diclofenac sodium (DF) in the treatment of MIA-induced knee OA in male Wistar rats.
rats. It was hypothesised that the combined administration of Sct and N-3 will not proffer better treatment than the single therapy or DF.

The results showed that Sct and N-3 had both additive and non-additive effects on the biochemical parameters that were considered in this study. The combined administration of both therapies offered better pharmacological benefits than the single or DF administration.

**Materials and methods**

**Drugs and chemicals**

Sct and MIA were purchased from Sigma-Aldrich (St. Louis, MO, USA), whereas omega-3 fatty acids were obtained from Gujarat Liqui Pharmcups, Ltd. (Vadodara, Gujarat, India). Sodium pentobarbital was procured from Nicholas Piramal, Ltd. (Thane, Maharashtra, India). In addition, DF was bought from Wuhan Grand Pharmaceutical Company (Wuhan, Hubei, China).

**Experimental animals and care**

Forty adult male Wistar rats, weighing between 180 and 220 g, were used for this research. The rats were obtained from the Animal Holding Unit of the Biochemistry Department, University of Ilorin, Ilorin, Nigeria. They were kept in wooden cages at a room temperature of about 27°C–30°C, and 12 h light-12 h dark photo-periodicity cycle. The rats were acclimatised for 1 week, afterwards, they were randomly allotted to separate groups, prior to their exposure to the various chemical agents that were used in the study. They were given standard pelleted diet (Ace Feed PLC Ibadan, Oyo, Nigeria) and water *ad libitum* daily, and were weighed weekly.

All the animals received humane care in accordance with the criteria outlined in the ‘Guide for the Care and Use of Laboratory Animals’ documented by the National Academy of Science [14], and approved by the Ethical Committee of the University of Ilorin, Ilorin, Nigeria.

**Experimental design**

The 40 adult male Wistar rats that were used for this study were divided into 8 groups, which included the following: group 1: Normal control; group 2: Osteoarthritic (OA) control; group 3: OA + Omega-3 fatty acids (N-3); group 4: OA + Low dose of Sct (Sct.Lw); group 5: OA + High dose of Sct (Sct.Hi); group 6: OA + N-3 + Sct.Lw; group 7: OA + N-3 + Sct.Hi and, group 8: OA + DF.

Low and high doses of Sct were administered at 2.5 IU/kg body weight (b.w.)/day and 5.0 IU/kg b.w./day (im) respectively. However, N-3 (eicosapentaenoic acid and docosahexaenoic acid, ratio – 3/2) and DF were administered at 200 mg/kg b.w./day and 1 mg/kg b.w./day (p.o.) respectively. Treatments started 9 days after the induction of the OA, and they lasted for 28 days.

**Induction of knee osteoarthritis**

Knee OA was induced with 4 mg of MIA in 40 μL of sterile saline. The solution was injected (using a 27-gauge needle) intra-articularly through the patellar ligament of the rats’ left knee joint while they were under sodium pentobarbital anaesthesia (40 mg/kg b.w., *ip*) [15]. Under the same procedure, the rats in the normal control group were injected intra-articularly with 40 μL of sterile saline.

**Preparation of salmon calcitonin**

Sct powder was dissolved in 0.9% of sodium chloride to obtain the desired doses [16]. The solution was stored in a refrigerator at 2°C–8°C for the maintenance of the hormone viability.

**Biochemical analyses**

At the end of the experiment, the rats were administered ketamine hydrochloride (50 mg/kg b.w., *im*) 12 h after treatments on the 28th day. Thereafter, blood was collected by cardiac puncture into the heparinised bottles, which were centrifuged at 4000 revolutions per min, for 15 min, at −4°C, using a cold centrifuge, model 8881 (Centurium Scientific, Chichester, West Sussex, UK). The separated plasma samples were collected in separate plain bottles for the assessment of some biochemical parameters.

The diagnostic kits for the determination of superoxide dismutase (SOD), catalase (CAT), malondialdehyde (MDA), and uric acid (UA) were obtained from Fortress Diagnostics, Ltd. (Belfast, Northern Ireland, UK). The analytic kits for the estimation of total alkaline phosphatase (TALP), interleukin-6 (IL-6), collagen type 2-C1 (C2M), and c-telopeptide of type 1 collagen (CTX-1) were purchased from Elabscience Biotechnology Company, Ltd. (Wuhan, Hubei, China). The analyses were performed according to the manufacturers’ instruction.

**Data analyses**

Statistical evaluations of the differences between the group mean values were tested by one way analysis of variance, followed by least significance difference *post-hoc* test for multiple comparisons, using statistical package for social sciences version 20.0. Statistical significance was considered at *p* < 0.05, and the results were presented as mean ± standard error of mean (SEM).

**Results**

**Effects of Sct and/or N-3 on TALP, CTX-1 and C2M in MIA-induced knee OA in rats**

There were significant (*p* < 0.05) elevations in TALP activity in the experimental animal groups 2–8 (OA control,
The induced knee OA caused a significant (p < 0.05) increase in the plasma level of the CTX-1 in the OA control group, when compared with the normal control (Figure 2). In the animal groups 3–8 (OA + N-3, OA + Sct.Lw, OA + Sct.Hi, OA + N-3 + Sct.Lw, OA + N-3 + Sct.Hi, and OA + DF), there was no significant difference in the CTX-1 level,
when compared with the normal control group. However, relative to the OA control group, there were significant decreases in CTX-1 level in groups 3–8.

Compared with the normal control group, there was a significant ($p < 0.05$) elevation in the C2M level in the OA control group (Figure 3). However, relative to the latter, there were significant reductions in the plasma level of C2M in groups 3–8 (OA + N-3, OA + Sct.Lw, OA + Sct.Hi, OA + N-3 + Sct.Lw, OA + N-3 + Sct.Hi, and OA + DF). Moreover, there were significant diminutions in C2M level in the OA + N-3 + Sct.Lw group, when compared with the OA + Sct.Lw group, and in the OA + N-3 + Sct.Hi group, relative to the OA + Sct.Hi group.

**Effects of Sct and/or N-3 on SOD, CAT and MDA in MIA-induced knee OA in rats**

Relative to the normal control group, there were significant ($p < 0.05$) decreases in SOD activity in the OA control and the OA + DF groups (Figure 4). However, significant increases in SOD activity were recorded in groups 3–7, (OA + N-3, OA + Sct.Lw, OA + Sct.Hi, OA + N-3 + Sct.Lw, and OA + N-3 + Sct.Hi), relative to the OA control group. Moreover, there were significant increases in SOD activity in groups 5–7 (OA + Sct.Hi, OA + N-3 + Sct.Lw, and OA + N-3 + Sct.Hi), when compared with the normal control and OA control groups. In addition, there was a significant elevation in SOD activity in OA + N-3 + Sct.Lw group, when compared with the OA + Sct.Lw group.

There was no significant difference in CAT activity in groups 2–5 (OA control, OA + N-3, OA + Sct.Lw, and OA + Sct.Hi) relative to the normal control group (Figure 5). In addition, compared with the normal control and OA control groups, there was a significant ($p < 0.05$) decrease in CAT activity in group 8 (OA + DF). Relative to the OA control group, a significant increase in CAT activity was recorded in group 6 (OA + N-3 + Sct.Lw). However, compared with the normal control and the OA + N-3 groups, a significant decrease in CAT activity was noted in group 7 (OA + N-3 + Sct.Hi).

There was a statistically significant ($p < 0.05$) increase in MDA level in group 2 (OA control), when compared with the normal control group (Figure 6). In groups 3–7 (OA + N-3, OA + Sct.Lw, OA + Sct.Hi, OA + N-3 + Sct.Lw and OA + N-3 + Sct.Hi), there were significant decreases in MDA level, relative to group 2 (OA control). There was also a significant decrease in the MDA level in group 7 (OA + N-3 + Sct.Hi), relative to groups 5 (OA + Sct.Hi) and 3 (OA + N-3).

**Effects of Sct and/or N-3 acids on UA and IL-6 in MIA-induced knee OA in rats**

A significant ($p < 0.05$) elevation in UA level was recorded in the OA control group, when compared with the normal control (Figure 7). However, in the animal groups 3–8 (OA + N-3, OA + Sct.Lw, OA + Sct.Hi,
OA + N-3 + Sct.Lw, OA + N-3 + Sct.Hi, and OA + DF), there were significant diminutions in UA level, relative to the OA control group.

The observed effects of Sct and/or N-3 on IL-6 level were similar to what was recorded in the determination of UA level. The induced OA caused a significant (p < 0.05)
Figure 6: Effects of Sct and/or N-3 on malondialdehyde level (µM) in MIA-induced knee OA in male Wistar rats.
Values are expressed as mean ± SEM. *p < 0.05 is significant compared with group 1 (normal control), *p < 0.05 is significant compared with group 2 (osteoarthritic control). #p < 0.05 is significant – OA + N-3 vs. OA + N-3 + Sct. Hi, *p < 0.05 is significant – OA + Sct. Hi vs. OA + N-3 + Sct. Hi. NB: OA, osteoarthritic; N-3, omega-3 fatty acids; Sct.Lw, low dose of Sct; Sct.Hi, high dose of Sct; DF, diclofenac sodium.

Figure 7: Effects of Sct and/or N-3 on uric acid level (mg/dL) in MIA-induced knee OA in male Wistar rats.
Values are expressed as mean ± SEM. *p < 0.05 is significant compared with group 1 (normal control), #p < 0.05 is significant compared with group 2 (osteoarthritic control). NB: OA, osteoarthritic; N-3, omega-3 fatty acids; Sct.Lw, low dose of Sct; Sct.Hi, high dose of Sct; DF, diclofenac sodium.

increase in IL-6 level in the OA control group, when compared with the normal control (Figure 8). However, relative to group 2 (OA control), significant decreases in IL-6 level were recorded in groups 3–8 (OA + N-3, OA + Sct. Lw, OA + Sct.Hi, OA + N-3 + Sct.Lw, OA + N-3 + Sct.Hi, and OA + DF).
Discussion

In the present study, the induced knee OA precipitated significant elevations in the bone formation marker (TALP) in groups 2–8, when compared with the normal control group. DF, Sct, and N-3 on single administration had no significant effects on bone formation process. Therefore, they tend not exacerbate the OA disease process [17]. No change in the plasma alkaline phosphatase activity was recorded in adult rats maintained on high dietary N-3 [18]. However, N-3 have also been noted to decrease [19], or increase [20, 21] the bone formation process. In favour of the induced OA, the co-administration of N-3 and low dose of Sct brought about a significant decrease in TALP activity in the OA + N-3 + Sct.Lw group, relative to the normal control, OA control, OA + N-3, and OA + N-3 + Sct.Hi groups. This finding suggests the possible beneficial effect of a low dose of Sct, compared with the high dose, as regards the bone formation process in the OA. Hamdy and Darley [22] reported that, a higher dose of Sct is not necessarily better than a lower dose. Nevertheless, they opined that the determination of the most favourable dose of Sct remains a challenge. Although it has been proposed that calcitonin receptors are present on osteoblasts, it remains uncertain whether Sct facilitates osteoblast-mediated bone formation process [23].

Increase in subchondral bone degradation characterise the early stage of OA; however, elevated bone formation process features in the late stage [24]. In the present study, the observed increase in both bone formation and bone degradation processes, showed that, the induced OA condition was at the mid-way between the early and the late stages of the disease. The induced OA caused a significant elevation in the plasma level of CTX-1. However, the single and combined administration of Sct and N-3, and also DF significantly reversed this effect. Although Sct and N-3 proved to have additive effects on the endogenous status of CTX-1, the effect of Sct was observed not to be dose graded. At therapeutic doses, Sct uncouples bone formation from bone resorption, impeding the latter, without disturbing the former [22]. Like Sct, N-3 have anti-resorptive effects on the bone tissue. They have been shown to inhibit the activity of osteoclasts, which instigates bone resorption [25].

Apart from the aforementioned increased bone formation and bone degradation processes, OA also features an increase in cartilage degradation [26]. In the OA control group, there was a significant increase in the plasma level of C2M, relative to the normal control. Although the single administration of N-3, the low and high doses of Sct, as well as DF brought about significant reductions in the plasma level of C2M, there was no significant disparity in the effects of these therapies. Sct has been documented

Figure 8: Effects of Sct and/or N-3 on interleukin-6 level (pg/mL) in – MIA-induced knee OA in male Wistar rats. Values are expressed as mean ± SEM. *p < 0.05 is significant compared with group 1 (normal control), †p < 0.05 is significant compared with group 2 (OA control). NB: OA, osteoarthritic; N-3, omega-3 fatty acids; Sct.Lw, low dose of Sct; Sct.Hi, high dose of Sct; DF, diclofenac sodium.
to increase cellular production of type II collagen in a dose-dependent manner [27, 28]. Even at a low dose, it attenuates the degradation of type II collagen [29]. As for N-3, Knotty and colleagues reported that Dunkin-Hartley guinea pigs maintained on N-3 diet showed a decreased degradation of type II collagen [19]. Although there was no significant difference in the C2M level in group 4 (OA + Sct.Lw), when compared with group 5 (OA + Sct.Hi), the significant reduction in the level of this marker in group 6 (OA + N-3 + Sct.Lw), relative to group 4 (OA + Sct.Lw), and in group 7 (OA + N-3 + Sct.Hi), relative to group 5 (OA + Sct. Hi) revealed the additive effect of the co-administration of Sct and N-3 on the C2M level. The co-administration of Sct and N-3 was found to provide a more beneficial effect than the single therapy or DF in attenuating the progression of cartilage degradation after the induction of OA. The anti-degradative effects of Sct, N-3, and DF on bone and cartilage tissues could be partly attributed to their anti-inflammatory actions [30–33]. As a result, these therapies effectively arrested the progress of MIA-induced knee OA condition in this study. MIA is a chemical substance that instigates an inflammatory process. Therefore, the advancement of OA as a result of the pro-inflammatory processes could be arrested by any of these therapies. In addition to the stated pathological changes accompanying OA, a decrease in the activities of endogenous enzymatic and non-enzymatic antioxidants [e.g. SOD, glutathione peroxidase (GPx), CAT, protein kinase C, and vitamins C and E] have also been reported [34, 35]. The resulting imbalance between the endogenous pro-oxidants and antioxidants indices tend to cause cellular oxidative stress, which is known to play a vital role in the progression of OA [36]. In this study, there was a significant decrease in SOD activity in the OA control group, relative to the normal control group. DF had no significant effect on SOD activity. However, Sct and N-3 showed an additive action in elevating SOD activity, and as such, both therapeutic agents could help redress the imbalance between pro-oxidant and antioxidant markers, in favour of the latter. The high dose of Sct was, however, observed to be more effective than the low dose in promoting SOD activity. In the determination of CAT activity, there was no significant difference in the activity of this enzyme in the OA control group, relative to the normal control. This could possibly due to some endogenous adaptive mechanisms or the duration of the experiment. Nevertheless, there was a slight evidence of the additive actions of Sct and N-3 on this parameter. DF significantly reduced the activity of CAT, relative to what was observed in the normal control and OA control groups. Although DF is well known for its analgesic and anti-inflammatory properties [33], its adverse effects have also been equally reported [37]. Sct abets lipid peroxidation, and therefore has a favourable effect on the endogenous antioxidant system [38]. Unlike Sct, there are incongruent reports about the effect of N-3 on oxidative stress. The consumption of N-3 has been shown to result in the elevation of reactive oxygen species (ROS) [39, 40]. In another study, it was documented that there was no change in ROS level after the administration of N-3 [41]. Yet, some other researchers reported that N-3 reduce lipid peroxidation, and so improve the activities of the antioxidant system [42]. The possible generation of free radicals in OA, and the accompanying lipid peroxidation are associated with the depression of the antioxidant system [36]. The anti-lipid peroxidative effect of Sct and/or N-3 was well-appreciated in the current study. There was an evidence of the additive action of Sct and N-3 in reducing MDA level. In contrast, DF showed no significant effect on this lipid peroxidative marker. Increasingly, OA is considered as an inflammatory condition [43], even though the extent of inflammation varies and is often moderate. Sct has been reported to have an anti-inflammatory property [30]. It also enhances the anti-inflammatory action of corticosteroids [44]. On the other hand, N-3 regulate the expression of inflammatory genes [31], and inhibit leukotriene-mediated inflammatory pathways [32]. The observed significant increases in inflammatory markers (UA and IL-6), which attended the induced OA, was abated by treatments with low and high doses of the Sct alike, as well as N-3 and DF. In addition, there was no evidence of the additive effect of the combined administration of Sct and N-3 on the endogenous level of these parameters.

In conclusion, the combined, and sometimes the single administration of Sct and N-3 proved to be better therapeutic options in the management of OA than DF, which was found to either reduce or have no effect on the endogenous antioxidant enzymes.

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References


Unauthenticated
the development and progression of knee osteoarthritis?


