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Effects of Salmon Calcitonin and Omega - 3 Fatty Acids on Glucoregulatory Indices, Lipid Profile and Antioxidant Markers in Experimental Knee Osteoarthritis in Wistar Rats

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Summary: It has been opined that a combined therapeutic approach should be considered in the optimal management of osteoarthritis (OA). Therefore, the study investigated the effects of salmon calcitonin (Sct) and/or omega-3 fatty acids (N-3), relative to diclofenac sodium (DF) on selected biochemical parameters in induced osteoarthritic rats. Forty (40) adult male Wistar rats were used for this study. The rats were divided into 8 groups (n=5), *viz*: Group 1-Normal control; Group 2-OA control; Group 3-OA+N-3 (200 mg/kg, *p.o.*); Group 4-OA + low dose of Sct (Sct.Lw-2.5 IU/kg, *i.m.*); Group 5-OA + high dose of SCT (Sct.Hi-5.0 IU/kg, *i.m.*); Group 6-OA+N-3+Sct.Lw; Group 7-OA+N-3+Sct.Hi; and, Group 8-OA+DF (1 mg/kg, *p.o.*). Osteoarthritis was induced with 4 mg of sodium monoiodoacetate in 40 µl of saline. The solution was injected intra-articularly into the left knee joint space of anaesthetised (sodium pentobarbital - 40 mg/kg, *i.p.*) rats. Nine (9) days afterwards, treatments started, and they lasted for 28 days. The results showed that Sct has hypocalcaemic, hypocortisolism, and anti-dyslipidaemic effects. It significantly inhibited nitric oxide (NO) production and insulin release. Like Sct, N-3 have hypocortisolism and anti-dyslipidaemic actions. Nevertheless, they caused significant increases in hepatic glycogen content and plasma levels of calcium ion, insulin and NO. Although DF was also observed to stimulate insulin release and NO synthesis, it significantly increased plasma level of LDL-c, but significantly decreased HDL-C. In conclusion, N-3 annul the undesirable effect of Sct, presenting it as a better anti-arthritic drug. Moreover, the combined administration of both pharmacological agents proffer preferable therapeutic benefits in OA condition relative the single or DF therapy.

Keywords*:* Salmon calcitonin; Omega - 3 fatty acids; Osteoarthritis; Glucose homeostasis; Lipid profile; Antioxidant

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INTRODUCTION

Osteoarthritis (OA) is a chronic disease that affects more than 10 % of the world's population (Suri *et al*., 2012). About 60 % of men and 70 % of women above the age of 65 years have been reported to suffer from this condition (Golding, 2006). Although this disease affects the hip, hand, spine, wrist and ankle (Arden and Nevitt, 2006), knee OA is the most predominant form of this condition (Symmons *et al*., 2006).

To enhance studies on the pathogenesis of OA and the efficacy of suggested anti-arthritic drugs, several animal models were developed (Bendele, 2001). Of these models, intra-articular injection of sodium monoiodoacetate (MIA) is the most widely used method (Smith *et al*., 2007). This chemical agent alters glycolytic process in chondrocytes, and hence causes cell death (Kalbhen, 1987). However, it should be noted that there is no gold standard animal model that truly represents the etiology of OA disease process in human.

Osteoarthritis has been associated with imbalance in glucose homeostasis and the precipitation of diabetes mellitus (Rahman *et al*., 2014). More so, studies in lipidomic indicated disorder in lipid metabolism in OA disease state (Castro-Perez *et al*., 2010). Considering only total cholesterol, about 32 % prevalence of dyslipidaemia was documented in OA patients (Singh *et al*., 2002). Apart from the reported dyslipidaemia that accompanies OA condition, diminution of endogenous antioxidants and hence increase peroxidation of lipids have been implicated in the pathogenesis and progression of OA disease process (Suprapaneni and Mohan, 2007).

Nevertheless, it has been opined that a combined therapeutic approach should be considered in the optimal management of OA (Sukhorebska *et al*., 2013). Calcitonin is a well-known anti-arthritic agent (Behets *et al*., 2004). Synthetic or recombinant calcitonin from different species, including human, eel, porcine, and salmon have been used for medical purposes. However, salmon calcitonin (Sct) is the most widely used calcitonin preparation in clinical practice, because of its 40-50 times higher intrinsic potency when compared to human calcitonin, and its improved analgesic action (Azria *et al*., 1995). The drug is commonly administered via intramuscular,

intravenous or subcutaneous route. Nevertheless, several investigations have been conducted on the delivery of Sct through other routes, such as, oral, vaginal, nasal, and rectal (Hoyer *et al*., 2010; Renukuntla *et al*., 2013). Calcitonin has hyperglycaemic action (Arisawa *et al*., 2008). Moreover, it effects on endogenous lipid profile (Nishizawa *et al*., 1988) and antioxidant/pro-oxidant balance (Ozgocmen *et al*., 2007) have been documented. Like calcitonin, omega - 3 fatty acids (N-3) have therapeutic effects in OA condition (Adeyemi and Olayaki, 2017). However, not all studies concluded that dietary supplementation with N-3 is of benefit in the treatment of OA (Rosenbaum *et al*., 2010). Omega - 3 fatty acids have favourable effects on lipid metabolism (McKenney and Sica, 2007). Nonetheless, there are contrasting reports in literature about its antioxidant (Kesavulu *et al*., 2002; Sarkadi-Nagy *et al*., 2003; Hatanaka *et al*., 2006; Obajimi *et al*., 2007) and gluco-regulatory (van Woudenbergh *et al*., 2009; Punithavathi *et al*., 2011) actions. Therefore, the aim of the present study was to determine the effects of Sct and/or N-3 (eicosapentaenoic acid and docosahexaenoic acid - ratio 3/2) relative to diclofenac sodium (a widely used anti-arthritic drug) on indices of glucose homeostasis, lipid profile, and antioxidant markers in experimentally-induced osteoarthritic rats.

MATERIALS AND METHODS

Drugs and chemicals

Salmon calcitonin and sodium monoiodoacetate were acquired for Sigma Aldrich, St. Louis, MO, USA, while omega - 3 fatty acids were purchased from Gujarat Liqui Pharmacaps Pvt. Ltd., Vadodara, Gujarat, India. Diclofenac sodium was purchased from Wuhan Grand Pharmaceutical Company, Wuhan, Hubei, China, while sodium pentobarbital was procured from Nicholas Piramal Ltd., Thane, Maharashtra, India.

Experimental animals and care

Forty (40) adult male Wistar rats weighing between 180 and 220 g were used for this research. The rats were acquired from the Animal Holding unit of the Biochemistry Department, University of Ilorin, Ilorin, Nigeria, and were kept in wooden cages at a room temperature of about 27–30 ^{0}C and photo-periodicity of 12hrs light/12hrs dark. After one week of acclimatisation, five (5) rats were randomly allotted to each of the group. Afterwards, they were introduced to the various chemical agents that were used in the study. The rats had free access to standard pelletised diet (Ace Feed PLC Ibadan, Nigeria) and water *ad libitum* daily, and were weighed weekly.

The rats used in the present study received humane care in accordance to the standard outlined in the 'Guide for the Care and Use of Laboratory Animals' prepared by the National Academy of Science (National Academy of Sciences, 2011) and approved by the Ethical Committee of the University of Ilorin, Ilorin, Nigeria.

Experimental Design

The forty (40) rats that were used for this study were divided into 8 groups of 5 rats each, *viz*: Group 1 - Normal control; Group 2 - Osteoarthritic (OA) control; Group 3 – OA + omega - 3 fatty acids (N-3); Group 4 - OA + Low dose of salmon calcitonin (Sct.Lw); Group 5 - OA + High dose of salmon calcitonin (Sct.Hi); Group 6 - OA+N-3+Sct.Lw; Group 7 - OA+N-3+Sct.Hi; and, Group 8 - OA + Diclofenac Sodium (DF). The normal and osteoarthritic control groups received normal saline (0.05 ml, *p.o.*; 0.05 ml *i.m.*) daily. Salmon calcitonin was administered at a low and high dose of 2.5 and 5.0 IU/kg body weight/day (*i.m.)* respectively, while, DF and N-3 (eicosapentaenoic acid and docosahexaenoic acid - ratio 3/2) were administered at 1 and 200 mg/kg body weight/day, (*p.o.)* respectively. Treatments commenced nine (9) days after the induction of OA, and they lasted for 28 days.

Induction of knee osteoarthritis

Knee OA was induced with 4 mg of MIA which was dissolved in 40 µl of sterile saline. The resultant solution was injected intra-articularly (using a 27-gauge needle), through the patellar ligament of the rats' left knee joints, while they were under sodium pentobarbital (40 mg/kg, *i.p.*) anaesthesia (Orita *et al*., 2011). Following the same procedure, the rats in the normal control group were administered 40 µl of sterile saline intra-articularly.

Preparation of salmon calcitonin injection

Salmon calcitonin powder was dissolved in 0.9 % of sodium chloride solution to obtain the desired doses (Berkoz *et al*., 2010). The solution was stored in a refrigerator at a temperature of about 2^{o}C - 8^{o}C for the sustainability of the hormone viability.

Biochemical measurements

Twelve (12) hours after treatment on the 28th day of the experiment, the rats were anaesthetised with sodium pentobarbital (40 mg/kg, *i.p.*). Afterwards, they were dissected prior to blood collection by cardiac puncture. Whole blood for the determination of serum insulin was collected in plain tubes, which were left undisturbed at room temperature for 30 minutes to clot. However, blood for the determination of the other biochemical parameters were collected into heparinised tubes, which were centrifuged at 4000 revolutions per minute, for 15 minutes, at – 4^{o}C, using a cold centrifuge (Bench top centrifuge, Bio-Gene Technology Ltd., Grandtech Centre, Shatin, Hong Kong). The separated serum and plasma samples were collected into separate plain tubes prior to the biochemical assays.

The analytic kit for the estimation of insulin was purchased from Elabscience Biotechnology Company Ltd., Wuhan, Hubei, China, while the diagnostic kits

for the determination of cortisol, calcium ion, total cholesterol, triglyceride, high density lipoprotein cholesterol, glutathione peroxidase, catalase, total bilirubin, total antioxidant capacity and lactate dehydrogenase were procured from Fortress Diagnostics Ltd., Belfast, Northern Ireland, United Kingdom. Non-enzymatic colorimetric assay kit for the determination of nitric oxide was purchased from Oxford Biomedical Research, Inc., Rochester Hills, Michigan, USA. In addition, liver glycogen was estimated by Hassid's and Abraham's method **(**Hassid and Abraham, 1957), while blood glucose was determined with the aid of Accu-Check Active glucometer (Roche Diagnostics, Pvt. Ltd., Mumbai, Maharashtra, India). The analyses were performed according to the manufacturers' instruction.

Determination of insulin resistance

Insulin resistance score was computed with the formula: Fasting blood glucose (mmol/l) x Fasting serum insulin (pmol/l) / 22.5 (Bonora *et al*., 2000)

Determination of low density lipoprotein cholesterol (LDL-c)

Low density lipoprotein cholesterol was calculated using the formula below:

LDL-c (mg/dl) = TC – (HDL-c - TG/5) (Friedewald *et al*., 1972)

Statistical Analysis

Statistical evaluations of the differences between the group mean values were tested by one way analysis of variance (ANOVA) following least significant difference (LSD) *post - hoc* test using statistical package for social sciences (SPSS) version 20.0. Statistical significance was considered at $p < 0.05$.

RESULTS

Effects of Sct and/or N-3 on calcium ion (Ca^{2+}), insulin, nitric oxide (NO), liver glycogen, and cortisol in induced knee osteoarthritis in male Wistar rats

Compared with OA control group, there were significant ($p < 0.05$) decreases in Ca^{2+} level in groups 4 (OA+Sct.Lw), 5 (OA+Sct.Hi), and 7 (OA+N-3+Sct.Hi). More so, there were significant reductions in Ca^{2+} in group 4 (OA+Sct.Lw), compared with group 6 (OA+N-3+Sct.Lw), and in group 3 (OA+N-3), relative to group 7 (OA+N-3+Sct.Hi).

Although insignificant ($p > 0.05$) differences were recorded in the terminal blood glucose and insulin resistance when comparisons were made among the different animal groups, this was not the case in the result of insulin concentration (Table 1). There were significant ($p < 0.05$) increases in insulin level in groups 3 (OA+N-3) and 8 (OA+DF), compared with normal control and OA control groups. Moreover, there were significant diminution in insulin concentration in groups 6 (OA+N-3+Sct.Lw) and 7 (OA+N-3+Sct.Hi), relative to group 3 (OA+N-3).

There were significant ($p < 0.05$) increases in NO level in OA+N-3, OA+N-3+Sct.Lw and OA+DF groups, compared with the normal and OA control groups (Table 1). More so, there was a significant elevation in the level of NO in OA+N-3 group, compared with OA+N-3+Sct.Hi group, and a significant decline in NO level in OA+Sct.Lw group, relative to OA+N-3+Sct.Lw group.

Significant ($p < 0.05$) increases in the liver glycogen content were observed in groups 3 (OA+N-3) and 6 (OA+N-3+Sct.Lw), compared with the normal and OA control groups (Table 1). Moreover, there was a significant increase in the hepatic glycogen content in group 7 (OA+N-3+Sct.Hi), relative to OA control group. In addition, there were significant increases in the hepatic glycogen content in group 6 (OA+N-3+Sct.Lw), compared with group 4 (OA+Sct.Lw), and in group 7 (OA+N-3+Sct.Hi), relative to group 5 (OA+Sct.Hi).

Compared with the normal control group, there were significant ($p < 0.05$) elevations in cortisol level in OA control and OA+DF groups (Table 1). Furthermore, there were significant decreases in cortisol level 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 OA control group.

Effects of Sct and/or N-3 on total cholesterol (TC), triglyceride (TG), low density lipoprotein cholesterol (LDL-C), and high density lipoprotein cholesterol (HDL-C) in induced knee osteoarthritis in male Wistar rats

Relative to the normal control group, there were significant ($p < 0.05$) increases in TC level in groups 2 (OA control) and 8 (OA+DF) (Table 2). In addition, significant decreases in TC were documented in groups 3 (OA+N-3), 4 (OA+Sct.Lw), 6 (OA+N-3+Sct.Lw), and 7 (OA+N-3+Sct.Hi), compared with the OA control group. There were significant ($p < 0.05$) elevations in TG level in animal groups 2-8 (OA control, OA+N-3, OA+Sct.Lw, OA+Sct.Hi, OA+N-3+Sct.Lw, OA+N-3+Sct.Hi, and OA+DF), compared with the normal control group (Table 2). Although an insignificant decrease in TG level was recorded in group 8 (OA+DF), compared with the OA control group, significant diminutions in the plasma level of this marker were noted 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 latter. Moreover, there was a significant decrease in TG level in group 5 (OA+Sct.Hi), compared with group 4 (OA+Sct.Lw).

Compared with the normal control group, there were significant ($p < 0.05$) increases in LDL-c level in groups 2 (OA control), 3 (OA+N-3), 5 (OA+Sct.Hi), and 8 (OA+DF) (Table 2). In addition, relative to the OA control group, there were significant decreases in

Table 1: Effects of salmon calcitonin and omega - 3 fatty acids on indices of glucose homeostasis in induced knee osteoarthritis in male Wistar rats

GROUPS	Calcium (mg/dl)	Blood glucose (mmol/l)	Insulin (pmol/l)	Insulin resistance	nitric oxide (μM)	liver glycogen (mg/g of wet tissue)	Cortisol (ng/ml)
Normal control	14.87±0.58	3.94±0.24	119.85±0.60	21.00±1.34	26.05±3.31	0.45±0.00	11.83±0.93
OA control	16.09±0.55	3.49±0.22	114.72±5.82	17.86±1.94	24.70±2.22	0.34±0.03	17.33±1.33*
OA+N-3	15.64±0.10^{b}	3.47±0.31	152.37±17.45$^{*\#}$	23.74±4.24	53.90±9.41$^{*\#b}$	0.62±0.04$^{*\#}$	10.00±1.53$^{\#}$
OA+Sct.Lw	13.38±0.80$^{\#\ d}$	3.58±0.31	105.91±0.52	16.83±1.38	26.54±1.86^{d}	0.38±0.01^{d}	9.33±1.86$^{\#}$
OA+Sct.Hi	12.96±0.65$^{\#}$	3.86±0.19	105.30±1.88	18.06±0.66	23.90±2.05	0.34±0.03^{e}	9.83±0.44$^{\#}$
OA+N-3+Sct.Lw	16.29±0.18	3.74±0.55	121.34±5.82^{a}	19.05±0.58	52.28±7.02$^{*\#}$	0.61±0.09$^{*\#}$	10.33±1.33$^{\#}$
OA+N-3+Sct.Hi	13.11±1.35$^{\#}$	2.90±0.03	123.80±12.41^{b}	21.47±5.55	35.37±2.02	0.59±0.04$^{\#}$	12.00±1.53$^{\#}$
OA+DF	15.16±0.80	3.94±0.24	214.66±4.59$^{*\#}$	27.71±0.89	43.58±0.27$^{*\#}$	0.39±0.02	15.67±1.01*

Values across the column are expressed as mean ± S.E.M. $^{*}p < 0.05$ is significant compared with normal control. $^{\#}p < 0.05$ is significant compared with osteoarthritic control. $^{a}p < 0.05$ is significant - OA+N-3 *vs* OA+N-3+Sct.Lw; $^{b}p < 0.05$ is significant – OA+N-3 *vs* OA+N-3+Sct.Hi; $^{c}p < 0.05$ is significant - OA+Sct.Lw *vs* OA+Sct.Hi; $^{d}p < 0.05$ is significant - OA+Sct.Lw *vs* OA+N-3+Sct.Lw; $^{e}p < 0.05$ is significant - OA+Sct.Hi *vs* OA+N-3+Sct. Hi; OA - Osteoarthritic; N-3 - Omega - 3 fatty acids; Sct.Lw - Low dose of salmon calcitonin; Sct.Hi - High dose of salmon calcitonin; DF- Diclofenac sodium

Table 2: Effects of salmon calcitonin and omega - 3 fatty acids on lipid profile in induced knee osteoarthritis in male Wistar rats

GROUPS	Total Cholesterol (mg/dl)	Triglyceride (mg/dl)	Low density lipoprotein cholesterol (mg/dl)	High density lipoprotein cholesterol (mg/dl)
Normal control	74.08±067	114.14±3.25	42.36±0.54	54.55±0.77
OA control	101.82±10.20*	222.31±2.74*	85.96±10.21*	55.44±3.13
OA+N-3	78.33±1.94$^{\#}$	190.13±6.36$^{*\#}$	60.24±3.43$^{*\#}$	53.79±3.58
OA+Sct.Lw.	77.10±1.68$^{\#}$	198.70±9.44$^{*\#\ c}$	55.71±2.34$^{\#}$	58.33±2.04
OA+Sct.Hi	86.99±0.99	173.85±1.21$^{*\#}$	64.40±0.93$^{*\#}$	59.48±1.69
OA+N-3+Sct.Lw	80.65±9.18$^{\#}$	196.53±1.12$^{*\#}$	54.35±8.60$^{\#}$	56.76±3.69
OA+N-3+Sct.Hi	81.38±8.10$^{\#}$	188.13±2.30$^{*\#}$	53.41±8.92$^{\#}$	60.57±5.04
OA+DF	96.72±8.39*	221.79±6.57*	102.11±8.46*	42.53±3.85$^{*\#}$

Values across the column are expressed as mean ± S.E.M. $^{*}p < 0.05$ is significant compared with normal control. $^{\#}p < 0.05$ is significant compared with osteoarthritic control. $^{c}p < 0.05$ is significant - OA+Sct.Lw *vs* OA+Sct.Hi. OA - Osteoarthritic; N-3 - Omega - 3 fatty acids; Sct.Lw - Low dose of salmon calcitonin; Sct.Hi - High dose of salmon calcitonin; DF - Diclofenac sodium.

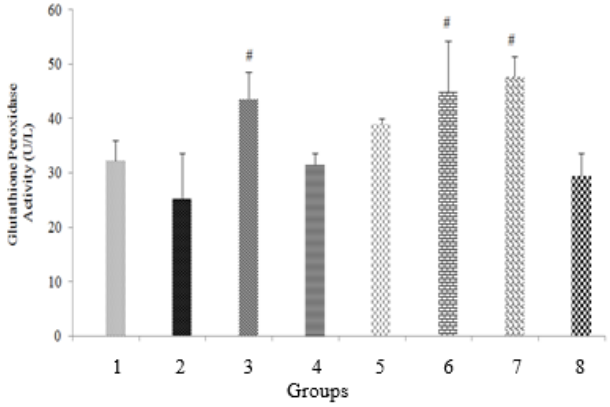

Fig. 1: Effects of salmon calcitonin and omega - 3 fatty acids on glutathione peroxidase activity (U/L) in induced knee osteoarthritis in male Wistar rats Values (n=5) are expressed as mean ± S.E.M. $^{*}p < 0.05$ is significant compared with normal control; $^{\#}p < 0.05$ is significant compared with osteoarthritic control. 1= Normal control, 2= OA control, 3= OA+N-3, 4= OA+Sct.Lw, 5= OA+Sct.Hi, 6= OA+N-3+Sct.Lw, 7= OA+N-3+Sct.Hi, 8= OA+DF

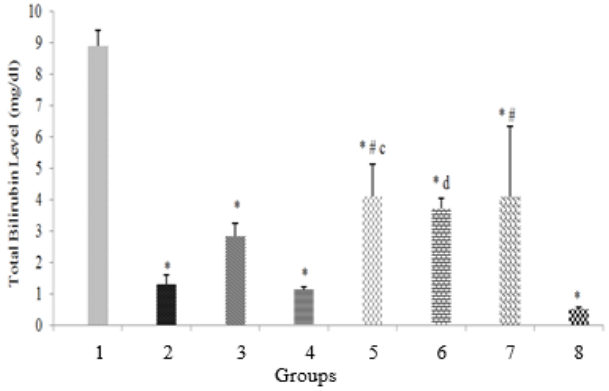

Fig. 2: Effects of salmon calcitonin and omega - 3 fatty acids on total bilirubin level (mg/dl) in induced knee osteoarthritis in male Wistar rats Values (n=5) are expressed as mean ± S.E.M. $^{*}p < 0.05$ is significant compared with normal control; $^{\#}p < 0.05$ is significant compared with osteoarthritic control; $^{c}p < 0.05$ is significant - OA+Sct.Lw *vs* OA+Sct.Hi; $^{d}p < 0.05$ is significant - OA+Sct.Lw *vs* OA+N-3+Sct.Lw. 1= Normal control, 2= OA control, 3= OA+N-3, 4= OA+Sct.Lw, 5= OA+Sct.Hi, 6= OA+N-3+Sct.Lw, 7= OA+N-3+Sct.Hi, 8= OA+DF

LDL-c level 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 insignificant ($p > 0.05$) differences in HDL-c level when comparisons were made among groups 1-7 (Normal control, OA control, OA+N-3, OA+Sct.Lw, OA+Sct.Hi, OA+N-3+Sct.Lw, and OA+N-3+Sct.Hi) (Table 2). Moreover, there was a significant ($p < 0.05$) diminution in HDL-c level in

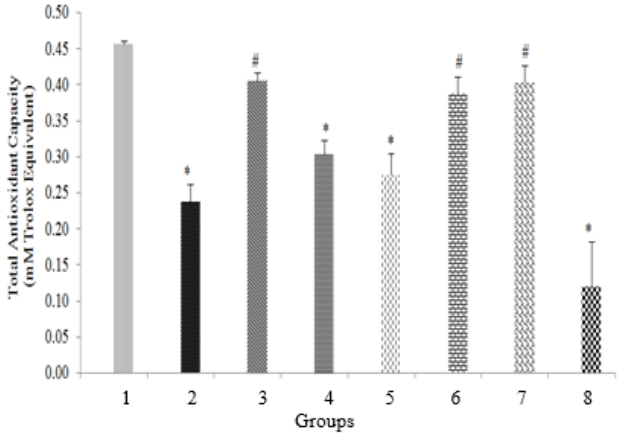

Fig. 3: Effects of salmon calcitonin and omega - 3 fatty acids on total antioxidant capacity (mM Trolox Equivalent) in induced knee osteoarthritis in male Wistar rat Values (n=5) are expressed as mean ± S.E.M. *$p < 0.05$ is significant compared with normal control; #$p < 0.05$ is significant compared with osteoarthritic control. 1= Normal control, 2= OA control, 3= OA+N-3, 4= OA+Sct.Lw, 5= OA+Sct.Hi, 6= OA+N-3+Sct.Lw, 7= OA+N-3+Sct.Hi, 8= OA+DF

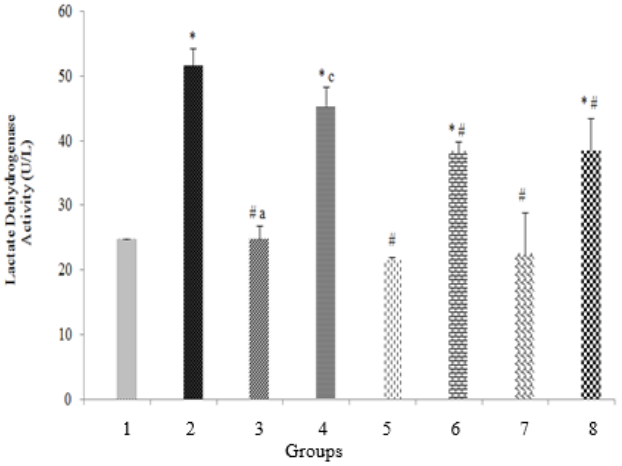

Fig. 4: Effects of salmon calcitonin and omega - 3 fatty acids on lactate dehydrogenase activity (U/L) in induced knee osteoarthritis in male Wistar rats.Values (n=5) are expressed as mean ± S.E.M. *$p < 0.05$ is significant compared with normal control; #$p < 0.05$ is significant compared with osteoarthritic control; a$p < 0.05$ is significant - OA+N-3 *vs* OA+N-3+Sct.Lw; c$p < 0.05$ is significant - OA+Sct.Lw *vs* OA+Sct.Hi. 1= Normal control, 2= OA control, 3= OA+N-3, 4= OA+Sct.Lw, 5= OA+Sct.Hi, 6= OA+N-3+Sct.Lw, 7= OA+N-3+Sct.Hi, 8= OA+DF

OA+DF group, relative to the normal and OA control groups.

Effects of Sct and/or N-3 on glutathione peroxidase (GPX), total bilirubin (TB), total antioxidant capacity (TAC), and lactate dehydrogenase (LDH) in induced knee osteoarthritis in male Wistar rats

Insignificant ($p > 0.05$) differences were recorded in GPX activity in groups 2-8 (OA control, OA+N-3, OA+Sct.Lw, OA+Sct.Hi, OA+N-3+Sct.Lw, OA+N-3+Sct.Hi, and OA+DF), compared with the normal control group (fig. 1). Furthermore, significant ($p < 0.05$) increases in the activity of GPX were documented in groups 3 (OA+N-3), 6 (OA+N-3+Sct.Lw) and 7 (OA+N-3+Sct.Hi), relative to the OA control group.

Relative to the normal control group, there were significant ($p < 0.05$) decreases in TB level in the other experimental animal groups (fig. 2). In addition, there were significant increases in TB level in OA+Sct.Hi and OA+N-3+Sct.Hi groups, relative to the OA control group, and in OA+N-3+Sct.Lw and OA+Sct.Hi groups, compared with OA+Sct.Lw group.

Significant ($p < 0.05$) decreases in the TAC were recorded in OA control, OA+Sct.Lw, OA+Sct.Hi, and OA+DF groups, compared with the normal control group (fig. 3). Moreover, significant elevations in TAC were observed in OA+N-3, OA+N-3+Sct.Lw, and OA+N-3+Sct.Hi groups, relative to OA control group.

Significant ($p < 0.05$) increases in LDH activity were recorded in the OA control, OA+Sct.Lw, OA+N-3+Sct.Lw, and OA+DF groups, compared with the normal control group (fig. 4). More so, relative to OA control group, there were significant decreases in LDH activity in groups 3 (OA+N-3), 5 (OA+Sct.Hi), 6 (OA+N-3+Sct.Lw), 7 (OA+N-3+Sct.Hi), and 8 (OA+DF). In addition, significant decreases in LDH activity was recorded in group 5 (OA+Sct.Hi), compared with group 4 (OA+Sct.Lw), and in group 3 (OA+N-3), relative to group 6 (OA+N-3+Sct.Lw).

DISCUSSION

Osteoarthritis has been reported to be accompanied with an increase bone resorption (Berry *et al*., 2010), which results in an elevated plasma Ca^{2+} level. In the present study, although there was an increase in the plasma level of Ca^{2+} in the OA control group, it was insignificant. Calcitonin lowers blood Ca^{2+} by inhibiting the action of osteoclast and possibly their number and secretory activity (Zaidi *et al*., 2002). As a result, it reduces bone resorption. The reduction of Ca^{2+} level by Sct was observed to be dose-dependent. In contrast, N-3 demonstrated hypercalcaemic action. Omega – 3 fatty acids up-regulate the absorption of calcium in the duodenum, and at the same time decrease calcium excretion (Haag *et al*., 2003). Despite the contradictory effects of Sct and N-3 on Ca^{2+}, there was an expression of the hypercalcaemic action of N-3 when it was co-administered with the high, but not the low dose of Sct.

A close negative correlation ($r = -0.813$, $P > 0.02$) has been established between blood Ca^{2+} and blood glucose level (Moisa and Nozdrachev, 2013). In spite of the noted effect of Sct and N-3 on the plasma Ca^{2+} level, there was no significant variation in the blood glucose level in all the experimental animal groups. This was also the case with the estimated insulin resistance. It could be suggested that Sct and N-3 have transient effect on the plasma Ca^{2+} level, therefore, they tend not to have a sustained effect on the

endogenous blood glucose status. Calcitonin has hyperglycaemic effect, however, it is not diabetogenic in action (Bulbul *et al*., 2008). It decreases the sensitivity of muscle and adipose tissues to insulin, and as a result, causes reduction in glucose consumption by these tissues under the action of insulin (Butakova and Nozdrachev, 2009). The suggested transient effect of Sct on the plasma level of Ca^{2+} and the accompanied insignificant effect of the chronic administration of this therapy on the blood glucose level, implies that Sct could be prescribed preferably at the lowest possible therapeutic dose to OA subjects who also suffer from diabetes mellitus. Rahman *et al*. (2014) reported that there exists a statistically significant relationship between OA and type 2 diabetes i.e. there tends to be the co-manifestation of both disease conditions in an individual.

Elevated plasma Ca^{2+} causes an increase in the intracellular Ca^{2+} concentration in the cytoplasm of pancreatic β cells, and as a result, promotes the release of insulin from the secretory granules of these cells (Parthemore and Deftos, 1978). Therefore, Sct with a hypocalcaemic effect could inhibit the release of insulin from β-cells, while N-3 with a hypercalcaemic action could precipitate a reverse action. In the presence of low and high doses of Sct, the insulin releasing effect of N-3 was not expressed. This was demonstrated by the significant decreases in insulin level in groups 6 (OA+N-3+Sct.Lw) and 7 (OA+N-3+Sct.Hi), relative to group 3 (OA+N-3). It is probable that in the presence of higher doses of N-3, the inhibition of the release of insulin by Sct could be overcome. Although DF was observed to cause a significant increase in serum insulin level, compared to what was observed in the normal and OA control groups, it has no significant effect on the blood glucose concentration and plasma Ca^{2+} level. Therefore, the analgesic and anti-inflammatory actions of DF, which are its primary therapeutic properties, could be linked with its ability to increase insulin production, and hence its beneficial effect in diabetic condition (Ashraf *et al*., 2014).

In addition to the stated pharmacological actions of N-3, they enhance endothelial function by promoting nitric oxide (NO) production and subsequently vasodilation (Morgan *et al*., 2006). On the other hand, Anderson and Ma (2009) reported that there are incongruent reports on the effect of N-3 on endothelial function hence, further investigations are required to confirm existing reports. In consonance with the findings of Morgan and colleagues, N-3 were observed in the present study to cause a significant increase in the production of endothelial NO. As for Sct, there are dearth of reports in literatures on its effect on endothelial function. It was observed in this study that Sct inhibited NO production. Moreover, N-3 - stimulated synthesis of NO was expressed when they were co-administered with the low but not the high dose of Sct. This affirms that Sct has a dose-dependent effect in inhibiting NO production – the high dose being more effective. In addition, the administration of Sct was not accompanied with hyperglycaemia. This observation could be dose-related. It can be suggested that one of the mechanisms by which Sct causes hyperglycaemia is by inhibiting endothelial NO production. As a result, Sct promotes vascular resistance, and so inhibits the uptake of glucose from the systemic circulation into the body tissues for the synthesis of glycogen and for energy production. To a considerable extent, DF mimicked the stimulatory effect of N-3 on endothelial NO production. This effect of DF could be associated with its stimulated increase in insulin release from the pancreatic β cells.

Despite the simile effects of DF and N-3 on insulin secretion and the production of endothelial NO, it was observed that unlike N-3, DF had no significant effect on the liver glycogen content. Omega – 3 fatty acids caused a significant elevation in the hepatic glycogen content in OA+N-3 group, compared to what was observed in the normal and OA control groups. In the presence of low and high doses of Sct, there was a manifestation of the glycogenetic action of N-3. The significant increase in the hepatic glycogen content that accompanied the administration of N-3, could be due to their reduction of vascular resistance by stimulating NO production. This results to an increase disposal of glucose from the systemic circulation (Sakamoto *et al*., 1998) into the hepatic tissue for the synthesis of glycogen. Contrary to N-3, calcitonin stimulates glycogenolysis in the hepatic tissue (Butakova and Nozdrachev, 2011). Nevertheless, in the present study, Sct showed no significant effect on the liver glycogen content. This observed action of SCT could be a dose-dependent response.

Notwithstanding the aforementioned antagonistic actions of Sct and N-3, it was observed that both therapies have a non-additive effect on the endogenous level of cortisol – a hyperglycaemic hormone, which has been associated with pain, which is one of the major signs of OA, and the primary reason pharmacological treatments are sought for by OA patients (Hawker *et al*., 2008). Specifically, OA-related pain has been linked with an increase cortisol level (Carlesso *et al*., 2016). Hypercortisolism is one of the results of pain in animals (Feldsien *et al*., 2010) and humans (Vachon-Presseau *et al*., 2013). However, there are incongruent reports in literature on the association of chronic pain with hypocortisolism and hypercortisolism (Vachon-Presseau *et al*., 2013). The reported analgesic action of calcitonin (Lyritis and Trovas, 2002) and N-3 (Galarraga *et al*., 2008), explains the reason for the significant reductions in cortisol level in the concerned treated groups, relative to what was documented in the OA control group. Despite the known analgesic property of DF, hypocortisolism was not observed in the animal group

that was post-treated with this drug. The reason for this could not be explained in this study. However, further studies could confirm this finding.

In the midst of the observed therapeutic benefits of DF in this study, it adverse actions were also noted, and these could have some cardiovascular implications. The administration of DF caused a significant reduction in HDL-C, relative to the normal and OA control groups. Moreover, it instigated significant increases in TC, TG, and LDL-C, relative to the normal control group. Although it has been reported that an altered lipid metabolism manifests in OA subjects, evidence centered on elevated serum cholesterol and triglyceride (Cheras *et al*., 1997; Stürmer *et al*., 1998). In the present study, there was no change in the plasma level of HDL-C in the OA control group, relative to the normal control group. Therefore, possible therapeutic benefits of Sct and N-3 on this lipid parameter could not be inferred. However, treatments with Sct and/or N-3 largely caused significant reductions in TC, TG and LDL-C in the concerned animal groups (Nishizawa *et al*., 1988; Harris and Bulchandani, 2006), relative to the normal and OA control groups. The beneficial effect of N-3 on lipid metabolism has been attributed to their reduction of lipid synthesis, their promotion of β – oxidation of fatty acids (Harris and Bulchandani, 2006), and their diminutions of hepatic production and secretion of very low density lipoprotein cholesterol (Harris, 1999). Contrarily, calcitonin promotes glucose production in the liver (Yamaguchi and Williamson, 1983), and as such, decreases the amount of substrates that are needed for fatty acids synthesis. Basically, in the present study, the observed effect of Sct and N-3 on lipid profile was found to be non-additive.

In addition to the disturbed lipid metabolism that accompanies OA, it is also characterized by imbalance in antioxidant/pro-oxidant status (Surapaneni and Venkataramana, 2007), and hence lipid peroxidation. Therefore, any therapeutic agent that could boost the endogenous level of enzymatic and non-enzymatic antioxidants, can be used in the management of OA disease, and in the prevention or delay of its chronic complications. The antioxidant effects of calcitonin (Ozgocmen *et al*., 2007) and N-3 (Ozen *et al*., 2008) have been reported in literature. Although there was no significant decrease in GPX activity in the OA control group, relative to the normal control group, there were significant diminutions in TB level and TAC in the former, relative to the latter. Apart from the reported anti-inflammatory effect of bilirubin (Stojanov *et al*., 2013), it also has antioxidant action (Sedlak and Snyder, 2004). In the present study, N-3 were found to cause significant increases in GPX activity and TAC, however, there was no corresponding increase in TB. Unlike N-3, low and high doses of Sct caused insignificant increases in GPX and TAC, relative to what was recorded in the OA control group. Moreover, there was an evidence of the additive actions of Sct and N-3 on the TB level. Unlike Sct and N-3, DF showed no significant effect on the endogenous status of GPX and TB.

The disturbed antioxidant profile that accompanied the experimental OA condition was thought to be responsible for the significant increase in the activity of LDH in the OA control group, relative to the normal control group. Lactate dehydrogenase has been considered as a marker of acute or chronic cell damage (Najeeb and Aziz, 2015). In the OA+DF group, there was a significant reduction in LDH activity, relative to the normal and OA control groups. This effect could be attributed to the anti-inflammatory action of DF. Moreover, the administration of high dose of Sct and/or N-3 caused a more significant reduction in LDH status, relative to DF. Therefore, these therapies could be said to have a more potent antioxidant (Ozgocmen *et al*., 2007; Ozen *et al*., 2008) and anti-inflammatory (Siamopoulos *et al*., 2001; Yates *et al*., 2014) actions.

In conclusion, Omega - 3 fatty acids annul the undesirable effect of Sct, presenting it as a better anti-arthritic drug. Moreover, the co-administration of both therapies demonstrated both non-additive and additive actions on the estimated biochemical parameters, and proffered better remedies in experimental OA, relative to the single or DF therapy.

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