PLASU Journal of Health Sciences (PLASUJHS) Vol. 2, 2026
A Publication of the Faculty of Health Sciences, Plateau State University, Bokkos – Nigeria
ISSN: 3121-982 https://journals.plasu.edu.ng

ANTI-INFLAMMATORY EFFECTS OF LYCOPENE ON CARRAGEENAN INDUCED KNEE OSTEOARTHRITIS IN MALE WISTAR RATS

Nsere Bature[1,2]*, AbdulWahab Alhassan[2], Abdullahi H. Umar[2]
[1]Faculty of Health Sciences, Plateau State University, Bokkos, Plateau State, Nigeria.
[2]Department of Human Physiology, College of Basic Medical Sciences, Ahmadu Bello University, Zaria, Kaduna State, Nigeria.
Email for correspondence: nserebature@plasu.edu.ng Phone- +2347032860914

Abstract
Osteoarthritis is an inflammatory joint disease that is characterized by loss of articular cartilage with concomitant structural and functional changes in the entire joint. The prescription drugs recommended by international guidelines for osteoarthritis management (such as Ibuprofen and Diclofenac) merely provide pain relief for symptoms, and the long-term use of these drugs is often associated with significant side effects such as nausea, vomiting, diarrhoea, dizziness or vertigo, stomach ache, loss of appetite, mild rash, tinnitus and toxicities. Lycopene is a natural pigment that has been reported to possess anti-inflammatory, anti-oxidant and anti-proliferative properties. The aim of this study was to evaluate the anti-inflammatory effect of lycopene administration on carrageenan-induced knee osteoarthritis in male Wistar rats. A total of thirty male Wistar rats were used for the study, and were divided into six groups (n=5) as follows: Group I received intra-articular injection of normal saline and 0.1 ml of olive oil which served as vehicle control. Group II received intra-articular injection of carrageenan (0.02ml of 1% w/v) and 0.1 ml of olive oil and served as negative control. Groups III, IV, V and VI received intra-articular injections of carrageenan and 10mg/Kg diclofenac sodium, 5 mg/Kg, 10 mg/Kg and 20 mg/Kg lycopene respectively orally for three weeks. The results showed significantly higher ($p<0.05$) knee joint diameter in a negative control compared to the vehicle control. Lycopene caused a decrease in knee joint diameter, Neutrophil-Lymphocyte Ratio and Serum Interleukin 1β, while increasing Serum Interleukin 10. It can be concluded that Lycopene has anti-inflammatory action that was effective in protecting against knee osteoarthritis.

Key Words: lycopene, carrageenan, knee osteoarthritis, inflammation

Introduction

Osteoarthritis (OA), which is also known as degenerative joint disease, is a degenerative disorder of joints that most frequently affects the knee, hip, spine and small joints of the hand (Hawkins & Barr, 2015). It is the most common form of arthritis worldwide and is characterised by chronic and painful disease of the synovium of the joints, which causes significant pain and disability (Zhang et al., 2016); it is characterized by progressive deterioration and loss of articular cartilage with concomitant structural and functional changes in the entire joint (Lotz et al., 2013). Osteoarthritis is a heterogeneous disease whose pathogenesis includes the contribution of biomechanical and metabolic factors associated with alteration in articular cartilage and subchondral bone homeostasis which determines the predominance of destructive over productive processes (Meng et al., 2018). The prescription drugs recommended by international guidelines for osteoarthritis management (such as Ibuprofen and Diclofenac) merely provide pain relief for symptoms, and the long-term use of these drugs is

often associated with significant side effects such as nausea, vomiting, diarrhoea, dizziness or vertigo, stomach ache, loss of appetite, mild rash, tinnitus and toxicities; with more than one-third of those taking treatment feel it is not very effective or not effective at all (Li et al., 2022).

Carrageenans are a family of water soluble, linear, sulphated galactans; The backbone of the polysaccharide is formed of D-galactose units linked alternately with α-(1-3) and β- (1-4) linkages (Popescu et al., 2007). The three principal types of industrial importance are kappa, iota and lambda carrageenans. Kappa (k) and iota (i) forms are gelling polymers, while lambda (λ) is a non-gelling, thickening agent. Carrageenans are used in the food industry for products such as frozen desserts, chocolate, cottage cheese, whipped cream, instant breakfasts, yoghurt, jellies, pet foods, relishes, sauces and syrups (Tojo & Prado, 2003).

Lycopene is a natural pigment synthesized by photosynthetic plants and microorganisms (Chen et al., 2015). Lycopene has been reported to have many therapeutic potentials that have been effective in the management of many disorders (Karakoy et al., 2022). The therapeutic activities of lycopene targeted in this study are the anti-oxidant and anti-inflammatory activities (Renju et al., 2013), as well as the possible regenerative ability in restoration of articular cartilage and other joint structures that are damaged in osteoarthritis, hence it could be of potential benefit in protecting against the development of symptoms of knee osteoarthritis and also prevent the formation of functional alterations in the joint structures.

Materials and Method

Thirty male (30) Wistar rats weighing between 120g-180g were obtained from the animal house, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria. The animals were kept in well-aerated individual laboratory cages in the animal house of the Department of Human Physiology. The experimental protocol was reviewed and certified by the Ahmadu Bello University Committee on Animal Use and Care (ABUCAUC), with approval number ABUCAUC/2023/163. Strict adherence to the Ethical Committee's directives was observed.

Induction of knee osteoarthritis

Knee osteoarthritis (OA) was induced by intra-articular injection of 0.02 ml of sterile 1% (w/v) carrageenan on days 1, 4, 7, 11, 14, 17, and 21 of the study, with the paw flexed to 90˚ as described by Hansra (Hansra et al., 2000). The carrageenan was dissolved in normal saline (0.9% NaCl, pH: 7.4) and injected in the right (hind) knee joints of the male Wistar rats. The rats in the group II received intra-articular injection of 0.02 ml normal saline in the right hind knee joint. The study lasted for 21 days.

Animal grouping

The animals were divided into 6 groups of 5 animals each as follows:

Group I: 0.1 ml/kg olive oil + Normal saline.

Group II: 0.1 ml/kg olive oil + OA.

Group III: Diclofenac sodium 10 mg/kg + OA (Hasani et al., 2011).

Group IV: 5 mg/kg lycopene + OA.

Group V: 10 mg/kg lycopene + OA (Ogundeji et al., 2013).

Group VI: 20 mg/kg lycopene + OA.

Measurement of knee joint diameter

Joint swelling was assessed by measuring the diameter of the right hind knee using digital vernier calliper and the results were expressed in millimetres (mm). To measure the knee joint diameter, hairs from the knee joints of the animals was shaved for the proper palpitation of the knee. Knee joint diameters were measured on days 0, 7, 14 and 21 (Renju et al., 2013).

Collection of blood sample

At the end of the three (3) weeks treatment period, the animals were anaesthetized by *ip* injection of ketamine hydrochloride (75 mg/kg) and diazepam (5 mg/kg). Blood samples were then collected via cardiac puncture using 5ml syringes and were stored in plain and EDTA sample bottles. The blood in the plain bottles was centrifuged at 1000 x *g* for 10 minutes. The serum was used for determining the level of IL-1β and IL-10. The blood in the EDTA bottle was used for hematological analysis.

Hematological analysis

Analysis of blood for total white blood cell (WBC), lymphocyte and neutrophil counts was done using hematological automated analyzer according to the manufacturer's instructions.

Biochemical analysis

The assay for the serum IL-1β and IL-10 levels were carried out using specific rat ELISA kits according to the manufacturer's instructions.

Data Analysis

All data collected were analyzed using SPSS version 25.0. Weekly changes in knee joint diameter were analysed using two- way mixed analysis of variance (ANOVA), while data for interleukin 1-beta and interleukin-10 were analysed using one-way ANOVA followed by Tukey's *post-hoc* test. All results were expressed as mean ± Standard Error of Mean (SEM) and values of $p < 0.05$ were considered statistically significant.

Results

Table 1: Effect of Lycopene on Knee Joint Diameter in Carrageenan-induced Knee Osteoarthritis in Male Wistar Rats

GROUPS	Week0 (mm)	Week1 (mm)	Week2 (mm)	Week3 (mm)
0.1ml/kg olive oil + NS	5.14±0.19	5.50±0.28^{a}	5.42±0.22^{acd}	5.30±0.32^{a}
0.1ml/kg olive oil + OA	5.28±0.66*	7.60±0.35$^{b\ \#}$	7.44±0.28$^{bc\ \#}$	7.10±0.30$^{b\#}$
10mg/Kg Dic + OA	5.44±0.75*	6.74±0.49$^{ab\ \#}$	5.08±0.42$^{ad\ *}$	5.38±0.12^{a*}
5mg/Kg LYC + OA	5.58±0.15*	6.42±0.28$^{ab*\ \#}$	6.70±0.10$^{cd\ \#}$	5.46±0.17^{a*}
10mg/Kg LYC + OA	5.58±0.19	6.14±0.27^{a}	6.16±0.22^{d}	5.66±0.20^{a}
20mg/Kg LYC + OA	4.98±0.17*	6.96±0.27$^{b\ \#}$	5.68±0.92$^{d\ *}$	5.00±0.16$^{a\ *}$

Means with superscripts of different letters (a, b, c, d) are statistically significant ($p<0.05$) between the groups while means with superscripts of different symbols (*, $^{\#}$) are statistically significant ($p<0.05$) across the weeks. NS= normal saline, OA= osteoarthritis, Dic= diclofenac sodium, LYC= Lycopene.

The results on table 1 showed the effect of lycopene on weekly knee joint diameters in carrageenan-induced knee osteoarthritis in male Wistar rats. Before the induction of osteoarthritis in week 0, there was no significant difference ($p>0.05$) in the knee joint diameter when all the groups were compared. However, in week 1, there

was a significant increase (p<0.05) in knee joint diameter in the osteoarthritic (OA) group administered 0.1ml olive oil (negative control) and osteoarthritic group administered 20 mg/Kg lycopene when compared with the vehicle control. In addition, the osteoarthritic rats treated with 10 mg/Kg lycopene showed a significant decrease (p<0.05) in the knee diameter when compared to the negative control. In week 2, the negative control and osteoarthritic rats administered 5 mg/Kg lycopene showed significant increase (p<0.05) in knee diameter compared to the vehicle control. The osteoarthritic rats treated with 10mg/Kg diclofenac sodium, 10 mg/Kg as well as 20 mg/Kg lycopene showed significant decrease (p<0.05) in knee diameter compared with the negative control. In the same week, the group treated with 5 mg/kg lycopene had a significantly higher knee joint diameter compared to the group that was given 10 mg/Kg diclofenac sodium. In week 3, the negative control had a higher knee joint diameter compared to the vehicle control, however, treatment with 10 mg/Kg diclofenac sodium as well as the three doses of lycopene used in this study, resulted in significant decrease (p<0.05) in knee joint diameter.

Across the weeks, the knee joint diameters of the vehicle control did not change significantly (p>0.05) in the weeks following the intra-articular injection of normal saline. The negative control however, had a significant increase (p<0.05) in the knee diameter in weeks 1, 2 and 3 compared to week 0. The group treated with 10 mg/Kg diclofenac sodium had a higher diameter in week 1 compared to week 0, with no other significant changes in the diameter in the other weeks. The group treated with 5 mg/Kg and lycopene had a significant increase (p<0.05) in knee diameter in week 2 compared to weeks 0 and 3; while in 20 mg/Kg lycopene group, the knee diameter was significantly higher (p<0.05) in week 1 compared to week 0, 2 and 3. The 10 mg/Kg lycopene group did not show any significant changes (p>0.05) across the weeks of treatment.

Table 2: Effect of Lycopene on Total White Blood Cell Count and Neutrophil- Lymphocyte Ratio in Carrageenan-induced Knee Osteoarthritis in Male Wistar Rats

GROUPS	WBC ($X10^3$ µL)	Neut ($X10^3$ µL)	Lymp ($X10^3$ µL)	Neut-Lymp Ratio
0.1ml/kg olive oil +NS	4.98±0.54	6.23±0.21	2.05±0.23^{a}	1.60±0.24^{a}
0.1ml/kg olive oil +OA	4.20±0.24	6.13±0.31	2.63±0.18^{a}	3..03±0.85^{b}
10mg/Kg Dic + OA	5.40±0.29	5.83±0.39	3.60±0.23^{b}	1.65±0.23^{a}
5mg/Kg LYC + OA	4.40±0.32	6.38±0.25	2.75±0.43^{a}	2.33±0.63^{ab}
10mg/Kg LYC + OA	5.13±0.46	5.60±0.54	3.30±0.19^{b}	1.75±0.25^{a}
20mg/Kg LYC + OA	4.65±0.20	6.28±0.32	3.70±0.11^{b}	1.65±0.20^{a}

Means with superscripts of different letters (a, b,) are statistically significant (p<0.05) between the groups.
NS= normal saline, OA= osteoarthritis, Dic= diclofenac sodium, LYC= Lycopene, WBC= white blood cells, Lymp= Lymphocytes, Neut= Neutrophils.

The results on table 2 showed the effect of lycopene on white blood cell count and –neutrophil-lymphocyte ratio in carrageenan-induced knee osteoarthritis in male Wistar rats. There were no significant changes (p>0.05) in the white blood cells and neutrophils counts in all the groups. There was a significant increase (p<0.05) in lymphocytes counts in the osteoarthritic rats administered 10 mg/Kg diclofenac sodium, 10 mg/Kg and 20 mg/Kg lycopene when compared with the vehicle control. The neutrophil-lymphocyte ratio was

significant higher (p<0.05) in the negative control when compared to the vehicle control. The groups treated with 10 mg/Kg diclofenac sodium, 10 mg/Kg and 20 mg/Kg lycopene had neutrophil-lymphocyte ratios significantly lower (p<0.05) than the negative control. There was no significant change (p>0.05) in the neutrophil-lymphocyte ratio of the group administered 5 mg/Kg lycopene when compared with the other groups.

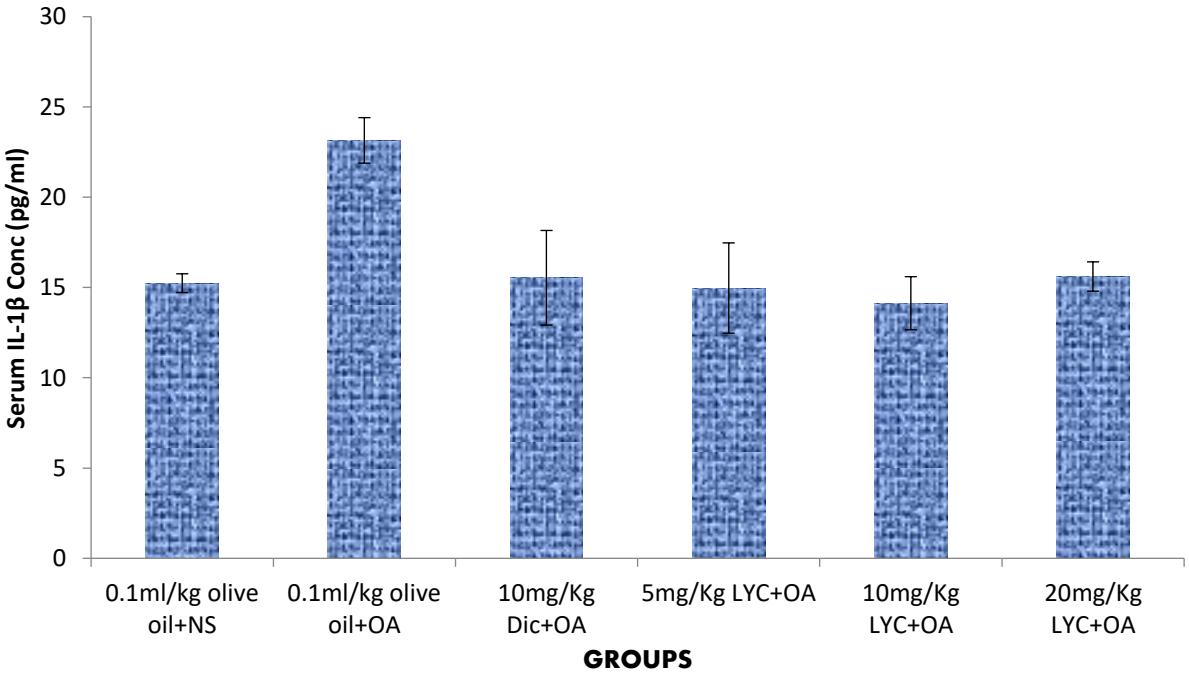

Figure 1: Effect of Lycopene on Serum Concentration of Interleukin-1β (IL-1β) in Carrageenan-induced Knee Osteoarthritis in Male Wistar Rats.

Means with superscripts of different letters (a, b) are statistically significant (p<0.05) between the groups. NS=Normal Saline, OA= osteoarthritis, Dic= diclofenac sodium, LYC= Lycopene.

The results in figure 1 showed the effect of lycopene on serum concentration of interleukin-1β (IL-1β) in carrageenan-induced knee osteoarthritis in male Wistar rats. There was a significant increase (p<0.05) in the serum concentration of IL-1β in the negative control when compared to the vehicle control. However, treatments with 10 mg/Kg diclofenac sodium, 5 mg/Kg, 10 mg/Kg and 20 mg/Kg of lycopene resulted in significant decrease (p<0.05) in the serum concentration of IL- 1β when compared to the negative control.

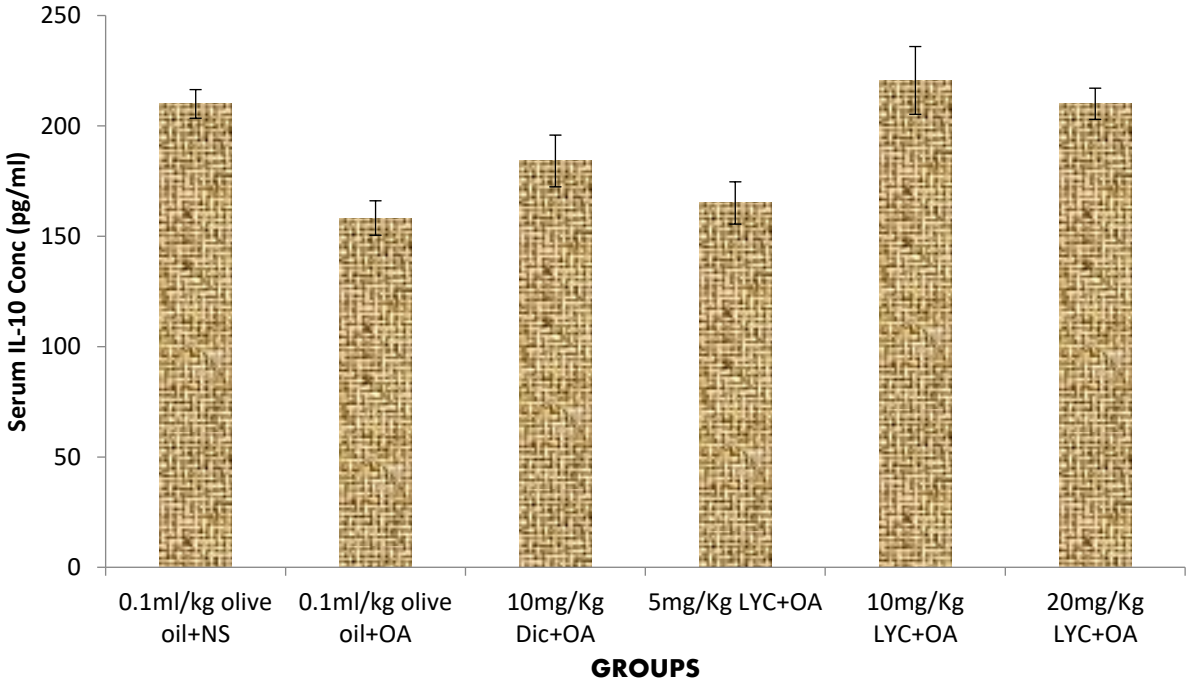

Figure 2: Effect of Lycopene on Serum Concentration of Interleukin-10 (IL-10) in Carrageenan-induced Knee Osteoarthritis in Male Wistar Rats.

Means with superscripts of different letters (a, b) are statistically significant (p<0.05) between the groups. NS=Normal Saline, OA= osteoarthritis, Dic= diclofenac sodium, LYC= Lycopene

The results in figure 2 showed the effect of lycopene on serum concentration of interleukin-10 (IL-10) in carrageenan-induced knee osteoarthritis in male Wistar rats. There was a significant decrease (p<0.05) in the serum concentration of IL-10 in the negative control when compared to the vehicle control. However, treatments with 10 mg/Kg as well as 20 mg/Kg of lycopene resulted in significant increase (p<0.05) in the serum concentration of IL- 10 when compared to the negative control. The groups treated with 10 mg/Kg diclofenac sodium and 5 mg/Kg lycopene showed no significant difference (p>0.05) compared to the other groups.

Discussion

Although considerable progress has been made in the development of variety of therapies for osteoarthritis, no disease-modifying drugs are currently available. The recommended drugs merely provide pain relief for

symptoms, and the long-term use of these drugs is often associated with significant side effects (Li et al., 2022). This study was carried out using the Wistar rat model in order to evaluate the possible anti-inflammatory potential of graded doses of lycopene in protecting against the development of typical inflammation of osteoarthritis which is characterized by joint swelling, increase in the level of pro-inflammatory cytokines and decrease in the level of anti-inflammatory cytokines.

In the present study, it was observed that intra-articular injection of carrageenan resulted in increased knee joint diameter which is an indication of the presence of joint swelling, a characteristic feature of osteoarthritis induced with carrageenan and asserted low-grade inflammation as the major driver of ongoing joint damage (Ashraf et al., 2018). Administration of graded doses of lycopene for three weeks was able to prevent the development of joint swelling in Wistar rats in the same degree as diclofenac sodium, the standard drug recommended for treatment of osteoarthritis with the highest dose of lycopene (20 mg/Kg) effective in decreasing the joint swelling across the weeks as the treatment progressed. This anti-inflammatory action of lycopene was attributed to its ability to decrease the migration of leukocytes in paw tissue and peritoneal cavity and lowering the concentration of myeloperoxidase (Van Steenwijk et al., 2020).

There was an increase in the neutrophil-lymphocyte ratio (NLR) in the osteoarthritic Wistar rats, although the total white blood cell counts did not differ significantly between the groups. Blood NLR is a promising inflammatory marker indicating the severity of knee osteoarthritis as osteoarthritis patients had higher NLR compared to healthy people and is associated with the severity of knee osteoarthritis (Al-Janaby, 2021). Treatment with lycopene at 10mg/Kg and 20mg/Kg doses in this study was able to lower the blood NLR compared to the osteoarthritic Wistar rats that were not treated.

The serum concentration of interleukin-1β was significantly higher in the osteoarthritc Wistar rats while the serum levels of interleukin-10 (IL-10) was significantly lower following intra-articular injections of carrageenan. Carrageenan provokes the release of some important pro-inflammatory cytokines such as tumor necrosis factor-α and interleukin-1β, which consequently activate neutrophil infiltration and these mediators are responsible to a great extent for the loss of metabolic homeostasis of tissues forming joints by promoting catabolic and destructive processes associated with elevated release of matrix metalloproteinases (MMPs) (Patel et al., 2015). Treatment with lycopene significantly decreased the serum levels of IL-1β while increasing the concentrations of anti-inflammatory cytokine IL-10. Lycopene possesses anti-inflammatory properties in its ability to elevate the levels of interleukin-10. IL-10 is involved in stimulating the synthesis of type II collagen, increase in proteoglycan synthesis and aggrecan and inhibiting the production of MMPs family of metalloproteinases. It also inhibits the apoptosis of chondrocytes, and likely result in the stimulation of the synthesis of IL-1β antagonist, which is IL-1Ra and the tissue inhibitor of metalloproteinases-1 (TIMP-1) as well as growth factors (Jansen et al., 2008).

Conclusion

It can be concluded that lycopene decreased knee joint diameter, decreased both the neutrophil-lymphocyte ratio and level of pro- inflammatory cytokine interleukin-1 beta and increased the level of anti-inflammatory cytokine interleukin 10 in carrageenan induced knee osteoarthritis in male Wistar rats.

Conflict of Interest

The authors declare that they do not have conflict of interest.

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