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Virgin coconut oil prevents and reverses the effects of pefloxacin - induced tendinopathy on the Achilles tendon in animal model

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Abstract. Pefloxacin (PEF) has been reported to cause most fluoroquinolone-associated tendinopathy while recent studies show that exogenous supplementation with micro-nutrients facilitate recovery of tendinopathy. Virgin coconut oil (VCO) has been reported to modulate antioxidant enzyme activities, pro-inflammation, and apoptosis. The aim of this study was to determine if VCO would prevent or reverse the damage of PEF on tendon morphology and structure. *Material and Method*. Fifty (50) male Sprague-Dawley rats were randomized into four main categories designated as Normal control, Experimental control, Prophylactic and Treatment groups. All animals in the experimental groups were orally administered PEF 400mg/kg of body weight in a fixed volume of 2.5 ml/kg body weight once daily for 6 consecutive days. The prophylactic groups had prior administration of VCO (V.PEF) at a dosage of 6.7 ml/kg body weight for 7 consecutive days while the treatment groups had the VCO after PEF (PEF.V). All the animals were serially sacrificed on days 8, 15 and 22 and the tendons excised and processed. Data were analyzed with RSTUDIO version 4.0.02 and data normality in the Control and Experimental groups were tested using Shapiro Wilk normality test. Level of significance was 0.5. *Results*. Kruskal Wallis showed significant differences ($p < 0.05$) in all the biomechanical parameters across all the groups. Days 8 and 15 comparisons of the biomechanics of the tendons in the experimental groups showed no significant ($p > 0.05$) differences except the day 22 groups ($p= 0.028$). The PEF and PEF.V tendons had severely compromised biomechanical properties on day 8, with improvements observed from day 15 while the V.PEF group showed better properties as early as day 8 which continued till 15th day. *Conclusion*: VCO given prophylactically ameliorated the damage of PEF on the tendon while it significantly improved biomechanical properties from day 15 when given post PEF.
***Key words**: fluoroquinolone, Pefloxacin, tendinopathy, virgin coconut oil.*

Introduction

The use of fluoroquinolones (FQs) has become popular because of their wide spectrum antibacterial activity despite their reported association with tendinopathy, especially of the Achilles tendon. (1) It has been demonstrated through a prior study that a single oral dose of FQ could induce lesions in the tendon of juvenile rats with Pefloxacin being responsible for most cases (37%) of fluoroquinolone-associated tendinopathy (2). Le Huec et al (3) had suggested that the mechanism of action may be associated with direct toxicity to collagen coupled with absence of inflammatory infiltrate similar to what is seen in in overuse conditions in athletes (4). Prior studies demonstrated cytotoxicity after fluoroquinolone exposure with stimulation of reactive oxygen species (ROS) production, resulting in induced oxidative damage of type I collagen (5, 6). Nitric Oxide (NO) has been found to be important in collagen synthesis and the volume of tissue synthesised during tendon healing, thus it has a beneficial effect on collagen organisation, tendon healing failure load and stress (load/area) (7). A study had reported a five-fold upregulation in Nitric Oxide Synthase (NOS) activity both in acute and chronic tendon injuries in rat Achilles tendon, with the peak activity at day 7 and return to baseline at day 14 (7). In tendinopathy there is hypercellularity due to increasee proliferation of tenocytes which are often altered in morphology when compared with healthy tenocytes, thereby resulting in a decrease in collagen type I with consequent decreased tensile strength and ability to withstand mechanical load (8, 9).

The current management of tendinopathy is mainly symptomatic resulting in poor outcomes, therefore, investigating modalities that can either prevent or reverse tendon degeneration by increasing the biosynthesis of extracellular matrix and collagen has become important (10, 11).

Recent studies have demonstrated that exogenous supplementation with micro-nutrients can facilitate recovery of tendinopathies and also protect against damage (12). Virgin coconut oil (VCO), the unrefined oil from the fresh coconut kernel (*Cocos nucifera*), enriched with medium chain saturated fatty acids and polyphenols has been reported to have antioxidant, anti-inflammatory, analgesic and anti-pyretic properties that modulate antioxidant enzyme activities, pro-inflammation, and apoptosis (13).
The purpose of this study was therefore to determine if VCO could prevent and/or reverse the damage of PEF on tendon morphology and structure.

Material and Method
Animals. Fifty (50) male Sprague-Dawley rats (6 weeks old) purchased from the institutional Animal House were used in all experiments. The animals were housed at five per wire-mesh cages in a well ventilated room with 12-hours photo-periodicity. The rats were fed with commercial pelleted rat chow and water *ad libitum.* Ethical approval was obtained from the institutional Health and Research Committee and all experimental procedures were performed in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80-23, revised in 1996). Tendon Harvest Process. The animals were sacrificed by cervical dislocation and the Achilles tendons excised. The tendons were processed for histology using previously described techniques (16). *Preparation of Pefloxacin*. Each 400mg Pefloxacin (PEF) tablet was suspended in distilled water and orally administered to the animals once daily for 6 consecutive days at a dose of 400mg/kg of body weight in a fixed volume of 2.5 ml/kg body weight. A prior study had reported that PEF at a dose of 300 mg/kg caused lesions of the Achilles tendon (2). Pefloxacin has been used clinically at dosages of 300 to 600 mg/kg/day, therefore 400mg/kg was chosen for the purpose of this study. *Preparation of Virgin coconut oil* (VCO). The solid endosperm of mature coconut purchased from an open market in Lagos, Nigeria in September, 2018 were used for the study. It was authenticated as *Cocos nucifera palmae* at the Federal Institute of Forestry Research Ibadan (Voucher number, 107825). The VCO was extracted by following a modification of the wet extraction method as previously described (14). The VCO was administered at a dosage of 6.7 ml/kg body weight (15).
A*nimal Grouping*. Fifty (50) male rats that weighed between 100-120g were randomized into four main categories designated as into four main categories designated as Normal control, Experimental control, Prophylactic and Treatment groups.
Control (Normal control): animals were neither administered PEF nor VCO.
Experimental Control: all the animals were administered PEF solution by gavage at a dosage of 2.5 ml/kg body weight for 6 consecutive days - PEF08 (Experimental control): the animals were sacrificed on day 8 post PEF administration; PEF15 (Experimental control): the animals were sacrificed on day 15 post PEF administration; PEF22 (Experimental control): the animals were sacrificed on day 22 post PEF administration.
The intervention groups were broadly divided into the prophylactic and treatment groups.
Prophylactic Groups: All the animals were fed VCO by gavage at a dosage of 6.7 ml/kg body weight once daily for 7 consecutive days. On day 8, the animals commenced administration of PEF solution at a dosage of 2.5 ml/kg body weight for 6 consecutive days. - V.PEF08 (Prophylactic group): the animals were sacrificed on day 8 post PEF administration; V.PEF15 (Prophylactic group): the animals were sacrificed on day 15 post PEF administration; V.PEF22 (Prophylactic group): the animals were sacrificed on day 22 post PEF administration.
Treatment Groups: all the animals were administered PEF solution at a dosage of 2.5 ml/kg body weight by gavage for 6 consecutive days and afterwards commenced VCO by gavage on day 7 at a dosage of 6.7 ml/kg body weight once daily for 7 consecutive days (16, 15). - PEF.V08 (Treatment group): the animals were sacrificed on day 8 post VCO administration; PEF.V15 (Treatment group): the animals were sacrificed on day 15 post VCO administration; PEF.V22 (Treatment group): the animals were sacrificed on day 22 post VCO administration.
Stereological Analysis. The slides were observed under a light microscope fitted with an ocular test grid at a magnification of 400x using the method of Cruz-Orive and Weibel (17). The tenoblast and tenocyte profiles identified were the nuclei. Fifty random values (10 per animal) were obtained for each group. The numerical density (NA) is the number of tenocyte profiles per unit area of field (18).

Estimation of Volume Fraction of Collagen. Estimation of collagen quantity was done in sections of tendon tissue embedded in paraffin and stained with Hematoxylin and Eosin (H&E) as previously described. (16) The volume fraction of the histological component was calculated as $V_v = P_p/P_t$, where V_v is the volume fraction, P is the tissue component under consideration, and Pp is the number of test points associated with P and Pt the number of points of the test system (19). *Preparation of Tendon Specimen for Biomechanical Testing.* The tensile test specimen preparation was conducted in accordance with the American Standard testing and measurement, method D412 (ASTM D4121983) (20). The specimen geometry was in a dumb-bell shape by weighing 50g of epoxy and 25g of hardener, which comprises of methyl ethyl ketone peroxide (MEKP). The composition was then mixed together rigorously which generated a little heat of about 30^{o}c. The mixture was then poured in an already made wooden pattern which was carved according to American standard ASTM D4121983, after which the tendon was buried inside the epoxy in the mould which then forms the tensile specimen. The specimen was allowed to cure for 24hrs at room temperature and the tensile specimen was removed from the wooden pattern (20). *Biomechanical Testing*. The tendon dimension of length (28.5mm), width (9.4mm), thickness (4.3mm) and cross-sectional area (CSA) were measured three times using a digital image of ultrasonography (US). The specimen were tightly clamped on the material testing system of Universal Testing Machine (UTM) (Instron -Series 3369, Instron Corporation, Norwood, MA, USA) and subjected to static axial load at speed (strain rate) of 1mms^{-1} and tested according to established protocol (21). The different biomechanical properties were estimated according to established procedures. *Nitric Oxide Synthase Estimation*. The test is based on enzyme linked immuno-absorbent assay-double antibody sandwich principle to assay Nitric Oxidase Synthase (NOS) level in the sample according to a prior established protocol (22).

Statistical Analysis. The RSTUDIO version 4.0.02 was used to perform statistical analysis. Descriptive statistics of central tendency (mean, *M*) and dispersion (standard deviation, *SD*) were used to summarise the sample parameters in the control and experimental groups. Data normality in the Control and experimental groups were tested using Shapiro Wilk (Shapiro.wilk) normality test. The R programming language of ggpubr and ggplot2 (23) were used to plot bar charts, do computation of one-way Analysis of Variance (ANOVA) and Kruskal Wallis rank sum. Post-hoc analysis of Tukey Honest Significant Difference (TukeyHSD) and Pairwise Wilcoxon Rank Sum Test were used for pairwise comparisons to identify mean(s) and median(s) that were different in the parameters of the experimental groups. Statistical level of significance of 5% was accepted. Pearson correlation was used to determine the linear relationship between NOS and the biomechanical and stereological properties in the experimental groups.

Results

Data summary of the biomechanical properties is presented in Table I

Table I. Biomechanical parameters of the Control and Experimental Groups (mean and standard deviation)

Groups	UTs (Mpa)	UTe (%)	Stiffness (N/mm)	E-M (Gpa)	LUs(N)	BTs (Mpa)	BTe (%)	LB (N)
Control	9.367(9.13)	2.37(1.37)	1053(761)	0.759(0.56)	378.6(369)	8.62(10.19)	3.33(0.0)	348.5(411.8)
PEF Only								
PEF08	0.942(0.59)	5.6(5.5)	259.2(332)	0.198(0.211)	38.06(24.1)	0.398(0.13)	10.1(3.1)	16.1(5.18)
PEF15	2.95(3.24)	2.63(0.98)	428.2(204.4)	0.302(0.145)	119.2(131)	0.65(0.28)	5.21(0.37)	26.25(11.2)
PEF22	3.1(1.08)	2.72(1.11)	525.6(58.29)	0.38(0.04)	125.3(43.5)	1.19(0.08)	9.7(3.6)	48.1(3.1)
Virgin Coconut Oil (VCO)								
V.PEF08	2.71(0.95)	1.84(0.62)	673.6(526)	0.448(0.33)	109.5(38.5)	2.1(0.55)	2.98(0.25)	85.04(22.3)
V.PEF15	3.48(1.0)	1.84(0.37)	671.3(9.1)	0.473(0.01)	140.8(40.4)	1.76(0.27)	3.1(0.35)	71.3(11)
V.PEF22	3.78(0.88)	1.6(0.25)	901(117)	0.603(0.13)	152.9(35.7)	2.24(0.4)	2.9(0.12)	90.5(16.2)
PEF.V08	0.829(0.0)	7.54(0.0)	62.4(0.0)	0.048(0.0)	33.52(0.0)	0.444(0.0)	15.5(0.0)	17.93(0.0)
PEF.V15	2.72(1.46)	1.22(0.0)	893(374.1)	0.63(0.26)	109.9(59)	0.81(0.62)	3.77(0.17)	32.6(25.2)
PEF.V22	3.12(1.11)	2.46(0.74)	578.7(69.8)	0.454(0.02)	126.2(44.7)	2.71(1.5)	3.51(0.25)	109.4(60.8)

Legend: UTs = Ultimate Tensile Stress, UTe = Ultimate Tensile Strain, E-M = Elastic (Young's) Modulus, LUs= Load at Ultimate Stress, BTs= Tensile Stress at Break, BTe = Tensile Strain at Break, LB = Load at Break. P8 = PEF only euthanized day 8, P15 = PEF only euthanized day 15, P22 = PEF only euthanized day 22,VPEF8= VCO for 7 days, then PEF treated for 6 days euthanized day 8, V.PEF15= euthanized day 15, VPEF22= euthanized day 22, PEFV8 = PEF 6 days, VCO treated for 7 days euthanized day 8, PEFV15: euthanized day 15, PEFV22: euthanized day 22.

Comparing all the groups with Kruskal Wallis showed significant differences in all the biomechanical parameters and pairwise comparisons are seen in figures 1-7. There were no significant differences (all $p > 0.05$) in the medians of all the biomechanical properties when the experimental groups were compared on days 8 and 15. However, ANOVA showed significant differences in the NOS, Young's Modulus and stiffness between the day 22 groups (Figure 7).

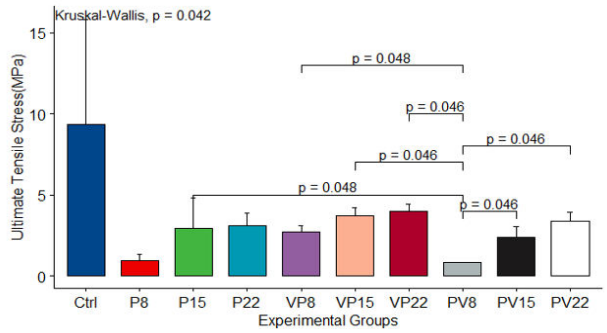


Figure 1:

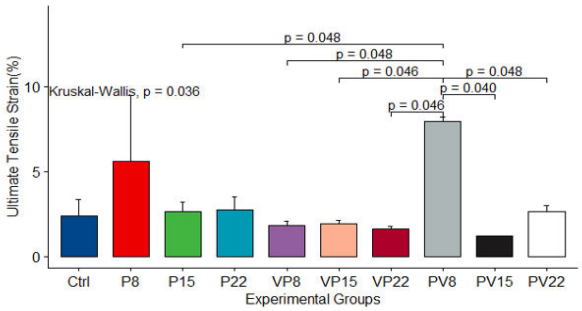


Figure 2:

Figure 1. Tensile Stress

Figure 2. Tensile Strain

P8, P15, P22 = PEF only euthanized days 8, 15, 22. VPEF8, VPEF15, VPEF22= VCO + PEF euthanized days 8, 15, 22. PEFV8, PEFV15, PEFV22 = PEF + VCO euthanized days 8,15, 22.

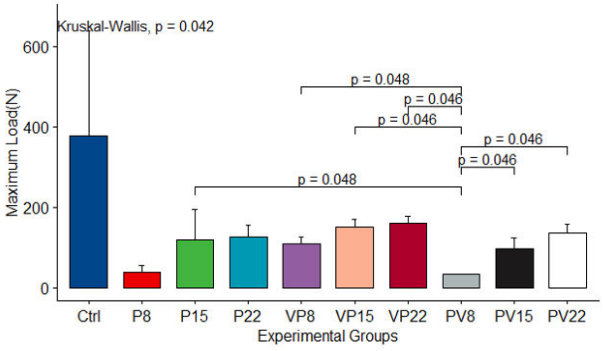


Figure 3

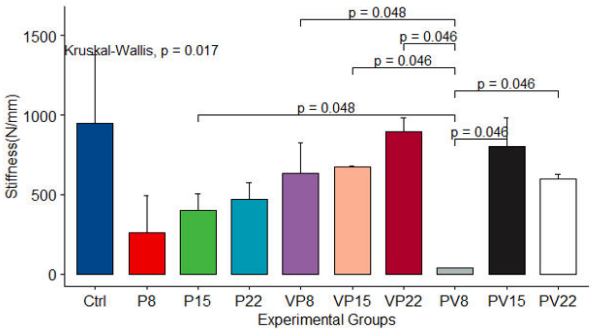


Figure 4

Figure 3. Maximum Load

Figure 4. Stiffness

P8, P15, P22 = PEF only euthanized days 8, 15, 22. VPEF8, VPEF15, VPEF22= VCO + PEF euthanized days 8, 15, 22. PEFV8, PEFV15, PEFV22 = PEF + VCO euthanized days 8,15, 22.

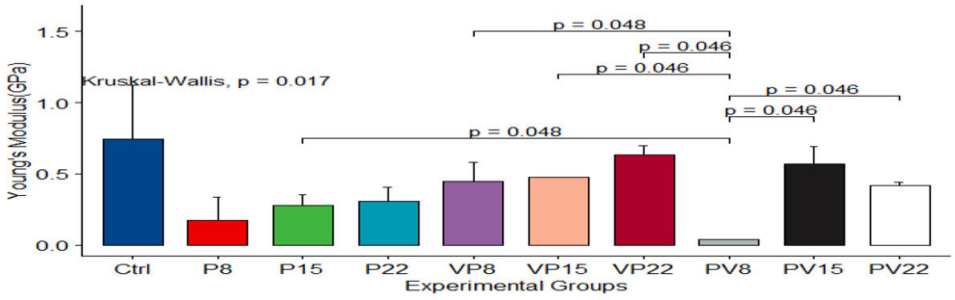


Figure 5:

Figure 5. Young's Modulus
P8, P15, P22 = PEF only euthanized days 8, 15, 22. VPEF8, VPEF15, VPEF22= VCO + PEF euthanized days 8, 15, 22
PEFV8, PEFV15, PEFV22 = PEF + VCO euthanized days 8,15, 22.

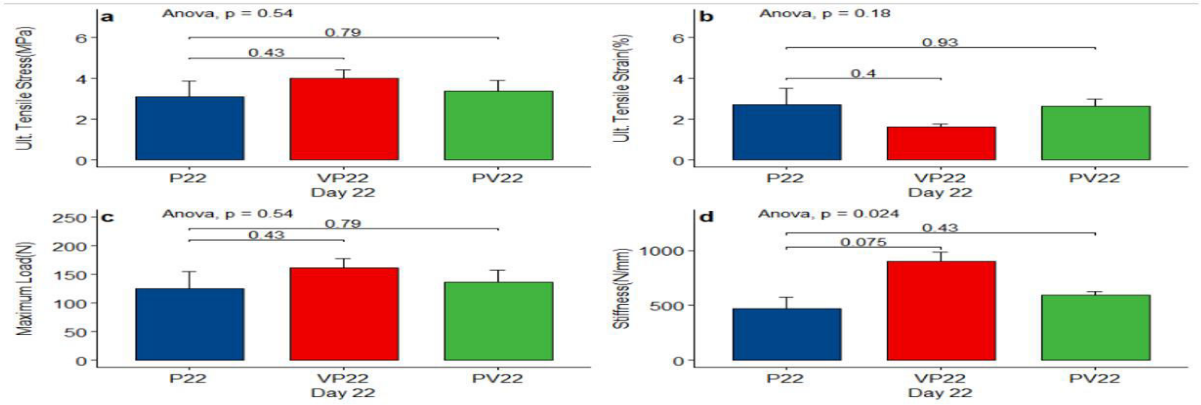


Figure 6

Figure 6. Biomechanical Properties on day 22
P22 = PEF only euthanized day 22. VPEF22= VCO + PEF euthanized day VP22. PEFV22 = PEF+ VCO euthanized day 22.

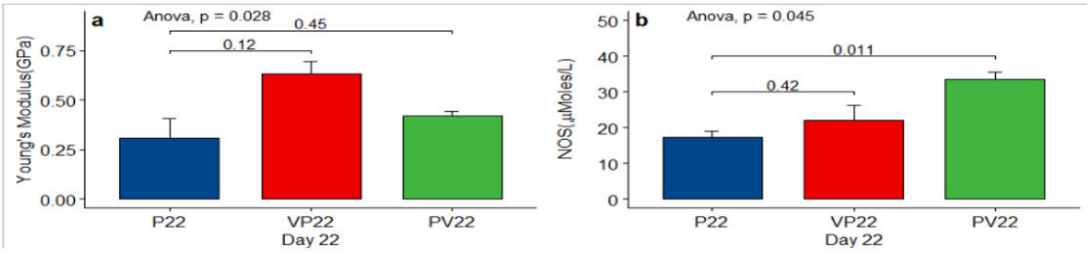


Figure 7

Figure 7. Young's Modulus and NOS day 22.
P22: PEF only. VPEF22: VCO + PEF. PEFV22: PEF +VCO.

The summary of the data, mean (M) and standard deviation (SD), of the biochemical and stereological parameters are presented in Table II. ANOVA showed significant differences across the groups only in NOS expressions on day 22 (figure 7).
The correlation of NOS with the biomechanical parameters are presented in table III. In the PEF only group, there was a significantly high positive association between NOS and ultimate strain ($r = 0.86$, $p = 0.012$) while in the VCO groups, negligible correlations were seen between NOS, ultimate stress and maximum load (all $|r| < 0.3$, $p > 0.05$).

Table II. ANOVA of the NOS, Tenocyte Population and Volume Fraction of Collagen across the Groups (mean and standard deviation)

Group	Tenocyte count/unit area	Vol Fraction of Collagen (%)	NOS (µmoles/l)
Control	38.3 (16.20	6.21 (0.69)	17.19 (8.42)
PEF08	33.6 (11.9)	5.56 (3.21)	27.39 (10.82)
PEF15	38.7 (6.3)	6.36 (4.17)	12.09 (3.90)
PEF22	40.2 (7.8)	6.52 (3.05)	17.44 (2.52)
VPEF08	38.3 (8.4)	6.19 (3.25)	19.45 (5.19)
VPEF15	36.2 (12.8)	5.87 (2.64)	11.80 (2.73)
VPEF22	46.7 (4.4)	7.66 (4.58)	22.00 (7.61)
PEFV08	39.3 (10.4)	6.63 (3.16)	28.45 (7.68)
PEFV15	34.7 (12.5)	5.32 (2.84)	14.92 (6.44)
PEFV22	45.5 (11.6)	7.45 (4.22)	33.62 (3.54)
p-value	*0.8985*	*0.9985*	*0.0362**

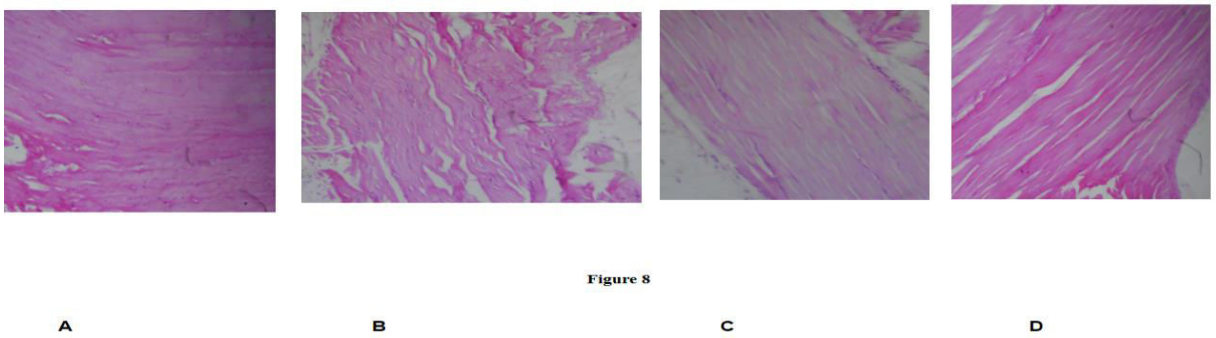
P8 = PEF only euthanized day 8, P15 = PEF only euthanized day 15, P22 = PEF only euthanized day 22, VPEF8= VCO for 7 days, then PEF treated for 6 days euthanized day 8, V.PEF15: euthanized day 15, VPEF22: euthanized day 22, PEFV8 = PEF 6 days, VCO treated for 7 days euthanized day 8, PEFV15: euthanized day 15, PEFV22: euthanized day 22.

Table III. Correlation between NOS and Biomechanical and Morphological Properties

Biomechanical Properties	**Experimental Groups**	
	PEF Only (r , p-value)	VCO Groups (r, p-value)
Ultimate Stress vs. NOS	-0.46, 0.297¶	-0.14, 0.556¶
Ultimate Strain vs. NOS	**0.86, 0.012****†	0.45, 0.054¶
Maximum Load vs. NOS	-0.46, 0.297¶	-0.15, 0.552¶
Stiffness vs. NOS	-0.73, 0.065†	-0.31, 0.191¶
Young's Modulus vs. NOS	-0.74, 0.06†	-0.31, 0.194¶
Morphological Properties		
%Vol. of Collagen vs. NOS	-0.36, 0.427¶	0.34, 0.152¶
Tenocyte Count/Area vs. NOS	-0.67, 0.102‡	0.35, 0.143¶

¶ = Low correlation coefficients with p-value > 0.05; † = High correlation coefficients with p-value > 0.05;
***†= High correlation coefficients with p-value < 0.05.*

Figure 8. Photomicrographs of the tendon, H&E x100 (A: P8 = PEF only showing poorly defined collagen fibers and abundant ground substance. B: PEFV8 = PEF + VCO. C: PEF8= VCO + PEF. D: P15= PEF only showing better aligned collagen fibers.)



Discussion and Conclusion

The use of FQs have become popular in clinical practice because of their wide spectrum antibacterial activity, despite their reported association with tendinopathy. FQs have been reported to cause damage to the tendon structure with consequent altered biomechanical properties through oxidative damage of type I collagen. (6) The mechanical integrity of tendons is important because they transmit the forces of contraction from the muscles to the bones to effect joint movement, thus any change in their morphology and elastic properties may affect their function during normal activities. Prior studies had suggested focusing on the morphological and biomechanical alterations in the tendon structure and investigating modalities that can either slow down or prevent the onset of tendon degeneration in tendinopathy (24).

The purpose of this study was therefore to investigate if VCO could prevent and/or reverse the deleterious effects of PEF on tendon morphology and structure.

Effect of VCO Tendon Biomechanics

From our findings, the animals given oral PEF alone had severely compromised biomechanical properties with high ultimate tensile strain on the 8th day when compared with the normal tendons, though the changes were not statistically significant. This insignificance, in spite of the profound biological changes seen may be due to the relatively small sample sizes of the specimens analyzed. For instance, on day 8, the normal tendon had an ultimate tensile stress of 9.367 while the PEF group had 0.942, a difference of 8.425 (90%). Also, there was an increase in ultimate tensile strain in the PEF tendons of 58% over the normal tendons. Further studies with much larger sample sizes are needed to corroborate these findings. (25) However, by the 15th and 22nd days, the biomechanical properties had improved, probably due to an upregulation in the expression of NO as seen on the 8th day in all the experimental groups (table II) because prior studies had reported a 5-fold increase in NO expression in chronic tendon injury which peaks at day 7 and returns to baseline by the 14th day post-injury (7). Studies have also shown NO to be important in both the synthesis of collagen and the volume synthesized thus indirectly improving the mechanical properties of the healing tendon (7).

It was observed also that the group that had PEF before being given VCO (PEF.V) had severely compromised biomechanical properties on day 8, just like those that had PEF only. Improvements in the mechanical properties of both groups were however observed from day 15, all the way to day 22 in support of some prior studies that suggested that tendinopathy changes in tendons may be reversible especially 2 weeks after a single oral dose of FQ (2). These two groups (PEF and PEF.V) had the lowest tensile stress, maximum load, stiffness and Young's modulus while exhibiting the highest ultimate tensile strain. This supports a prior study (26) that reported alterations in tendon tissue composition and structure as one of the tenotoxic effects of FQs resulting in degradation of the tendon matrix. (27). This subjects the tendon to higher strain because a less stiff tendon is normally subjected to higher strains, potentially resulting in microscopic disruptions of collagen fibers which confirm the toxic effect of PEF on the tendon structure (28). These findings also suggests that VCO is not effective initially in reversing the deleterious effect of PEF on the tendon structure, at least by the 8th day Post-PEF administration. The altered biomechanical

properties of the tendons in these two groups is expected as it has been reported that the integrity of the ECM which is mainly composed of collagen is a major determinant of the mechanical properties of the tendon (29).

On the other hand, compared with the PEF only and the PEF.V groups, the animals given VCO prophylactically before PEF (V.PEF) had significantly higher ultimate tensile stress, maximum load, stiffness and Young's modulus while exhibiting significantly lower ultimate tensile strain as early as the 8th day post PEF administration which continued till 15th day. An interesting observation is that the V.PEF group had marginal improvements from day 8 over the next 2 weeks and on day 22, compared with the PEF.V group had better mechanical properties in all the parameters investigated. In essence, the prophylactic effect of VCO on the tendon was more profound than its effect after administration of PEF. However, for the PEF.V group, the biomechanical properties of the tendon had significantly improved in all the study parameters by day 15 except for the fact that the properties were biomechanically inferior to the prophylactic (V.PEF) group. For instance, in the PEF.V treatment group, the ultimate tensile strain had reduced significantly ($p=0.046$) between days 8 and 15 (Fig 2). The high positive correlation between the tensile strain and NO expression in the PEF group suggested that the increase strain in the tendon was accompanied by increase NO to counteract its effect thereby resulting in improvement in other biomechanical parameters. The same pattern is seen in the VCO groups, though with less significant correlation coefficients, probably due to the natural antioxidant properties of VCO.

These effects of VCO on the tendinopathic tendon could be attributed to the documented restorative effect of VCO on antioxidant enzymes against toxicity (13). A number of recent studies have demonstrated that exogenous supplementation with micro-nutrients can facilitate recovery of tendinopathies (12). Existing literature indicates that the phenolic profile of VCO contains several natural compounds that modulate antioxidant enzyme activities, pro-inflammation, and apoptosis (13).

Effect on Tendon Morphology

Our findings also show that there are no significant changes in the tenocyte population and the volume fraction of collagen when compared with the normal tendons though there are numerical increases in both parameters in all the day 22 groups. This could be due to reported increased tenocyte activity in tendinopathy resulting in cell proliferation though the morphology of the cells is altered (8). There is also an increase in the volume of the collagen synthesized though with mechanically weaker tendons as FQs have been reported to promote the production of type III collagen over type I collagen with disorganization of the ECM as seen in figure 8 (28). This results in weakened tendon and subsequent decreased tensile strength and the ability to withstand mechanical load (9) as evident by our findings on the mechanical properties of the tendons.

From our histological findings, the ECM of the PEF only tendons had poorly defined collagen fibers with abundant ground substance (figure 8). However by day 15, there was improvement with better aligned collagen fibers which could be responsible for the improved biomechanical properties in the the PEF tendons. This could be attributed to the fact that as the percentage of aligned collagen fibers increase, the modulus of elasticity and stiffness of the tendon begin to normalize as reported in a prior study (30). The V.PEF/22 and PEF.V/15 tendons exhibited the highest Young's modulus among the experimental groups which indicate a greater number of properly aligned and healthy collagen fibers (25). This is reflected as the lowest Ultimate tensile strain in all the V.PEF groups and the PEF.V/15 and PEF.V/22 groups. The findings of this study suggest that PEF treated tendons had severely compromised mechanical properties on day 8 which improved by days 15 and 22, though not significantly while the PEF.V tendons had very poor mechanical properties on day 8 which improved significantly by days 15 and 2. The V.PEF tendons on the other hand showed significantly improved mechanical properties on day 8 which increased marginally from day 15 through to day 22.

VCO given prophylactically ameliorated the deleterious effect of PEF on the tendon from the onset, while when given for treatment reversed the effect of PEF from day 15 with significantly improved biomechanical properties. In essence, the prophylactic effect of VCO on the tendon was more profound than its effect after administration of PEF.

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