Intrasound Therapy Improves the Mechanical Properties and the Morphology of Tendons in Fluoroquinolone-Induced Achilles Tendinopathy

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ABSTRACT: Fluoroquinolones (FQs), especially Pefloxacin (PEF) have been reported to increase risk of Achilles tendon rupture. However, prior studies showed that intrasound therapy (ITR) significantly improved the morphology of the healing tendon. This study aimed to compare the effects of 7, 14 and 21 days intrasound treatment on the mechanical properties and morphology of the Achilles tendon in fifty (50) male Sprague-Dawley rats induced with FQ and randomized into three main categories designated as Normal control (A), Experimental control (B)and Treatment groups (C). All animals in groups B and C were orally administered PEF 400mg/kg in a fixed volume of 2.5 ml/kg daily for 6 consecutive days.The Achilles tendon of both hind limbs in group C were treated with the lowest intensity ITR 5 minutes daily for 7, 14 and 21 consecutive days. 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 the level of significance set at 0.05. Kruskal Wallis showed no significant differences (p >0.05) in all the biomechanical parameters across all the groups. Compared with group B, ITR 14 days treatment had the best improvement in all biomechanical variables, though insignificant (p>0.05). In the Intrasound groups, there were significant positive correlations of Nitric Oxide Synthase (NOS) with ultimate tensile stress and tensile load (p= 0.005; p=0.003) respectively. Intrasound treatment to the Achilles tendon for 14 days improved the mechanical properties and morphology of tendons in Fluoroquinolone-induced tendinopathy.

Fluoroquinolones (FQs) are broad-spectrum antibiotics that have been reported to have very potent cytotoxic effects on human connective tissue (Aranha *et al.,* 2000). Studies have shown they are responsible for a 3 to 6 times increased risk of developing tendinitis or rupture of the Achilles tendon in patients (Sode *et al.,* 2007). In an evaluation of more than 11 000 patients, rates of 2.4 incidences per 10 000 patient prescriptions for tendinitis and 1.2 per 10 000 for tendon rupture were cited (Van der Linden *et al.,* 2007). Recent studies have estimated the incidence of FQ-associated tendinopathy to be between 0.14% and 0.4%. Mostly involving sporting or very physically active persons (Stephenson 2013; Budny & Ley, 2015). Pefloxacin which is highly prescribed has been reported to be one of the FQs that induced the greatest number of lesions with the strongest toxic potential (Desbordes *et al.,* 1993; Kashida & Kato, 1997). One proposed mechanism of tendon injury is the accumulation of free radicals during the inflammatory process secondary to antibiotic use (Seeger *et al.,* 2006). These substances are toxic to tendon cells and

can lead to necrosis (Childs, 2007). With frequent use, there are histologic changes and disorganization of the extracellular matrix (ECM) leading to a remodeling process that weakens the tendon matrix eventually as demonstrated also in fluoroquinolone-treated rats (Kato *et al.,* 1997; Shakibaei & Stahlmann, 2001). The caspase and nitric oxide (NO) pathways play a role in tendon apoptosis as nitric oxide synthase (NOS) was found to be elevated in tendinopathy (Hashimoto *et al.,* 1999). Treatment of tendon injuries and tendinopathies currently remains a clinical challenge, with repair often resulting in the production of inferior tissue and long-term complications and morbidity in patients. The need for treatment strategies that address the underlying pathophysiology of the damaged tissue is thus pertinent (Walden *et al.,* 2017).The intrasound device produces mix frequency Intrasonic waves which are sound waves within the hearing threshold for humans (16 -20,000Hz). Some prior studies on acute tendon injury reported that intrasound therapy either in the low or high intensity mode resulted in significant proliferation of tenoblasts in the

proliferative phase of healing with organized and advanced healing of the tendon by the 14th day post-injury (Aiyegbusi *et al.,* 2012), however, no prior study had been done on its effect on tendinopathy. Rats were used for this study because prior studies had established that both humans and rats exhibit common responses including lesions and edema to the use of fluoroquinolones, specifically Pefloxacin (PEF) (Szomo *et al.,* 2001). The results of this study are important especially in managing FQ-induced tendinopathy in athletes who have been reported to use oral antibiotics twice as frequently as age-matched controls (2.7% vs 1.3%) (Alaranta *et al.,* 2008). The purpose of this study was to determine and compare the effects of 7, 14 and 21 days intrasound therapy in improving the mechanical properties of the Achilles tendon in FQ-induced tendinopathy in animal models.

MATERIAL AND METHODS

Animals: The animals for this study were Fifty (50) male, 6 weeks old Sprague-Dawley rats purchased from the institutional Animal house. The animals were housed by five per wire-mesh cages with access to commercial pelleted rat chow and water *ad libitum*. Ethical approval was obtained from the institutional Health and Research Committee. 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).

Preparation of Pefloxacin: Pefloxacin (PEF) tablet 400mg was dissolved in distilled water and orally administered to the animals by gavage 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 dosage of 400mg was chosen as a prior study had reported that PFX at a dose of 300 mg/kg caused lesions of the Achilles tendon (Kato *et al.,* 1995).

Instrumentation: The Novasonic -Novafon Intrasonic device from Denmark was used for this study.

Experimental Protocol
Animal Grouping: Fifty (50) male rats that weighed between 100gm -120gms were randomized into the following groups:
Group 1 (Normal control): Animals were neither administered PEF nor ITR

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.
Group 2: (Experimental control): The animals were sacrificed on day 8 post PEF - (PEF0 8)

Group 3 (Experimental control): The animals were sacrificed on day 15 post PEF- (PEF015)
Group 4 (Experimental control): The animals were sacrificed on day 22 post PEF- (PEF0 22)

Treatment groups: Animals were administered PEF solution at a dosage of 2.5 ml/kg body weight by gavage for 6 consecutive days and afterwards commenced Intrasound therapy for 5 minutes once daily for the next consecutive 7, 14 and 21 days depending on the group
Group 5: Animals had ITR for 7 days and were sacrificed of day 8, post ITR - (PI7 8)
Group 6: Animals had ITR for 7 days and were sacrificed of day 15, post ITR - (PI7 15)
Group 7: Animals had ITR for 7 days and were sacrificed of day 22, post ITR - (PI7 22)
Group 8: Animals had ITR for 14 days and were sacrificed of day 15, post ITR - (PI14 15)
Group 9: Animals had ITR for 14 days and were sacrificed of day 22, post ITR - PI14 22)
Group 10: Animals had ITR for 21 days and were sacrificed of day 22, post ITR - (PI 21 22)

Intrasound Therapy: The Achilles tendon of both hind limbs of each rat in the experimental groups were treated once daily with the lowest intensity of ITR using the small treatment head applied over the Achilles tendon for 5 min with sterile K-Y jelly as a coupling medium (Potawski & Watson, 2007). Treatment was given post- PFX administration for 7, 14 and 21 consecutive days depending on the respective groups (Aiyegbusi *et al.,* 2012).
Tendon Harvest Process: The animals were sacrificed and the Achilles tendons excised. The tendons were processed for histology using the technique of Stereology described by Young and Dyson (1990).

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 (1990).The tenocytes were identified by their nuclei and fifty random values (10 per animal) were obtained for each group. The numerical density (*NA*) was calculated as the number of tenocyte profiles per unit area of field (Gundersen, 1986).

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 (Avwioro, 2014). 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 (Aiyegbusi *et al.,* 2012).

Preparation of Tendon Specimen for Biomechanical Evaluation: The tensile test specimen preparation was conducted in accordance with the American Standard testing and measurement, method D412 (ASTM D4121983). 50g of epoxy and 25g of hardener, which comprises of methyl ethyl ketone peroxide (MEKP) were used and the tendon immersed in the epoxy to form a tensile specimen (ASTM D412, 19830.

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 (Davis, 2004). The different biomechanical properties were estimated according to established procedures.

Nitric Oxide Synthase Estimation: Nitric Oxidase Synthase (NOS) level was evaluated with prior documented protocol based on enzyme linked immunosorbent assay-double antibody sandwich principle (Nathan *et al.,* 2007).

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 (Alboukadel, 2020) 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 (pairwise. Wilcox. 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 AND DISCUSSION

The summary of data of the biomechanical properties of the Achilles tendon is presented in table1. There were no statistically significant differences (all p > 0.05) in the medians of all the biomechanical properties when the control and PEF only treated tendons were compared on days 8, 15 and day 22. Similarly, there were no substantial differences (all p > 0.05) in the middle values of biomechanical properties between the PEF only and ITR groups on days 8, 15 and 22. Table 2 shows the percentage changes in biomechanical parameters when the PFx only groups were compared with the treatment groups at the different timelines of 8, 15 and 22 days post treatment. The 7 days treatment had substantial improvement in the biomechanical parameters by the 8th day though not significant statistically.

Table1: Biomechanical parameters of the Control and Experimental Groups

Groups	UTs (MPa) M(SE)	UTe (%) M(SE)	Stiffness (N/mm) M(SE)	E-M (GPa) M(SE)	LUs(N) M(SE)
Control	9.37(6.46)	2.37(0.97)	946.2(431.12)	0.74(0.38)	378.6(261.1)
	Experiment A: PEF Only				
P8	0.92(0.40)	5.6(3.89)	259.5(235.1)	0.18(0.16)	38.1(17)
P15	2.95(1.87)	2.63(0.57)	397.8(106.5)	0.28(0.08)	119.2(75.6)
P22	3.1(0.78)	2.72(0.78)	467.5(106.2)	0.31(0.1)	125.3(30.8)
	Experiment B: PEF and Intra-sound				
PI78	5.13(0.94)	3.9(2.5)	559.9(21.2)	0.45(0.13)	200 (37.3)
PI715	1.55(0.06)	2.10(0.06)	284.7(4.2)	0.199 (0.0)	62.2 (0.7)
PI722	3.98(0.1)	3.10 (0.3)	543.5(17.9)	0.395 (0)	165.5 (1.3)
PI1415	2.39(0.05)	2.22(0.26)	519.8(165.3)	0.366(0.12)	96.6 (8.1)
PI1422	4.00 (1.34)	3.42(0.78)	583.4(113.5)	0.411(0.08)	161.6 (54.7)
PI2122	3.49(0.44)	2.11(0.31)	645.8(124)	0.455(0.09)	141.1(17.7)

UTs = Ultimate Tensile Stress, UTe = Ultimate Tensile Strain, E-M = Elastic (Young's) Modulus, LUs= Load at Ultimate Stress,
P8= PEF only 7 days euthanized day 8, P15= PI78 = EF, Intra-sound treated for 7 days, euthanized day 15, P22= PEF only for 7 days, euthanized day 22
PI78 =PEF, Intra-sound treated for 7 days, euthanized day 8, PI715 = PEF, Intra-sound treated for 7 days, euthanized day 15, PI722 = PEF, Intra-sound treated for 7 days, euthanized day 21
PI1415 = PEF, Intra-sound treated for 14 days, euthanized day 15, PI14 22 = PEF, Intra-sound treated for 14 days, euthanized day 22 PI2122 = PEF, Intra-sound treated for 21days, euthanized day 22.

Table 2: Changes in Selected Biomechanical Parameters in Timelines across the Pefloxacin Only and Intrasound Treatment Groups

		UTs	UTe	Stiffness	E-M	LUs
Day 8	P8	+458%	-30.4%	+116%	+150%	+425%
	PI78	p= 0.2	p = 0.2	p = 0.2	p = 0.2	p = 0.2
Day 15	P15	-47.5%	-20.2%	-28.4%	-29%	-47.8%
	PI715	p = 0.66	p = 0.66	p = 0.66	p = 0.66	p = 0.66
	P15	-19%	-16%	+30.7%	+30.7%	-23.4%
	PI1415	p = 0.66	p = 0.82	p = 0.66	p = 0.66	p = 0.66
	P22	+28.4%	+14%	+16.3%	+27.4%	32.1%
	PI722	p = 0.095	p = 1	p = 1	p = 1	p = 0.095
Day 22	P22	+29%	+25.7%	+24.8%	+32.6%	+29%
	PI1422	p = 0.67	p = 0.67	p = 0.6	p = 0.67	p = 0.67
	P22	+12.6%	-22.4%	+38.1%	+46.8%	+12.6%
	PI2122	p = 0.8	p = 0.53	p = 0.53	p = 0.53	p = 0.8

UTs = Ultimate Tensile Stress, UTe = Ultimate Tensile Strain, E-M = Elastic (Young's) Modulus, LUs= Load at Ultimate Stress,
P8= PEF only 7 days euthanized day 8, P15= PI78 = EF, Intra-sound treated for 7 days, euthanized day 15, P22= PEF only for 7 days, euthanized day 22
PI78 =PEF, Intra-sound treated for 7 days, euthanized day 8, PI715 = PEF, Intra-sound treated for 7 days, euthanized day 15, PI722 = PEF, Intra-sound treated for 7 days, euthanized day 21
PI1415 = PEF, Intra-sound treated for 14 days, euthanized day 15, PI14 22 = PEF, Intra-sound treated for 14 days, euthanized day 22 PI2122 = PEF, Intra-sound treated for 21days, euthanized day 22.

Table 3: Biochemical and Stereological Parameters of the Control and Experimental Groups

Group	NOS (µmoles/l) M(SD)	Tenocyte count /unit area M(SD)	% Vol Fraction of Collagen M(SD)
Control	17.19 (8.42)	38.3 (16.20	6.21(0.69)
PEF08	27.39 (10.82)	33.6 (11.9)	5.56 (3.21)
PEF015	12.09 (3.9)	38.7 (6.3)	6.36 (4.17)
PEF021	17.44 (2.52)	40.2 (7.8)	6.52 (3.05)
PEF78	12.32 (8.16)	34.8 (11.2)	5.7 (1.12)
PEF715	3.59 (2.55)	44.7 (8.6)	7.34 (4.28)
PEF721	15.06 (8.4)	40.2 (16.6)	6.5 (3.21)
PEF1415	10.06 (6.67)	49.7 (10.3)	8.17(1.41)
PEF1421	14.64 (8.42)	45.3 (7.2)	7.48 (0.15)
PEF2122	11.24 (1.2)	53.6 (11.6)	8.8 (4.32)
p-value	0.0744	0.774	0.9684

P8= PEF only 7 days euthanized day 8, P15= PI78 = EF, Intra-sound treated for 7 days, euthanized day 15, P22= PEF only for 7 days, euthanized day 22
PI78 =PEF, Intra-sound treated for 7 days, euthanized day 8, PI715 = PEF, Intra-sound treated for 7 days, euthanized day 15, PI722 = PEF, Intra-sound treated for 7 days, euthanized day 21
PI1415 = PEF, Intra-sound treated for 14 days, euthanized day 15, PI14 22 = PEF, Intra-sound treated for 14 days, euthanized day 22
PI2122 = PEF, Intra-sound treated for 21days, euthanized day 22.

The 14 days treatment appeared to have more sustainable effects and by day 22 as seen in figures 1 and 2, there were no significant differences in the biomechanical variables among the PEF only and treatment groups. The summary of the biochemical and stereology data in mean (M) and standard error (SE) is presented in table 3. There were no statistically significant differences (all p > 0.05) in the medians of the biochemical and stereology parameters of NOS, tenocyte population and volume fraction of collagen when the control and experimental groups were compared. On table 4 is shown the correlations between NOS expression and the different variables evaluated.

In the PEF only groups, all the variables had negative correlations with NOS except the Ultimate tensile strain which had significant strong positive correlation. In the Intrasound groups on the other hand, there are significant and strong positive correlations of NOS with ultimate tensile stress and tensile load and moderate negative association with the tenocyte population while there are weak insignificant negative correlations with the ultimate strain and volume fraction of collagen. Disorders of the Achilles tendon rank among the most frequently reported overuse injuries and have been reported to afflict both athletes and sedentary persons (Huttunen *et al.,* 2014). This has been worsened by the popular

use of fluoroquinolones to treat infections because the use of this brand of antibiotics has been linked to a number of adverse effects, including FQ-associated tendinopathy which was first reported in literature in 1983 (Bailet *et al.,* 1983). This study is pertinent because it has been reported that several cases of FQ-associated tendinopathy involve sporting or very physically active people due to the combined adverse effects of exercise and regular use of fluoroquinolones even as athletes have been found to use oral antibiotics twice as frequently as age-matched controls (2.7% vs 1.3%) (Ng and Naughton, 2007; Alaranta, 2008; Lewis, 2009). The choice of PEF for this study was premised on the fact that it is categorized as one of the newer FQs found to be more more toxic for cartilages and tendons while also being one of the most

frequently prescribed antibiotic (Kashida and Kato, 1997; Jeffrey & Aronson, 2016). The treatment of tendon disorders, particularly tendinopathies have been challenging clinically with the healing tendon having biomechanical properties which are inferior to the normal tendon, thereby posing a risk of re-injury and subsequent tendon rupture. Moreover, it has been reported that recovery from fluoroquinolone-related tendinopathy is sometimes slower than from other types of tendinopathy (Trevor & Cook, 2014). It becomes pertinent therefore to investigate modalities that may augment healing with better biomechanical properties even as it has been earlier reported that the closer the Elastic modulus of a tendon is to the normal value, the more it will respond normally under loading (Khayyeri *et al.,* 2017).

Table 4: Correlation of NO with Biomechanical and Morphological Variables

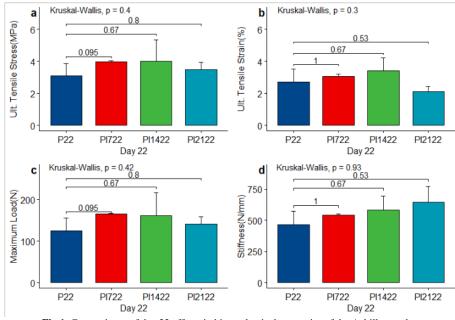| Biomechanical Properties	PEF Only	PEF and INTRASOUND
	(r , p-value)	(r, p-value)
Ultimate Stress vs. NOS	-0.46, 0.297¶	**0.62, 0.005****‡
Ultimate Strain vs. NOS	**0.86, 0.012****†	-0.01, 0.978
Maximum Load vs. NOS	-0.46, 0.297¶	**0.64, 0.003****‡
Stiffness vs. NOS	-0.73, 0.065†	0.29, 0.232
Young's Modulus vs. NOS	-0.74, 0.06†	0.26, 0.278
Biochemical & Stereology Properties		
%Vol. of Collagen vs. NOS	-0.36, 0.427¶	-0.25, 0.301
Tenocyte Count/Area vs. NOS	-0.67, 0.102‡	**-0.56, 0.012****‡

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

This study was therefore carried out as part of an on-going attempt to investigate modalities that could improve and reverse the biomechanical and morphological changes seen in tendinopathy. Intrasound therapy has been reported by prior studies to augment and improve the morphological properties of the healing tendon following acute tendon injury (Aiyegbusi *et al.,* 2012). Though no study has been done on its effect on the biomechanical properties in tendinopathies, hence the need for this study. Prior studies had established that both humans and rats exhibit common responses including lesions and edema to the use of fluoroquinolones, specifically Pefloxacin (Kashida & Kato, 1997). However, it has been reported that the injection of fluoroquinolones in rats does not lead to tendon rupture as it does in humans (Ribard *et al.,* 1992) and a lower dosage in humans seems to cause more severe effects than in rats, even as Kato *et al.,* (1997) had demonstrated that a single oral dose of fluoroquinolones in juvenile rats induced reversible lesions 2 weeks after administration. Going by this assertion, it is expected that the 400mg/kg dosage used in this study will have more severe effect in humans, especially as PEF is usually used at a higher daily dosage of 800 mg to treat infections in humans and the majority of cases of tendinopathy occurred with a PEF dosage of 800 mg

per day (Meyboom *et al.,* 1994; Khaliq and Zhanel, 2003).

The results of this study showed that the tendons that had PEF administered without treatment had their mechanical integrity compromised by day 8 post-PEF even as a prior study had reported 6 days as the median onset of tendinopathy following FQ treatment (Corrao et al 2006). PEF treatment has been reported to induce oxidative damage of type 1 collagen by direct toxicity (Van der Linden *et al.,* 2001; Pouzaud *et al.,* 2004). The altered biomechanical properties of the PEF - only treated tendons was evident by the highest ultimate tensile strain and reduced stiffness observed in this group especially on the 8th day post PEF while the other biomechanical properties were much reduced when compared with the control, though the changes were not statistically significant. The statistically insignificant changes in the variables in the experimental groups in spite of the evident biological changes are likely due to the relatively small sample sizes used in this study. It was however observed that by day 22 Post PFx, these properties had significantly improved when compared with the values on days 8 and 15 post-PFx. A prior study had reported that lower dosage in humans seems to cause more severe effects than in rats which could explain the fact that

AIYEGBUSI, AI; AJIBOLA, OA; SAMUEL, TA; BALOGUN, OJ; DURU, FI

tendinopathic lesions seen were not too pronounced and by day 22, there was ample recovery as seen in table 2 where all the biomechanical properties had improved by then, though with inferior biomechanical properties when compared with the normal tendon. Further studies are needed with much higher dosage, such as 800mg/kg body weight as given in clinical settings. Also, the expression of NOS was highest in the PEF-only group on day 8 post-PEF to stimulate extracellular matrix synthesis as reported in earlier studies that the expression of NO is up-regulated in tendinopathy to enhance and promote collagen synthesis with a return to baseline by the 14th day (Xia *et al.,* 2006; Murrell, 2007). Our results show a negative correlation between the biomechanical properties and NO expression in the PEF only group

except for the Ultimate tensile strain which showed a significant ($p<0.05$) high correlation. This may be indicative of the fact that NO is normally not regulated in a normal tendon so as the biomechanical properties are altered, NO is increased to stimulate healing, even as it seen in table 3 that the expression of NO was highest on the 8th day Post-PEF (Xia *et al.,* 2006). The need for modalities that will augment healing in tendinopathies cannot be over-emphasized as significant structural changes have been shown to persist in animal studies for as long as 20 weeks post - FQ exposure with clinical symptoms reported to manifest as late as 6 months after exposure (Khaliq and Zhanel, 2003; Shakibaei *et al.,* 2007).This could make sportsmen and physically active individuals prone to re-injury and the risk of tendon rupture.

Fig 1: Comparisons of day 22 effects in biomechanical properties of the Achilles tendon.
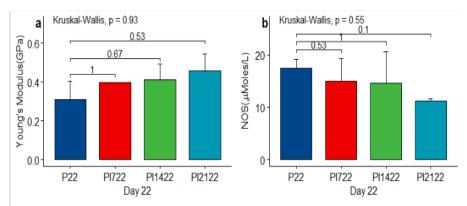

Fig 2: Comparisons of day 22 effects of Young's modulus and NOS of the Achilles tendon.

The animals that had intrasound treatment post-PFx exhibited varied responses as seen from our results. The 7 day- treatment showed markedly improved biomechanical parameters by day 15 post -PFX which however were reduced, though not significantly by day 22. As seen in table 2, there was significant (p<0.05) increase in all the biomechanical variables analysed in the tendons in this group when compared with the experimental control. However, the effects of the 7-day treatment was not sustainable till day 22. The 14 days treatment on the other hand had the best biomechanical qualities by the end of the 22nd day when compared with the PFx only tendons, though these qualities were still inferior to the normal tendons. The Young modulus (E-M) is described as a measure of material resistance (stiffness) to elastic deformation under load and the closer the elastic modulus of a loaded tendon is to that of a control experiment, the more similarly it will behave under mechanical stress (Khayyer, 2017)**.** Our results showed that the 14 and 21 days treated tendons had the nearest E-M to the normal tendon. The inferior mechanical properties seen in the PEF only group can also be seen as markedly reduced stiffness of the tendons. Stiffness, which is an important constituent of tendon mechanical properties has a significant influence on force transmission as an optimal level of tendon stiffness is critical for effective muscle-tendon interactions (Bojsen-Moller *et al.,* 2005; Reeves *et al.,* 2003). Our results show that by day 22, the tendons treated for 14 and 21 days had the closest value of stiffness to the control group. However, from figures 1 &2 which showed the biomechanical properties of the tendons across the groups on day 22, it can be seen that the 14 days treatment group had the best biomechanical properties.

The significant positive correlations between NOS expression and some of the biomechanical properties in the intrasound group may suggest that ITR regulates NOS expression during tendon healing which may be responsible for the improved properties seen in this group as it has been previously reported that the addition of NO had a protective or beneficial effect on collagen organisation, tendon healing failure load and stress (load/area) (Murrell *et al.,* 1997; Bokhari & Murrell, 2011). Comparing the tenocyte population and Volume fraction of Collagen across the control and experimental groups show no significant differences (p > 0.05). This is expected as it has been reported that tendinopathic tendons are thicker, but with reduced energy-storing capacity, which implies that the tendons exhibit higher strains for the same load than healthy tendons thus exhibiting a decline in both the structural and material properties, as reported also by a prior study (Helland *et al.,* 2013;

Alice *et al.,* 2014). This is seen from our results where the Ultimate tensile stress of the PEF tendon on day 8 was about a tenth of the normal value, while the volume fraction of collagen was almost the same as the control group. However, the quality and types of collagen fibres in the various treatment groups were not ascertained in this study even as prior studies have demonstrated an increase in volume and size of tendon tissue in tendinopathy but with inferior mechanical properties due to increase production of type 3 collagen fibres with small diameters over type 1 which is synthesized in abundance during the repair process in degenerative tendons (Chan *et al.,* 2008; Thorpe *et al.,* 2015). This is pertinent as a prior study had reported a relationship between different collagen fibril diameters in tendons and variations in tendon strength and elasticity (Biancalana *et al.,* 2010). Since the remodelling phase of tendon healing which begins from day 21 is characterized by greater structural organization, we hypothesize that the tendons treated with ITR could demonstrate greater biomechanical resistance in the later stages of the repair process. Further studies are recommended to evaluate the effects of ITR in the later stages of this phase.

Conclusion: Intrasound treatment to the Achilles tendon for 14 days improved the mechanical properties and morphology of tendons in Fluoroquinolone-induced tendinopathy probably by regulating the expression of NOS.

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