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Oral Vitamin C Intake Ameliorates Crude Oil-Polluted Water-Induced Jejunal Contractile Dysfunctions in Wistar rats

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ABSTRACT: Inhabitants of rural Niger-Delta oil communities in Nigeria often, unintentionally, consume crude oil polluted water (COCW) due to crude oil spills in the region. The impact of vitamin C supplementation during COCW ingestion on the contractile mechanism of the jejunum is not fully known. Hence, this study investigates the outcomes of COCW water and vitamin C intake on the smooth muscle activity of the jejunum in Wistar rats using standard techniques. Data obtained showed that jejunal tissue SOD concentration was significantly reduced, while jejunal tissue MDA concentration was significantly increased in the COCW-only treated group. Contraction mediated by acetylcholine was significantly increased in the COCW-only treated rats. Calcium and potassium ion influx significantly increased jejunal contraction in the COCW-only rats. Incubation of the jejunal tissue in nifedipine, indomethacin, and L-NAME significantly increased acetylcholine-mediated contractions in the jejunal tissue of the COCW-only treated rats when compared to other groups. COCW causes free radical-induced jejunal damage that results in impaired jejunal contractile activity mediated by the M_2 muscarinic receptor, nitric oxide synthase activity, voltage-gated large-conductance calcium channels, potassium channels, and prostaglandins. However, the oral intake of Vitamin C supplementation significantly ameliorated impairments by enhancing jejunal antioxidant activity.

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Keywords: crude oil-polluted water; jejunal tissue; impaired contractile mechanism; amelioration; Vitamin C

Abbreviations: COCW: crude oil-polluted water, L-NAME: N-nitro-L-arginine methyl ester, MDA: malondialdehyde concentration, SOD: superoxide dismutase

Crude oil is essential for fueling cars, tractors, and electric power generation (Ohanmu *et al*., 2019). The chemical composition of crude oil and petroleum products can affect different organisms in the ecosystem (Overton *et al*., 1994). Oil spills usually lead to heavy metal contamination in the environment (Egbe and Thompson, 2010). Spillage of crude oil causes the introduction of petroleum hydrocarbon environmental contaminants into the environment (Wang *et al*., 2012). Nigeria is a foremost crude oil exporter in Africa and experiences multiple oil spills that have damaged agricultural lands and plant growth and development in the impacted areas (Agbogidi *et al*., 2005). Studies have shown that oil spills in the Niger Delta and other tropical regions can have long-term environmental impacts (Amadi *et al*., 1996). Several millions of barrels of oil have been reported leaked into the Niger Delta ecosystem over the years

(Onyena and Sam, 2020). Polycyclic aromatic hydrocarbons are major components of crude oil that induce nausea, vomiting, and skin and eye irritation after acute, high-level exposures. (Kim *et al*., 2013). Crude oil spills are a common event in Nigeria, and often unreported (Emuedo *et al*., 2014). Concrete efforts are delayed or not done to remediate the ecosystem even when reported (Linden and Palsson, 2013). Rural dwellers of the Niger Delta, where there are frequent incidences of crude oil spillage, are more exposed to the hazards as they mostly drink from crude oil polluted water sources. Exposure to crude oil and contaminants in water usually causes stomach upset, stomach cramping, nausea, vomiting, and diarrhea. Obidike (2012) reported that oral ingestion of benzene-contaminated water resulted in impaired motility and cytohistology of the ileum. Ingestion of crude oil was reported to impact otter digestive function by reducing the retention time of food and causing malabsorption of ingested oil (Ormseth & Ben-David, 2000). Exposure to Bonny light crude oil was reported to cause derangement in antioxidant system in rats' testis and sperm (Farombi *et al*., 2010). Dietary vitamin C and E supplementation was however, reported to reduce toxic effect caused by petroleum exposure (Achuba and Otuya, 2006). Recently, vitamin C supplementation was reported to ameliorate crude oil contaminated water induce gravid uterine contractile dysfunctions (Salami *et al*., 2023a). Vitamin C augmentation was also reported to be beneficial to adult erectile functions after prepubertal crude oil-polluted water ingestion (Salami *et al*., 2023b). While the deleterious impact of crude oil polluted water on gastrointestinal functions is not debatable, our understanding of the basis and mechanism as they relate to the contractile mechanisms of specific small intestinal sites is limited. The understanding of the influence of antioxidant supplementation during ingestion on the contractile mechanisms is also not fully known. The fact that the jejunal area experiences intense peristaltic activity relative to the duodenum and ileum (Sanders, 2019) also makes this investigation relevant. This study investigates the outcomes of COCW water and vitamin C intake on the smooth muscle activity of the jejunum in Wistar rats.

MATERIALS AND METHODS

Animal: Female Wistar rats (15) were kept in the LASUCOM Animal House. The rats were maintained at room temperature with a 12-hour dark/light cycle. The animals were fed standard rat chow and water for the duration of the study. The animal ethics committee at the College granted approval for the study with approval number AREC/2022/044.

Experimental design, treatment protocol and COCW: The fifteen female Wistar rats (150–200 g) were divided into three groups of five rats each. Group 1 (control) received normal saline; group 2 ingested 2.5 ml of COCW; and group 3 consumed COCW (2.5 ml) and 10 mg/kg of vitamin C. The animals were administered through the oral route once daily and lasted for 6 weeks. The crude oil polluted water from Ogbia local government area in Bayelsa State (in the Niger Delta region) was used in this study. The crude oil polluted water was retrieved in March 2022. Precaution was taken to ensure adequate mixing (by shaking) of the COCW before administration. We also ensured that enough COCW was taken once at a specific community water source. The constituents of the COCW analyzed by gas chromatography – mass spectrometry was as earlier reported (Salami *et al*., 2023a).

Preparation of the jejunum and constitution of the Physiological solution: When the six weeks of treatment were completed, the animals were fasted overnight and sacrificed (cervical dislocation) after sodium pentobarbital (30 mg/kg) administration. About 10 cm of the jejunum from each animal in each group was cut, flushed, and placed in a Tyrode solution. The constituents of the Tyrode solution were as previously described (Salami *et al*., 2021). The PH of the solution in the chamber was maintained at 7.40 (37^0 C) and perfused continuously with 5% of $C0_2$ and 95% of 0_2. To prepare a Ca^{2+}-free Tyrode solution, EDTA (0.5 mM) was added in lieu of $CaCl_2$. The jejunal tissues were placed in the tissue bath chamber (50 ml) and connected to a transducer. The connection of the jejunal tissue to the transducer and the determination of isometric contractions from the jejunal tissues were as previously described (Salami *et al*., 2021).

Contractile activity experiment on the jejunum: The jejunal tissues were made to stabilize for 30 minutes in the chamber, and thereafter, they were subjected to the following:

The influence of vitamin C supplementation during COCW ingestion on voltage-operated calcium channel activity in the jejunal tissues across treatment groups was determined. Cumulative doses of $CaCl_2$ (Tocris Biotechne, UK) were added to the Ca^{2+}-free Tyrode solution, and contractions in the jejunal tissues were recorded.

Jejunal tissues in the organ chamber were incubated for 15 minutes with nifedipine procured from Unicure Pharmaceuticals Nigeria. A graded amount of acetylcholine (10^{-9} – 10^{-5} M) was injected, and the contractions in the jejunal tissues were observed and recorded.

The action of the nitric oxide synthase in the jejunal tissues was investigated by incubating the jejunal tissues in L-NAME (AK Scientific, Inc., CA, USA) for 15 minutes. A graded amount of acetylcholine (10^{-9} – 10^{-5} M) was injected, and the contractions in the jejunal tissue were recorded.

The effect of vitamin C intake during COCW ingestion on the activity of the K^+ channels in the jejunal tissues was investigated by adding a graded amount of potassium chloride (Tocris Biotechne, UK) to the jejunal tissues in the potassium-free tissue chamber.

The activity of muscarinic receptors in the jejunal tissue was investigated by adding a graded dose of acetylcholine (10^{-9} – 10^{-5} M) to the jejunal tissue in the organ chamber. The contractions were then recorded.

Jejunal tissues were incubated with atropine (a competitive muscarinic antagonist) obtained from AK Scientific USA for 15 minutes. The contraction of the jejunal tissues to graded doses of acetylcholine was subsequently recorded.

The ATP-sensitive K+ channel (K^+ATP) and the cyclo-oxygenase enzyme activity in the jejunal tissues were investigated by incubating them in glibenclamide (AK Scientific, USA) and indomethacin (Jiangxi Pharmaceutical, China), respectively. A graded amount of acetylcholine was then added, and the contractions in the jejunal tissues were recorded.

*Homogenization of jejunal tissue and assay of jejunal superoxide dismutase and MDA concentration***:** The jejunal tissues obtained during sacrifice were homogenized in a cold phosphate buffer solution (Ph 7.4). The homogenates were then centrifuged at 2500 rpm for 20 minutes. The supernatant was carefully removed and used for the assay of superoxide dismutase and malondialdehyde concentration. The concentration of superoxide dismutase in jejunal homogenate was determined using the method of Marklund and Marklund (1974). The malondialdehyde concentration in the jejunum homogenate was determined by the method previously described (Buege and Aust 1978).

Statistical Analysis: The data in this study were presented as the mean ± standard error of the mean (SEM). A One- or two-way analysis of variance (ANOVA) was carried out where appropriate using statistical software (Prism Graph Pad, version 8.0). A statistical difference was inferred when $p < 0.05$.

RESULTS AND DISCUSSION

The constituents in COCW obtained by GC-MS analysis: The major bio-constituents obtained include 21.4 % of methylene chloride, 19.1 % of pentamethylnaphthalene, 5.5 % of 11,13-dimethyl-12-tetradecen-1-ol acetate, 4.9 % of cyclohexene, 4.1 % of octadecane,1-chloro-, 8.4 % of bicyclo [3.1.1]heptane,2,6,6,trimethyl, 3.3 % of 7-pentadecyne, 3.3 % of 2,4-di-tert-butylphenol, 3.2 % of 1-methylbicyclo[3.2.1]octane, 2.8 % of 2,6,10-trimethyltridecane, 2.5 % of phthalic acid, 2.5 % of neopentyl 2-propyl ester, 2.4 % of 6,11-undecadiene, 1-acetoxy-3,7-dimethyl-, 6.2 % of 1-docosene, and 2.3 % of D-Homoandrostane (Figure 1) (Salami *et al*., 2023a) of the different bio-constituents, their retention time and relative abundance.

Effect of COCW and vitamin C intake on body weights and jejunal concentrations of SOD and MDA: Jejunal tissue SOD concentration was significantly reduced, while jejunal tissue MDA concentration was significantly increased in the COCW group when compared to the control and vitamin C-supplemented groups (Table 1). Averagely, the body weights reduced slightly ($p > 0.05$) in the COCW-only rats (Table 1).

Table 1: Jejunal tissue SOD, MDA concentration, and body weight on the 4th week of the animals

Parameters	Control	COCW	COCW+ vit C
SOD (µmol/ml)	14.3 ± 0.94	7.5± 0.44*	11.0 ± 0.60
MDA (µmol/ml)	0.18 ± 0.05	1.09 ± 0.11*	0.39 ± 0.03
Body weight (g)	149.3 ± 4.6	138.5 ± 6.7	159.8 ± 5.9

N=5, * = p <0.05, COCW= contaminated water-only, COCW+VIT C = contaminated water + Vitamin C-treated

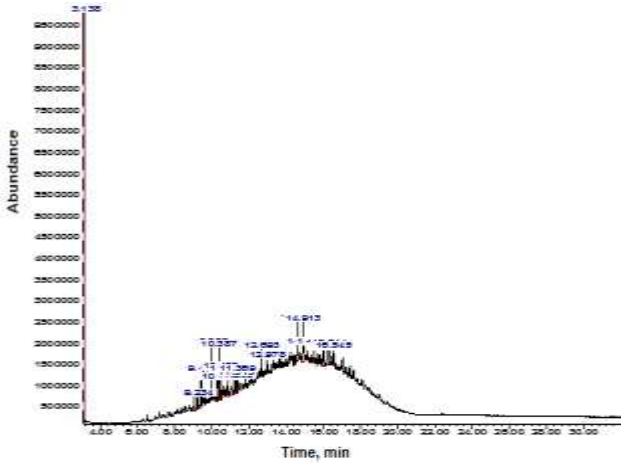

Fig 1: Chromatogram of COCW

Effect of COCW and vitamin C intake on the jejunal contractile mechanisms: Jejunal tissues from the COCW-only treated group showed significantly increased acetylcholine-mediated contraction as

compared to the group co-treated with vitamin C (Figure 2). Furthermore, calcium and potassium ion influx via voltage-operated calcium and K^+ -channels respectively into jejunal tissues caused significantly increased contraction in the COCW-only treated group (Figures 3 and 4).

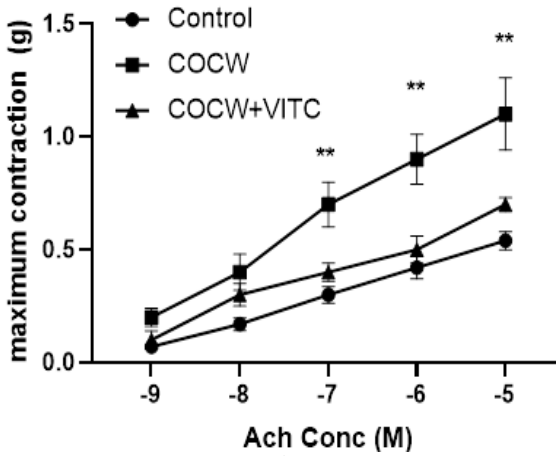

Fig 2: Maximum contraction of jejunal tissues in response to graded acetylcholine (10^{-9}-10^{-5} M). Where n = 5, ** = p < 0.01, COCW= contaminated water-only, COCW+VIT C = contaminated water + Vitamin C-treated

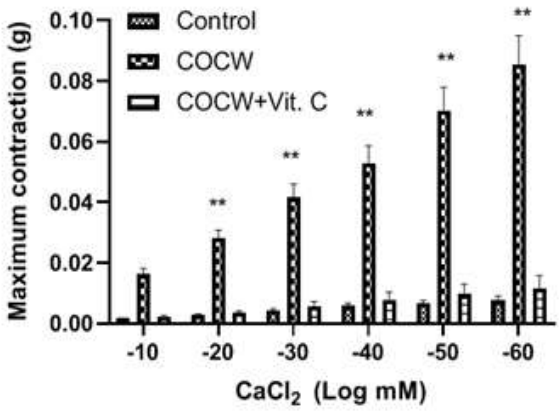

Fig 3: Maximum contraction of jejunal tissues to graded calcium chloride (10 - 60 mM). Where n = 5, ** = p < 0.01, COCW = contaminated water-only, COCW+VIT C = contaminated water + Vitamin C-treated

Acetylcholine-mediated contractions in the jejunal tissues were not significantly altered across groups with the incubation of the jejunal tissues in a competitive muscarinic receptor antagonist (atropine) and ATP-sensitive K^+ channel inhibitor (glibenclamide) (Tables 2 and 3). Jejunal tissues of the COCW-only group incubated in large conductance voltage activated Ca^{2+} channel inhibitor (nifedipine), non-specific cyclooxygenase inhibitor (indomethacin), and nitric oxide synthase inhibitor (L-NAME), respectively, showed a significant increase in acetylcholine-mediated contraction when compared to the other groups (Tables 4-5 and Figure 5, respectively).

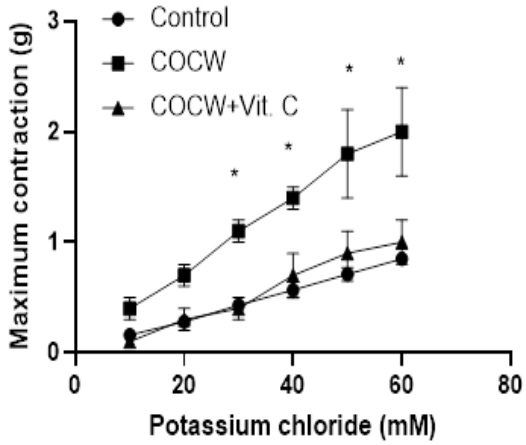

Fig 4: Maximum contraction of jejunal tissue to graded potassium chloride (10-60 mM). Where n = 5, * = p <0.05, COCW = contaminated water-only, COCW+VIT C = contaminated water + Vitamin C-treated

Table 2: Maximum contraction of **j**ejunal tissues to graded acetylcholine (10^{-9} – 10^{-5} M) post-incubation with atropine at 10^{-5} M

Acetylcholine (M)	Control	COCW	COCW+ vit C
-9	0.07 ± 0.001	0.11± 0.04	0.17± 0.05
-8	0.14 ± 0.02	0.21 ± 0.07	0.27 ± 0.06
-7	0.17 ± 0.02	0.37 ± 0.09	0.35 ± 0.08
-6	0.27 ± 0.02	0.52 ± 0.15	0.40 ± 0.09
-5	0.37 ± 0.05	0.64 ± 0.19	0.45 ± 0.09

N=5. Values expressed as mean SEM, COCW = contaminated water-only, COCW+VIT C = contaminated water + Vitamin C-treated

Table 3: Maximum contraction of jejunal tissues to graded acetylcholine (10^{-9} – 10^{-5} M) post-incubation with glibenclamide at10^{-4} M

Acetylcholine (M)	Control	COCW	COCW+ vit C
-9	0.14 ± 0.03	0.17± 0.04	0.08± 0.00
-8	0.35 ± 0.06	0.29 ± 0.03	0.16 ± 0.03
-7	0.50 ± 0.10	0.39 ± 0.03	0.23 ± 0.05
-6	0.65 ± 0.15	0.46 ± 0.03	0.39 ± 0.05
-5	0.74 ± 0.04	0.60 ± 0.07	0.51 ± 0.07

N=5. Values expressed as mean SEM, COCW = contaminated water-only, COCW+VIT C = contaminated water + Vitamin C-treated

Table 4: Maximum contraction of jejunal tissue to graded acetylcholine (10^{-9} – 10^{-5} M) post-incubation with nifedipine at 10^{-4}M

Acetylcholine (M)	Control	COCW	COCW+ vit C
-9	0.15 ± 0.04	0.24± 0.06	0.19± 0.04
-8	0.24 ± 0.05	0.42 ± 0.09	0.36 ± 0.08
-7	0.36 ± 0.01	0.67 ± 0.11	0.52 ± 0.07
-6	0.43 ± 0.00	0.82 ± 0.09*	0.66 ± 0.09
-5	0.49 ± 0.00	0.95 ± 0.10*	0.78 ± 0.10

Where n = 5, * = p <0.05, COCW = contaminated water-only, COCW+VIT C = contaminated water + Vitamin C-treated

Table 5: Maximum contraction of jejunal tissues to graded acetylcholine (10^{-9} – 10^{-5} M) post-incubation with indomethacin at 10^{-4} M

Acetylcholine (M)	Control	COCW	COCW+ vit C
-9	0.18 ± 0.02	0.13± 0.02	0.13± 0.01
-8	0.36 ± 0.00	0.26 ± 0.02	0.23 ± 0.04
-7	0.51 ± 0.03	0.36 ± 0.04	0.37 ± 0.06
-6	0.60 ± 0.04	0.46 ± 0.05*	0.55 ± 0.08
-5	0.77 ± 0.06	0.59 ± 0.08	0.64 ± 0.08

Where n = 5, and * = p <0.05, COCW = contaminated water-only, COCW+VIT C = contaminated water + Vitamin C-treated

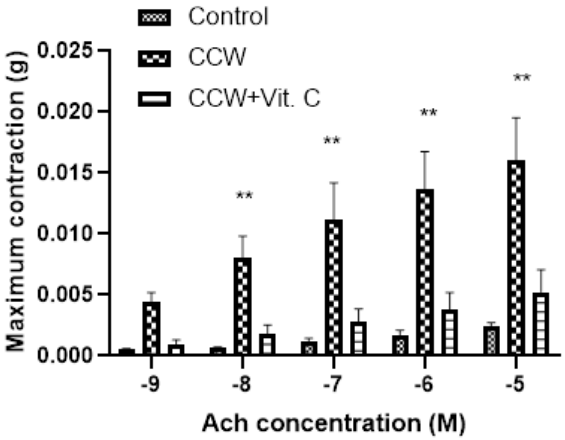

Fig 5: Maximum contraction of **j**ejunal tissues to graded acetylcholine (10^{-9} – 10^{-5} M) post incubation with L-NAME at 10^{-4} M. N = 5, ** = p < 0.01, CCW = contaminated water-only, CCW+VIT C = contaminated water + Vitamin C-treated

The jejunum is an important part of the small intestinal area. It primarily absorbs lipids, monosaccharides, peptides, amino acids, and several vitamins and minerals (Coulston *et al*., 2013). It also has an extensive blood supply (Drenckhahn & Waschke, 2008) and constitutes about 40% of the entirety of the small intestine (Welsch, 2006). The use of the jejunum in this study is also germane, as it has intensive peristaltic activity relative to the duodenum and ileum (Sanders, 2019). Therefore, impairments in small intestinal contractile activity due to COCW ingestion and the potential role of vitamin C supplementation on contractile activity are justifiably investigated in the jejunum. This study found that the jejunal tissue SOD concentration was significantly reduced while the jejunal tissue MDA concentration was significantly increased in the COCW-only treated rats compared with other groups (Table 1). This outcome signifies that COCW ingestion exacerbated free radical accumulation in the jejunal tissue. This study also shows that concomitant supplementation with vitamin C during COCW consumption offers significant protection in the jejunal tissue by elevating the antioxidant activity of SOD. Recent studies have also reported on the protective ability of antioxidant supplementation during crude oil, crude oil by-products, or crude oil polluted water exposure on other body tissues and organs (Achuba and Awhin, 2009; Ita *et al*., 2014). The impact of the free radical scavenging activity in the jejunal tissues of vitamin C-co-treated rats during COCW ingestion was also identified in several unique contractile mechanisms of the jejunal tissue in this study. COCW-only-treated rats showed a significant increase in acetylcholine-mediated contraction of the jejunum when compared to the vitamin C-co-treated rats (Figure 1). The contraction in the gut is facilitated by the highly expressed M2 acetylcholine receptor subtype in smooth muscle tissues in the gastrointestinal tract (Iino & Nojyo, 2006). The fact that the expression of this receptor subtype was not determined in this study is a limitation in asserting that their over-expression resulted in the exaggerated contraction in the jejunal tissue of the COCW-treated group in this study. However, this line of thinking is suggested due to an earlier report by Ormseth and Ben-David (2000), who found that ingestion of crude oil in otters decreased food retention time. Atropine is a competitive muscarinic receptor antagonist that usually inhibits acetylcholine-induced contraction (Singh and Mandal, 2013). Incubation of jejunal tissues in atropine in this study (Table 2) increased acetylcholine-stimulated contraction in the COCW group. However, vitamin C supplementation slightly reduced the contraction, similar to that of the control.

Furthermore, jejunal tissues from the COCW-only group showed a significant increase in contraction during Ca^{2+} influx into jejunal tissues when compared to vitamin C-co-treated rats (Figure 3). This suggests the likely overexpression of the Ca^{2+} sensing receptor in the COCW group. The calcium sensing receptor has been hypothesized to guide calcium influx-mediated contraction in the small intestine and is expressed in fish, birds, amphibians, mammals, and humans (Kirchhoff & Geibel, 2006). Incidentally, jejunal tissue incubation with nifedipine (a large-conductance Ca^{2+} channel inhibitor) caused a significantly increased acetylcholine-mediated contraction in the COCW-only rats (Table 5). This observation further buttresses our postulation of the amplification of the activity of the Ca^{2+} sensing receptor in the COCW group.

According to Lamarca *et al*., (2006), KCl-stimulated contraction in the rabbit small intestine is mediated by the inward rectifier K+ channels, voltage-dependent K+ channels, HERG K+ channels, and high-conductance Ca^{2+}-activated K^+ channels. The significant increase in the contraction of the jejunal tissue in the COCW group during KCl influx in this study (Figure 4) may have been mediated by the

amplification of the activity or expression of any of these aforementioned channels. Incubation of the jejunal tissue with glibenclamide (an ATP-sensitive K^+ channel) failed (Table 3) to significantly change the acetylcholine-mediated contraction across groups. This suggests that the channel may not be affected by COCW exposure.

Vitamin C supplementation during the ingestion of COCW modulated the exaggerated contractions observed in the COCW group in this study. This can be explained by the reported ability of Vitamin C administration to cause decreased intestinal contraction by stimulating adrenergic receptors (Wawrzeska, 1987).

Indomethacin is known to suppress contractile activity in the small intestine in rats. The mechanism is reported to involve increased edema development and inflammatory mediators (Lenard *et al*., 2015). Incubation of jejunal tissue with indomethacin in this study significantly reduced contraction in the COCW-only rats when compared to other groups (Table 5). This result showed that COCW ingestion impacted the prostaglandin mechanism in the jejunal tissue, and vitamin C supplementation seemed to ameliorate the impact.

The inhibition of nitric oxide release by L-NAME usually increases jejunal contraction, as reported by Alemayehu *et al*. (1994). The increase in contraction after L-NAME incubation is caused by the inhibition of NO synthase activity and, subsequently, NO release. This results in improved autonomic cholinergic function and augmented neurotransmitter release (Diab, 2008). The activity of nitric oxide synthase was severely impaired in the COCW-treated group in this study, and vitamin C supplementation significantly ameliorated the impairment. Incubation of jejunal tissues with L-NAME caused a significant increase in contraction in the COCW-only group when compared to the other groups (Figure 5). This shows the vital importance of vitamin C supplementation in attenuating impaired NO synthase activity during COCW ingestion.

It is appropriate to suggest that compounds identified in the COCW (Figure 1) via GCMS analysis are biologically active and should have synergistically caused the impaired contractile activity observed in this study. Coincidentally, most of the compounds are similar to compounds previously reported as environmental contaminants in water after oil spillages (Ifelebuegu *et al*., 2017; Salami *et al*., 2023a).

There was a slight reduction in the weight of the COCW group compared to the control and the vitamin C-supplemented group. Ormseth & Ben-David (2000) reported that crude oil ingestion in otters reduced food retention time and caused impaired digestion and malabsorption. Both of these can impair body weight, as observed in this study. Another plausible explanation for the reduction in weight of the COCW group in this study is the laxative effect of the crude oil polluted water, which decreases the bioavailability of nutrients, as explained by Fisher *et al*. (2006).

Conclusion: The intake of crude oil-polluted water produced free radical-induced jejunal damage that results in impaired jejunal contractile activity mediated by the M2 muscarinic receptor subtype, nitric oxide synthase activity, voltage-gated large-conductance calcium channel activity, potassium channels, and prostaglandins. Vitamin C supplementation significantly ameliorated these impairments by enhancing jejunal antioxidant activity.

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