PRINT ISSN 1119-8362
Electronic ISSN 2659-1502

Full-text Available Online at
https://www.ajol.info/index.php/jasem
https://www.bioline.org.br/ja

J. Appl. Sci. Environ. Manage.
Vol. 27 (12) 2783-2792 December 2023

Moringa leifera Leaf Improves Crude Oil-Polluted Water-Induced Altered Cavernosa Functions by Elevating Testosterone and Phosphodiesterase-5 Activity in Male Wistar Rats

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ABSTRACT: Ingestion of crude oil released into the environment is reported to cause oxidative stress-induced reproductive impairments. This study investigates the effect of *Moringa oleifera* leaf (MO) treatment on cavernosa contractile activity in crude oil-contaminated water (CCW)-exposed male Wistar rats. Cavernosa tissues excised from distilled water, CCW, CCW+MO, and MO treated rats were subjected to contractile functions studies using acetylcholine, calcium chloride, potassium chloride, sodium nitroprusside, glibenclamide, nifedipine, methyl blue and barium chloride. Serum testosterone, cavernosa oxidative markers, and phosphodiesterase 5 were also determined using standard techniques. Serum testosterone, body weight, and testicular, epididymal and cavernosa weights were significantly reduced in the CCW group as compared to CCW+MO group. MDA concentration increased significantly in CCW group as compared to CCW+MO and MO groups. *Moringa oleifera* improved the relaxation response to acetylcholine and sodium nitroprusside in CCW+MO group, and relaxation was not significantly affected by incubation in nifedipine, methyl blue and barium chloride when compared to the CCW group. MO treatment ameliorated crude oil-contaminated water-induced cavernosa dysfunctions by increasing testosterone, phosphodiesterase 5 activity, and its cytoprotective antioxidant properties.

DOI: https://dx.doi.org/10.4314/jasem.v27i12.16

Cite this paper as: SALAMI, S. A; ALABI, A. A; ALLEN, M. O; SALAHDEEN, H. M; MURTALA, B. A (2023). *Moringa oleifera* leaf improves crude oil-polluted water-induced altered cavernosa functions by elevating testosterone and phosphodiesterase-5 activity in male Wistar rats. *J. Appl. Sci. Environ. Manage.* 27 (12) 2783-2792

Dates: Received: 12 November 2023; Revised: 10 December 2023; Accepted: 20 December 2023 Published: 30 December 2023

Keywords: crude oil-contaminated water; oxidative stress; cavernosa activity, *Moringa oleifera;* cavernosa

The primary source of foreign exchange for Nigeria is crude oil, which is primarily produced in the Niger Delta region, where oil spillage frequently threatens both the ecosystem and public health (Oruambo and Jones, 2007). The entire aquatic ecosystem is heavily contaminated with petroleum hydrocarbons as a result of human and industrial activities like drilling, manufacturing, storing, transporting, managing oil waste, and sabotaging of oil pipelines (Zabbey *et al*., 2017). These present significant risks to public health and the socioeconomic system. The people in the heavily oil-producing Niger Delta region of Nigeria live in a purely agrarian society, relying heavily on farming and fishing to survive (Nriagu *et al*., 2016). According to Adeyemi *et al*. (2016), hazardous substances accumulate in seafood and farm produce when crude oil and petroleum hydrocarbons contaminate the aquatic ecosystem. This eventually affects people and other animals throughout the food chain. Achuba *et al*. (2016) reported that crude oil consumption causes oxidative damage, which predisposes animals to a variety of illnesses as well as organ and tissue damage. Erectile smooth muscle, and intra-cavernous arteries are impacted by oxidative

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stress. It has been demonstrated that reactive oxygen species (ROS), like superoxide, usually oxidize NO, slowing down its bioavailability (Azadzoi *et al*., 2005). The cavernosa tissue is capable of producing excess free radicals, and oxidative stress has been connected to erectile dysfunction (ED). The availability of NO, which is needed for penile erection, is decreased by oxidative stress in ED. This is due to increased levels of arginase activity in ED, which limits NO synthase activity, decreases NO biosynthesis, and increases arginine degradation (Jeremy *et al*., 2000). Superoxide and other free radicals can be eliminated by the presence of antioxidants, allowing NO to persist long enough to exert its biological effects (Oboh and Rocha, 2007). The medicinal plant *Moringa oleifera* (Moringaceae), also known as drumstick, is cultivated extensively in tropical and subtropical areas. The ancient world was familiar with *Moringa oleifera*, but it wasn't until recently that it was rediscovered as a versatile tree with a variety of potential uses (Mishra *et al*., 2021). The leaves of the plant are utilized as a nutritional supplement and considered to boost the immune system (Siddhuraju and Becker, 2003). Reports have shown that *Moringa oleifera* leaves possess various pharmacological properties such as antiatherosclerosis, anti-inflammatory, antihypertensive, and antioxidant effects (Verma *et al.,* 2009). *Moringa oleifera* is also known to have various medicinal benefits and these have been attributed to its phytochemicals such as phenolic compounds (Vongsak *et al.,* 2013). Reports have revealed that phenolic contents of medicinal plants are related to their antioxidant capacity (Chu *et al*., 2002). Antioxidant activity plays an important role in the protection against cell oxidation (Sreeramulu *et al.,* 2013). The *Moringa oleifera* leaf extract has been shown to preserve sexual function under stressful conditions due to its antioxidant property (Prabsattroo *et al.,* 2015) and might have beneficial effects on male sexual dysfunction induced by stress (Sreelatha and Padma, 2009). *Moringa oleifera* extracts from both mature and tender leaves have high antioxidant activity against free radicals, prevent oxidative damage to major biomolecules, and provide significant antioxidant protection (AbdullRazis *et al.,* 2014).

Previous studies have shown the deleterious impact of crude oil-contaminated water ingestion on the contractile function and activity of the corpus cavernosum in male Wistar rats and the uterine tissues of gravid female Wistar rats (Salami *et al*., 2023a; Salami *et al*., 2023b). However, the understanding of the influence of antioxidant supplementation provided by the ingestion of *Moringa oleifera* extract on the contractile mechanisms has not been investigated. This study investigates the outcomes of crude oil-contaminated water and *Moringa oleifera* extract ingestion on the contractile functions of the corpus cavernosum of male Wistar rats.

MATERIALS AND METHODS

Plant preparation and extraction of plant: Mature *Moringa oleifera* leaves were collected from the local areas in Lagos, Nigeria, between September and December 2022. The *Moringa* plant and leaves were appropriately identified at the Department of Botany, Lagos State University. The air-dried leaves were extracted in a 70% hydro-ethanolic solution (Vongsak et al., 2013) using the maceration technique for 72 hours at ambient temperature with intermittent shaking. The extract was then filtered using Whatman filter paper. The yield from the extraction was 17.2% after drying in the oven.

Gas chromatography- mass spectrometry (GC-MS) of the Moringa oleifera extract: An Agilent 7820a gas chromatograph that was coupled to a 5975c inert mass spectrometer was used for the GC-MS analysis of the *Moringa oleifera* extract. Helium gas was used as the carrier at a constant flow rate of 1.4871 ml/min. A measure of 1µl of the samples was injected in spitless mode at an injection temperature of 300°C. The run time was 32.7 min with a 5 min solvent delay.

Experimental animals: Twenty male Wistar rats were used in the study, and the animals were kept in a standard condition (12-hour light–dark cycle at 18 - 26◦C and relative humidity of (30 % -70 %). The standard procedure (NIH guide) for the safety and use of laboratory animals was adhered to throughout the study. The procedures for animal use were also certified by the Lagos State University College of Medicine Animal Ethics Committee, Ref. No: AREC/2022/058.

Experimental design and treatment: The rats were randomly divided into four groups of five rats in each group. Rats in Group 1 (the control) were fed with normal rat chow and water only. Group 2 rats were treated with 2.5 ml of crude oil-contaminated water (CCW) obtained from the Ogbia Community in Bayelsa, Nigeria. Group 3 rats were treated with 2.5 ml of crude oil-contaminated water and *Moringa oleifera* (250 mg/kg body weight) (CCW+MO). Rats in group 4 received only *Moringa oleifera* (250 mg/kg body weight). All treatments were administered via oral gavage for 6 weeks. The period of withdrawal of the CCW, analysis of its constituents, and the precautions taken in its administration were as previously reported (Salami *et al*., 2023c).

Drugs and chemicals: Chemicals used for the preparation of physiological salt solution (PSS) include calcium chloride, glucose, magnesium sulphate, potassium dihydrogen phosphate, potassium carbonate, sodium bicarbonate, and sodium chloride (purchased from Lazco Chemical, Lagos). Drugs used for the dose response of the tissues include potassium chloride (KCl), acetylcholine (ACh), nifedipine, methyl blue, barium chloride, glibenclamide, sodium nitroprusside (SNP), $CaCl_2$ and phenylephrine (purchased from Tocris, UK). All chemicals and drugs used were of the highest analytical grade.

The preparation of the corpus cavernosum for homogenization and for contractile function experiments: At the end of the experiment, the rats were fasted overnight and sacrificed using cervical dislocation. The testis, epididymis, and corpus cavernosum were excised, and the weights were determined. The penis was surgically removed completely and carefully placed in a petri dish containing a physiological salt solution (PSS). The corpus cavernosa strip was prepared following the procedure described by Salami *et al*., (2018). A section of the cavernosa tissue was stored at -20^0 C for homogenization, while the other was attached to a hook in the organ chamber on one end and a force isometric transducer on the other (model 7004; Ugo Basile Varese, Italy). The isometric transducer was connected to a Data capsule acquisition system for recording isometric contractions.

Serum sample collection: Blood samples were collected from the heart with a 5 ml syringe and needle into plain sample bottles. The samples were centrifuged at 3000 rpm for about 15 mins. The serum was aspirated and immediately refrigerated at -4˚C for the testosterone assay.

Assay of cavernosa tissue superoxide dismutase (SOD), catalase, malondialdehyde (MDA), phosphodiesterase 5, and serum testosterone: The corpus cavernosum tissue was homogenized using a laboratory mortar and pestle in a cold phosphate buffer solution (pH 7.2). The homogenate was then centrifuged at 2500 rpm for 20 minutes. The supernatant was carefully removed to assay for SOD, catalase, MDA, and PDE 5. The assay of SOD was as described by Marklund and Marklund, (1974). It is based on the autooxidation of pyrogallol in a basic medium. One SOD activity was defined as the amount of SOD producing 50 % of inhibition of the autooxidation of pyrogallol. The VIS spectrophotometer (model 720 China) was used, and the absorbance was read at a wavelength of 420 nm. Catalase activity was determined following the method of Sinha, (1972). It was assayed colorimetrically. The reaction mixture contains 1.0 ml of 0.01 M phosphate buffer, 0.1 ml of tissue homogenate, and 0.4 ml of 2M H_20_2. Malondialdehyde an index of lipid peroxidation, was determined using the method of Buege and Aust, (1978). About 1.0 ml of the tissue homogenate was added to 2 ml of TCA-TBA-HCL reagent (trichloroacetic acid (15 %), thiobarbituric acid (0.37 %), & 0.24 N hydrochloric acid). This was boiled at 100^0 C for 15 minutes and allowed to cool. MDA was calculated from the molar extinction coefficient. The level of PDE 5 was determined using the PDE-Glo phosphodiesterase assay kit (Promega Corp., Thailand) following the kit instructions. Finally, serum testosterone was determined using the testosterone ELISA Kit (Accu-Bind ELISA, Microbind Inc., Lake Forest, USA).

Contractile functions experiments on the excised corpus cavernosum. Cumulative responses to phenylephrine, potassium chloride and calcium chloride: The tissue was allowed to equilibrate in the physiological solution for 90 minutes. The corpus cavernosum tissue was contracted with phenylephrine (10^{-9} – 10^{-5} M). Response to potassium was determined by adding potassium chloride (10–60 mM), progressively, to the potassium-free solution in the organ chamber, while response to calcium was determined by adding calcium chloride (10–60 mM) progressively, to the calcium-free solution in the organ chamber. The addition of calcium and potassium chloride to calcium- and potassium-free solution was conducted to assess the effect of calcium influx through voltage-dependent calcium channels and the inhibitory effect on receptor-operated calcium channels, respectively.

Contractile responses of corpus cavernous strips to cumulative doses of acetylcholine (ACH) and sodium nitroprusside (SNP): The tissue was contracted with phenylephrine (10^{-7} M), and the dose–response of the tissue to acetylcholine (10^{-9} – 10^{-5} M) and SNP (10^{-9} – 10^{-5} M) were then determined, respectively. Acetylcholine and SNP were used to investigate corpus cavernosa relaxation activity mediated through NO-dependent pathways across all treatment groups.

Contractile activity experiments on corpus cavernosum strips after pre-incubation: The activity of guanylate cyclase was investigated in the cavernosa strips from each group by incubating the cavernosa strips in methylene blue (10^{-4} M) for 15 minutes before adding cumulative doses of acetylcholine (10^{-9} - 10^{-5} M) and recording the contractile activity. In each group, the activity of the ATP-sensitive potassium (K^{ATP}) channel was investigated by incubating the

cavernosa tissues for 15 minutes in glibenclamide. The contractile activity at cumulative doses of acetylcholine (10^{-9} - 10^{-5} M) was then recorded. The role of K-channel inward rectifiers in the cavernosa strips of each group was studied by incubating the cavernosa tissues in barium chloride (10^{-4} M) for 15 minutes. Then, cumulative dosages of acetylcholine (10^{-9} - 10^{-5} M) were administered, and the contractile activity was recorded. The activity of voltage-gated large-conductance calcium channels was evaluated in each group by incubating the cavernosa tissue with nifedipine (10^{-4} M) for 15 minutes. The contractile activity at cumulative doses of acetylcholine (10^{-9} - 10^{-5} M) was then recorded. To ensure control, responses were allowed to stabilize before the addition of further doses. In addition, the tissues were washed three times before the use of a fresh drug

Statistical analysis and data representation: All the data were expressed as the mean ± SEM. The data were analyzed using Prism Graph Pad (version 8) statistical software. One-way and two-way analyses of variance (ANOVA) were carried out using Turkey's multiple comparisons. The statistical difference was taken at $P < 0.05$.

RESULTS AND DISCUSSION

Gas chromatography and mass spectrometry (GC-MS) analysis of Moringa oleifera leaf extract: Table 1 shows twenty-five (25) compounds that were identified in the *Moringa oleifera* extract through gas chromatography and mass spectrometry analysis. Methanamine, N-methoxy- and p-Dioxane-2,3-diol constitute 66.91% and 6.13% respectively but with low quality (5 % and 47 % respectively). Compounds with high quality in the extract include Hexadecanoic acid (99 %), methyl stearate (96 %), phenol (94 %), octadecanoic acid (97 %) and cyclononasiloxane (91 %).

Table 1: Gas Chromatography-Mass Spectrometry study showing the compound name, retention time, percentage of total area and quality of each bio-constituent found in *Moringa oleifera* leaf extract

S/N	% of Total Area	Retention time (min)	Compound Name	Quality
1	0.76	4.326	Cyclopentasiloxane, decamethyl-	70
2	0.55	4.855	Cyanogen chloride	5
3	66.91	5.193	Methanamine, N-methoxy-	5
4	6.13	5.521	p-Dioxane-2,3-diol	47
5	0.61	6.087	Cyclohexasiloxane, dodecamethyl-	91
6	0.51	6.607	Benzyl alcohol, TMS derivative	42
7	1.13	7.616	Cycloheptasiloxane, tetradecamethyl	91
8	0.99	9.014	2,5 Dihydroxybenzoic acid, 3TMS derivative	37
9	0.85	10.240	Cyclononasiloxane, octadecamethyl-	70
10	0.97	11.326	Trisiloxane, 1,1,1,5,5,5-hexamethy l-3,3-bis[(trimethylsilyl)oxy]-	53
11	0.70	11.421	Methenamine	91
12	1.54	11.796	Phenol	94
13	1.15	12.320	p-Cresol	97
14	1.05	12.344	Cyclononasiloxane, octadecamethyl-	59
15	5.42	13.168	Hexadecanoic acid, methyl ester	99
16	1.24	13.295	2,5-Dihydroxybenzoic acid, 3TMS derivative	46
17	0.98	13.389	Hexadecanoic acid, ethyl ester	98
18	1.48	14.184	Cyclononasiloxane, octadecamethyl-	87
19	0.35	14.291	Benzofuran, 2,3-dihydro-	55
20	0.66	14.427	Methyl stearate	96
21	1.24	14.569	12-Octadecenoic acid, methyl ester	97
22	1.65	15.045	Cyclononasiloxane, octadecamethyl-	53
23	0.92	15.308	(9Z,12Z,15Z)-2,3-Dimethoxypropyl octadeca-9,12,15-trienoate	86
24	0.90	15.533	9,12,15-Octadecatrienoic acid, ethyl ester, (Z,Z,Z)-	93
25	1.33	16.201	Cyclononasiloxane, octadecamethyl-	38

Effect of Moringa oleifera ethanol extract treatment on the body/organ weights, cavernosa tissue oxidative biomarkers, PDE 5 and serum testosterone in Wistar rats: By the 6th week of treatments, there was a significant reduction ($p<0.05$) in the body weight in the CCW group when compared to the control, CCW+MO, and MO co-treated groups, as shown in Table 2. There were significant decreases in the relative mean weights of the testis, epididymis, and corpus cavernosum in the CCW group when compared to the CCW + MO and MO co-treated groups (Table 2). As also shown in Table 2, there was a significant decrease in serum testosterone level in the CCW group (0.30 ng/ml), while there was a significant increase in serum testosterone level in the CCW+MO group (3.87 ng/ml), and MO group (3.73 ng/ml), when compared to the CCW group. There was a significant increase in the MDA activity of the crude oil-contaminated water group when compared to the control, CCW + MO and MO groups. Table 2 further showed that there was a

statistically significant increase ($p<0.0001$) in the SOD activity in the CCW group compared to the other groups. The concentration of catalase was significantly higher ($p<0.01$) in the CCW group (7.91) compared to the control (5.18), CCW+MO (2.95) and MO group (1.97). It was observed that the concentration of PDE 5 was reduced in the CCW-only group as compared to the other groups (Table 2).

Table 2: Body and organ weight, cavernosa tissue SOD, catalase, MDA, PDE 5 and serum testosterone after ethanol extract of *Moringa oleifera* treatment

PARAMETERS/GROUPS	CONTROL	CCW	CCW+MO	MO
Body weight changes(g) after the 6th week	24.67 ± 1.45	7.80 ± 3.32*	13.80 ± 0.58	18.52 ± 1.69
Testes (g)	1.36 ± 0.05	1.25 ± 0.11*	1.62 ± 0.03	1.35 ± 0.03
Epididymis (g)	0.62 ± 0.03	0.53 ± 0.03*	0.71 ± 0.03	0.63 ± 0.03
Corpus cavernosum (g)	0.10 ± 0.004	0.08 ± 0.008*	0.12 ± 0.007	0.10 ± 0.004
MDA	2.81 ± 0.11	14.22 ± 2.27***	8.00 ± 0.19*	3.01± 0.13
SOD	3.37 ± 0.65	10.40 ± 0.65***	2.66 ± 0.19	1.82 ± 0.18
CATALASE	5.18 ± 0.18	7.91 ± 1.31**	2.95 ± 0.06	1.97 ± 0.10
PDE 5	3.79 ± 0.51	2.65 ± 0.37	3.02 ± 0.09	2.83 ± 0.12
Testosterone	1.73 ± 0.35	0.30 ± 0.06**	3.87 ± 0.58**	3.73 ± 0.58**

*N= 5. Values expressed as Mean ± SEM. CCW = Crude oil contaminated water group, CCW+MO = Crude oil contaminated water and Moringa oleifera group, MO = Moringa oleifera group. * = p< 0.05, ** = p< 0.01, *** = p< 0.001, MDA = Malondialdehyde, SOD = Superoxide dismutase. PDE5 = Phosphodiesterase 5*

Contractile response of corpus cavernosa strip to cumulative doses of phenylephrine, KCl and $CaCl_2$: It was observed that the maximum response of contraction of the corpus cavernosa to cumulative doses of phenylephrine in the CCW+MO group (0.004, 0.007, 0.009, 0,011) was significantly higher ($p<0.05$) compared to the CCW-only group (0.002, 0.004, 0.006, 0.008) (Figure 1A). As shown in Figure 1B, the contraction of the corpus cavernosa to cumulative doses of potassium chloride in the potassium-free solution was significantly higher in the CCW+MO group compared to the other groups. There was a significant increase in the contractile response of the corpus cavernosa strip to cumulative doses of $CaCl_2$ in the calcium free solution in the MO group compared to the CCW+MO group and CCW group (Figure 1C).

Relaxation response of corpus cavernosa strip to cumulative doses of acetylcholine (10^{-9} – 10^{-5} M) and sodium nitroprusside (10^{-9} – 10^{-5} M) after pre-contraction in phenylephrine (10^{-7} M): The relaxation (%) of the corpus cavernosa to cumulative doses of acetylcholine was observed to be significantly higher in the MO (33.83%, 47.47%, 56.01%, 69.82%) and the CCW+MO (23.46%, 40%, 54.42%, 57.92%) groups than in the CCW group (14.83%, 23.83%, 36.10%, 43.91%) as shown in Fig 2A. After pre-contraction in phenylephrine, cumulative doses of SNP did not result in any significant difference in relaxation (%) of the corpus cavernosa between the CCW+MO group and the CCW group, as shown in Figure 2B.

For many years, *Moringa oleifera* has been used traditionally for treatment of several ailments. The GC-MS analysis (Table 1) showed that the plants contain various chemicals similar to previous reports (Igwe *et al*., 2015; Bhalla *et al*., 2021) some of which have been reported for their important medicinal properties such as anti-inflammatory, antioxidants, and anticancer activities (Enas and Duha, 2014). There was a significant decrease in the relative body weight changes in the sixth week (Table 2) and the relative weight of the testes, epididymis, and corpus cavernosa in the CCW group compared to the other groups (Table 2). This shows that the exposure of the rats to crude oil-contaminated water decreased their body weight as a sign of a toxic effect similar to what was observed by Ogara *et al.*, (2017) in animals fed a crude oil-contaminated diet. The observed decrease in the weight of the testes, epididymis, and corpus cavernosa is also consistent with the report by Nodu and Ohimain (2014), that female rabbits fed with crude oil-contaminated forage have a decrease in ovary size and weight, heart, liver, and kidney weight, indicating the negative effect of ingesting crude oil-contaminated water. The amelioration of the reduced body and organ weights in the *Moringa* co-treated groups corroborated studies that have reported the cytoprotective and antioxidant effects of *Moringa oleifera* leaves (Adeyemi and Elebiyo, 2014; Abdel *et al*., 2020; Melebary and Elnaggar, 2023). The serum testosterone level in the CCW group was significantly reduced, as shown in Table 2. This may be due to heavy metals that have been reported in crude oil, such as lead and mercury (Ifelebuegu *et al*., 2017), which have been linked to decreased testosterone production, and these heavy metals may aggregate in the testes of rats and restrict testosterone synthesis (Adedara *et al*., 2012). However, serum testosterone was increased in the CCW+MO group. This may be attributed to the presence of saponin in the plant extract, which may increase the testosterone level in the body (Shukla and Khanuja, 2005).

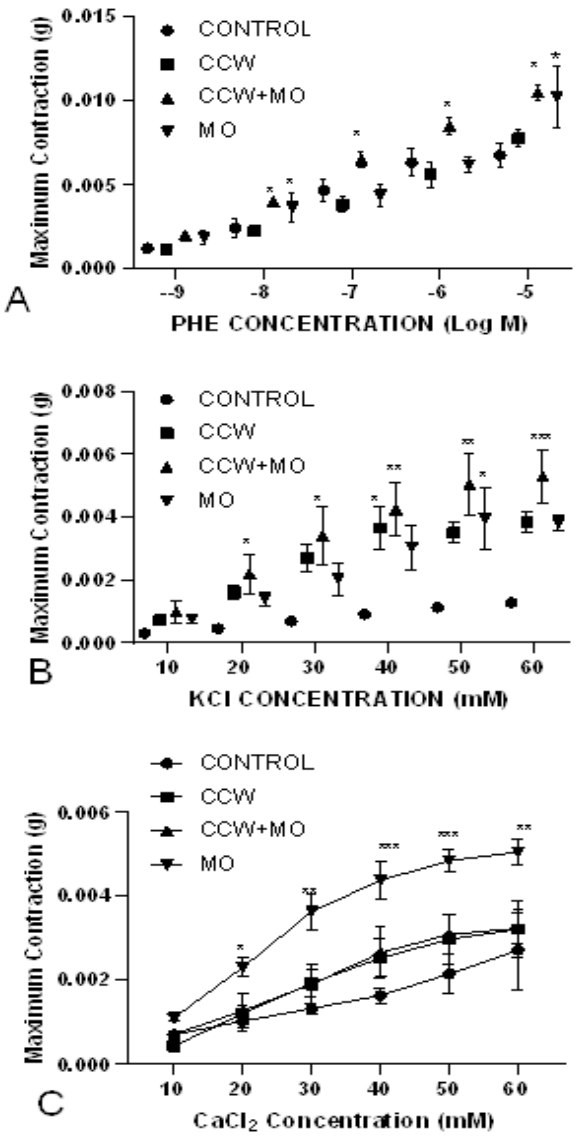

Fig 1: Contractile response of corpus cavernosa strip to cumulative dose of (A) phenylephrine (10^{-9}-10^{-5} M), (B) KCl (10 to 60 mM), (C) $CaCl_2$ (10 to 60 mM). N= 5, values expressed as Mean ± SEM. CCW = Crude oil contaminated water group, CCW+MO = Crude oil contaminated water and *Moringa oleifera* group, MO = *Moringa oleifera* group, * = p< 0.05, ** = p< 0.01, *** = p< 0.001

Moringa oleifera has been reported to improve male sexual functions and testosterone levels following exposure to different environmental contaminants such as cadmium (Siddhuraju and Becker, 2003; Ododo *et al*., 2019; Elblehi *et al*., 2019), lead (Owolabi *et al*., 2012), chemicals such as hydroxyurea (Saalu *et al*., 2011), and paroxetine (El-Sheikh *et al*., 2016). Studies have revealed that excess reactive species play an important role in causing erectile dysfunction by increasing MDA concentrations (Ho et al., 2013). As shown in Table 2, the MDA activity was significantly increased in the crude oil-contaminated water-treated group (p<0.001) when compared to the groups supplemented with *Moringa oleifera* extract. The significant increase in MDA activity in the CCW group could be due to the generation of ROS following CCW ingestion.

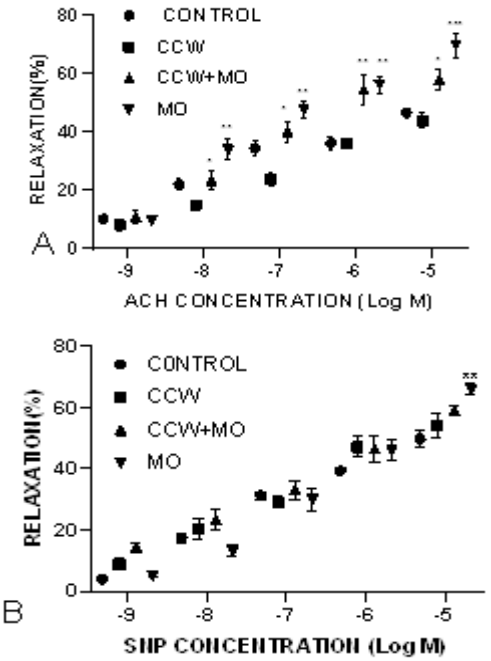

Fig 2: Relaxation response (%) of corpus cavernosa strip to cumulative doses of (A) acetylcholine (10^{-9} – 10^{-5} M), (B) sodium nitroprusside (10^{-9} – 10^{-5} M) after pre-contraction in phenylephrine (10^{-7} M). N= 5, values expressed as mean ± SEM. CCW = Crude oil contaminated water group, CCW+MO = Crude oil contaminated water and *Moringa oleifera* group, MO = *Moringa oleifera* group, * = p< 0.05, ** = p< 0.01, *** = p< 0.001

This was ameliorated in the Moringa oleifera co-treated group due to its antioxidant and free radical scavenging efficacy and properties, as reported by Achuba *et al*. (2016), Ododo *et al*. (2019), and Bhalla *et al.* (2021). There was a significant increase in SOD and catalase levels in the CCW group, as shown in Table 2. This may be due to the compensatory mechanisms of the body. Antioxidants play a role in the homeostasis of ROS. The compensatory attempt in the CCW group to equalize the continuous production of free radicals might be responsible for the increased levels of antioxidant enzyme production in the CCW group. This postulate is in agreement with the report of Sharifi-Rad *et al*., (2020). Superoxide dismutase and catalase are considered primary endogenous antioxidants that provide major antioxidant defense against ROS (Vona *et al*., 2021). This explains the elevated increase in their level in this study. The ratio of the antioxidants produced to the free radicals was, however, limited, as observed by the increased free radical levels in the CCW group.

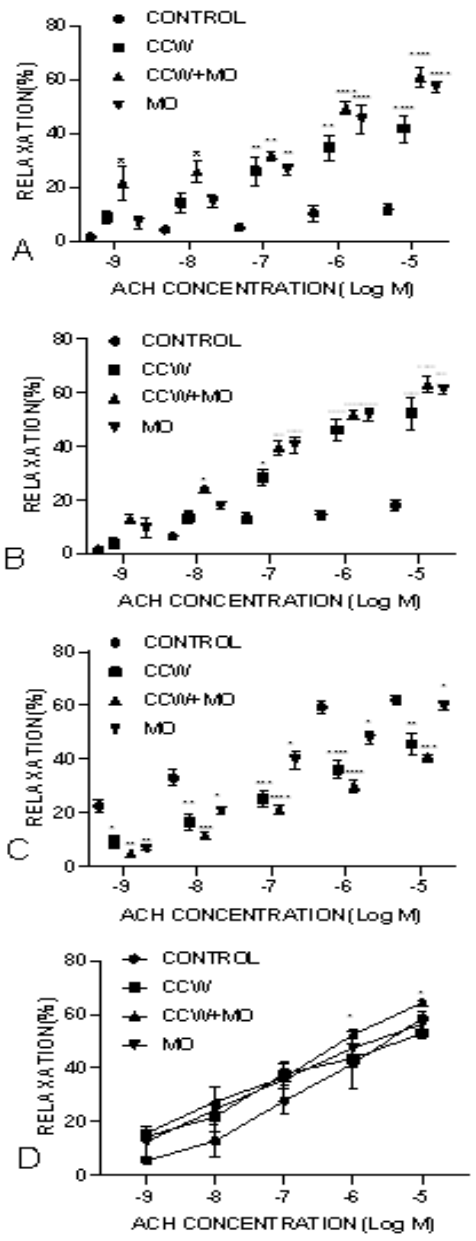

Fig 3: Relaxation response (%) of corpus cavernosa strip to cumulative dose of acetylcholine (10^{-9}- 10^{-5} M) after incubation with (A) methylene blue (10^{-4} M), (B) nifedipine (10^{-4} M), (C) Barium Chloride (10^{-4} M), and (D) glibenclamide (10^{-4} M). Value expressed as Mean ± SEM. CCW = Crude oil contaminated water group, CCW+MO = Crude oil contaminated water and *Moringa oleifera* group, MO = *Moringa oleifera* group * = p< 0.05, ** = p< 0.01, *** = p< 0.001, **** = p< 0.0001

Relaxation response of corpus cavernosa strip to cumulative dose of acetylcholine (10^{-9} -10^{-5} M) after incubation: As shown in Figure 3A, relaxation (%) of the corpus cavernosa to cumulative doses of acetylcholine after incubation with methylene blue was significantly higher in the *Moringa oleifera* co-treated group (21.41%, 25.76%, 31.75%, 49.43%, 60.95%) compared to the CCW group (8.79%, 14.33%, 25.92%, 34.71%, 41.80%). The relaxation (%) of corpus cavernosa to the cumulative dose of acetylcholine after incubation with nifedipine was higher in the CCW+MO group (12.96%, 24.46%, 39.66%, 51.68%, and 63.16%) and MO group (9.50%, 17.83%, 40.77%, 51.87%, 61.29%) compared to the CCW group (3.94%, 13.60%, 28.35%, 46.22%, and 52.43%) (Figure 3B). In addition, the relaxation (%) in corpus cavernosa to the cumulative dose of acetylcholine after incubation with barium chloride was significantly higher ($p<0.05$) in the MO group compared to the CCW+MO and CCW groups (Figure 3C). There was no significant difference in the relaxation (%) of the corpus cavernosa to cumulative doses of acetylcholine after incubation with glibenclamide in the CCW+MO (15.68%, 27.49%, 52.42%, 64.39%) group compared to the CCW group (14.45%, 21.89%, 43.63%, 52.91%) at doses 10^{-9} -10^{-7} M. However, there was a significant difference ($p<0.05$) at doses 10^{-6} M and 10^{-5} M (Figure 3D).

Phosphodiesterase 5 is expressed in a variety of tissues, particularly in the penile corpus cavernosum (Calogero *et al*., 2018). It is observed in this study that the level of PDE 5 is lower ($P > 0.05$) in the CCW compared to other groups (Table 2). PDE 5 can be regulated at protein and gene levels. In the penis, changes in PDE 5 activity have been linked to its phosphorylation status, and downregulation of PDE 5 expression has been associated with hypoxia (Lin, 2009). Hypoxia caused by the constituents of the CCW can be suggested to be responsible for the low level of PDE 5, which was ameliorated by *Moringa oleifera*; further confirming the antioxidant benefits of the extract. The contractile response to phenylephrine, potassium chloride, and calcium chloride (Figure 1) was high in the CCW+MO group and MO group compared to the CCW group.

The decrease in contractile response to PHE and $CaCl_2$ in the CCW group can be caused by CCW-induced impaired contractile activity of the corpus cavernosa, similar to the observed negative effect on excitation-contraction coupling due to crude oil exposure reported by Brette *et al.*, (2014). Acetylcholine and sodium nitroprusside-mediated relaxations were enhanced in the cavernosa tissues of rats co-treated with MO compared to rats treated with crude oil-contaminated water (Figure 2). This suggests the direct release of nitric oxide from the corpus cavernosa, and the generation of a nitric oxide free radical that activates the cytosolic (soluble) isoenzyme form of guanylate cyclase. This dual mechanism was reported in mesenteric artery relaxation (Aekthammarat *et al*., 2020a) and in *Moringa oleifera*-induced endothelium-derived NO release causing

vasorelaxation (Aekthammarat *et al*., 2020b). A similar conclusion was reported by Salami et al., (2018) with an aqueous extract of *Tridax procumbens*. Guanylate cyclase converts guanosine triphosphate (GTP) to cyclic guanosine monophosphate (cGMP), and cGMP plays a role in the relaxation of corpus cavernosum (Andersson, 2001). The relaxation of corpus cavernosa tissue to cumulative doses of acetylcholine after incubation in methylene blue (a guanylate cyclase inhibitor) was reduced in the CCW group as compared to the Moringa co-treated groups. This suggests that methylene blue inhibitor activity is exacerbated by crude oil-contaminated water exposure. It has been reported that crude oil-derived chemicals can activate or inhibit the estrogen, androgen, glucocorticoid, progesterone, and thyroid receptors (Kassotis *et al.*, 2016). Our current study suggests inhibition of cholinergic receptors by the CCW. Nifedipine is considered to selectively block calcium entry through L-type calcium channels (Khan *et al.*, 2023). The relaxation of corpus cavernosa to cumulative doses of acetylcholine after incubation in nifedipine (Figure 3) was significantly higher in the CCW+MO group and MO group when compared to the CCW group.

This shows that the relaxant effect of MO was not inhibited by the activity of the L-type calcium channel blocker and suggests that MO might be inhibiting the mobilization of sarcolemma Ca^{2+} via inositol trisphosphate receptor Ca^{2+} channels, similar to reports by Aekthammarat *et al.*, (2020a) rather than just the L-type calcium channels. There was no significant difference between the CCW and CCW+MO groups in the relaxation of corpus cavernosa to cumulative doses of acetylcholine after incubation with barium chloride, a potassium channel inward rectifier blocker, and glibenclamide, an ATP-sensitive potassium channel antagonist.

However, at higher doses (though not significant), the CCW+MO showed a higher relaxation response in the gibenclamide-inhibited experiment. The crude oil-contaminated water might be responsible for the low relaxation response observed in the CCW group to ACH in the presence of the various blockers.

Conclusion: Moringa oleifera exhibited cytoprotective action on the testes, epididymis, and corpus cavernosum through its free radical scavenging effect during the ingestion of crude oil-contaminated water. It also improved testosterone levels in the Moringa oleifera-treated groups. This, we suggest, is responsible for the improved erectile activity and the maintenance of the phosphodiesterase 5 level in the corpus cavernosum in this study.

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