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Omega-3 fatty acids prevented codeine-induced kidney damage by increasing antioxidant activity, reducing inflammation, and inhibiting apoptosis.

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Abstract

Background: The abuse of drugs including codeine has been increasingly documented among women. Furthermore, the misuse of codeine has been associated with deleterious effect on organ including the kidney.Omega-3 fatty acid has shown antioxidant function and benefits for preventing organ damage, nevertheless, there are limited studies to show its effect on codeine-induced renal dysfunction on female wistar rats.
Objectives: The current study therefore investigated the effect of omega-3 fatty acid on codeine induced renal dysfunction in female wistar rats.
Materials and methods: twenty female Wistar rats ranging between 160- 180 g were randomized into four groups (n=5/group): Group 1 [Control (0.5 mL)]; Group 2 [Codeine (10 mg/kg BW)]; Group 3 [Omega-3 fatty acid(300 mg/kg BW)] and Group 4 [Codeine (10 mg/kg BW) and Omega-3 fatty acid (300 mg/kg BW)] respectively. Administrations (via oral) was done for eight weeks (56 days).
Results: Data reveals that codeine administration resulted in significant increase of urea, creatinine, serum electrolytes(Na^+,HCO_3^-), oxidative stress, injury markers, kidney indices, inflammatory markers, apoptotic markers thereby causing decrease in renal blood flow, increase hydrostatic pressure, hypertension, metabolic alkalosis and acidosis, edema, diabetes, mitochondria dysfunction and a significant reduction in K^+, antioxidant activities and the

relative body weight gain causing cases such as hypokalemia, thereby leading to structural and functional disruption of the kidney tissues of female rats while enhancing kidney injury, potentially through oxidative stress, inflammation, apoptotic mechanisms. However, co-administration of Omega 3 fatty acids with codeine demonstrated a protective effect against codeine-induced renal dysfunctions by upregulating antioxidant activities and reducing inflammation and apoptosis mechanisms.
Conclusions: Thus, this present study demonstrates the effect of omega-3 fatty acids on codeine- induced renal damage in female wistar rats.
Keywords: Renal injury, Codeine, Omega-3 fatty acid, Oxidative stress, Apoptosis.

INTRODUCTION

Drug abuse among adolescents and adults is an increasing public health challenge globally as recreational drug intake had been associated with a range of medical consequences (1). These abusive drugs and their metabolites are excreted through the kidneys and are thus associated with so many reported cases of renal complications ranging from glomerular, interstitial and vascular diseases which ultimately results to acute or chronic renal failure. Over the years, codeine had been reported to be among the leading drugs commonly abused by both genders and belongs to a class of drugs known as opioids (2, 3). Medically, it is used as an analgesic, antitussive and anti-diarrheal (2, 3). However, it had been reported to caused detrimental health issues such as testicular dysfunction (4), hepatic impairment (5), cardiac and renal injury (5,6).

Opioids drugs use have been associated with reduction of the glucose level in plasma and blood pressure (7). Opioids abuse specifically affect the glomeruli, causing severe impairment to renal functions. Kidneys are especially vulnerable to the toxic effects of these substances, as they are involved in filtration, concentration and metabolism of the potentially toxic substances (8). Distinct clinical manifestations known to precede acute and chronic kidney injury have been reported for different drugs of abuse (9). The involvements of increased oxidative stress, inflammation, mitochondria-mediated induction of apoptosis, or activation of the renin-angiotensin system contribute to kidney cell damage (9). Alterations induced directly or indirectly by drugs of abuse and/or their metabolites may result in distinct clinical manifestations which may lead to acute tubular necrosis, the main cause of acute kidney injury (8, 9). Codeine has been reported to cause the formation of crystals that obstruct the kidney tubules (9).

Omega-3 fatty acids are polyunsaturated fatty acids (PUFAs) (10), particularly eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), have been recognized for their potential health benefits, including anti-inflammatory and cardioprotective properties (11, 12). Several studies have reported the protective effects of omega-3 fatty acid against various cardiovascular and renal conditions (13, 14) as its supplementation has been demonstrated in its ability to attenuate oxidative stress markers, reduce inflammation, improve endothelial function, regulate blood pressure, and enhance renal blood flow (15). Studies have shown that Omega-3 fatty acid can modulate multiple signaling pathways including the nuclear factor-kappa B (NF-κB) and mitogen-activated protein kinase (MAPK) pathways, leading to regulation of lipid metabolism, preservation of endothelial function, reduction in pro-inflammatory cytokines and oxidative stress markers (16). These molecular mechanisms contributed to the overall improvement in cardiovascular and renal function (17). Omega-3 fatty acids play a crucial role in maintaining the normal structure and function of nephron cells and it is a beneficial supplement in chronic

kidney diseases management (18). While the adverse effect of codeine on renal function has been established, reports have shown that there is scarcity of data in studies evaluating the protective effect of omega-3 fatty acid on codeine induced renal injury on female Wistar rat, hence this study.

MATERIALS AND METHOD

The study was carried out at the Animal house, Department of Physiology, College of Medicine, Ladoke Akintola University of Technology, Ogbomoso, Nigeria (Latitude N8°, 10 and Longitude E4°, 16). Codeine was kindly given by the National Drug Law Enforcement Agency (NDLEA) with (Ref Number: NDLEA/OSSC/13/Vol.111/78) while Omega-3 fatty acid fatty acids was obtained from a standard pharmaceutical company. This study was carried out according to the National Institutes of Health Guide for Care and Use of Laboratory Animals. Approval for this study was sought from the Ethics Review Committee of the Faculty of Basic Medical Sciences, LAUTECH, Ogbomoso, Oyo state with reference number ERCFBMSLAUTECH: 026/03/2024.

Animals

Twenty (20) female Wistar rats weighing between 160–180 g with age range of 10 – 12 weeks was purchased and used for the study. The animals were housed in the animal holding of the Department of Physiology, Ladoke Akintola University of Technology, Ogbomoso, at standard ambient temperature. These animals were kept under natural conditions of humidity, accommodated in dry, aerated plastic cages (5 rats per group) under standard laboratory conditions and acclimatized for two (2) weeks while they received adequate care with constant changing of beddings. All the animals had unrestricted access to standard rat pellets and water *ad libitum*.

Experimental Procedure

After acclimatization, the rats were weighed, recorded as the initial body weight and then randomly allotted into four groups (n = 5) and received treatment orally, once daily for fifty-six (56) days using oro-pharyngeal cannula as follows. CTRL (0.5 ml normal saline), CO (10 $mg/kg\ BW$ of codeine), Ω3 (400 $mg/kg\ BW$ of Omega-3); CO + Ω3 (Codeine 10 $mg/kg\ BW$ and Omega-3 (400 $mg/kg\ BW$) respectively. The codeine dose and Omega-3 was as reported (19).

Organ Collections

Twenty-four hours after last administration, the animals were weight and recorded as the final body weight, then anaesthetized via intraperitoneal ketamine (40 mg/kg) injection and cut open. Blood sample was collected via cardiac puncture into plain bottles and centrifuged at 3000 rpm for 5 minutes to obtain serum for urea, creatinine, and electrolytes (Na^+, K^+, Cl^-, HCO_3^-) estimation. The left and right kidneys were harvested, weighed using a sensitive digital weighing scale, and recorded. The percentage change in body weight was calculated using the following expression:

$$\text{Percentage Weight Change} = \frac{\text{Final Body weight (g)} - \text{Initial Body Weight (g)}}{Final\ Body\ Weight\ (g)} \times 100$$

The relative paired kidney weight was expressed as

$$\text{Relative paired kidney weight} = \frac{sum\ of\ both\ kidneys\ (g)}{Final\ Body\ Weight\ (g)} \times 100.$$

The left kidney was homogenized in Phosphate Buffer Saline (PBS: 1:5 w/w; PH 7.4) and centrifuged at 4000 revolutions for 5minutes to obtained homogenate used for biochemical assays (MDA, SOD, Catalase, NO, MPO, Pyruvate, Lactate, caspase 3) (20, 21). The right kidney was used for the histological examination using the Hematoxylin and Eosin stain (22).

Estimation of biochemical parameters

The serum urea, creatinine, and electrolytes (Na^+, K^+, Cl^-, HCO_3^-) concentration were estimated using assay kit purchased from

Randox Laboratories Ltd., United Kingdom.

Kidney tissue malondialdehyde (MDA) level was determined using the kidney homogenate assay (23-25).

The activities of SOD enzymes in the kidney tissues were assayed as previously reported (19,). Also, the catalase activity of kidney tissues was assayed as previously reported (21).

The nitric oxide (NO) concentration in the kidney homogenate was determined using Griess Reaction as previously documented (26), while the myeloperoxidase (MPO) activity was determined as previously reported (27).

RESULTS

Body and Organ Weights

Figure 1 shows the effect of Omega-3 fatty acid on the relative body weight gain of codeine exposed female rats. There is a significant reduction ($p < 0.05$) in the relative body weight gain in the rats treated with CO, omega-3 fatty acid, and the codeine and omega-3 fatty acid treated rats compared to the Normal saline and olive oil treated rats while Figure 2 shows no significant change in the relative paired kidney weight of the treated rats

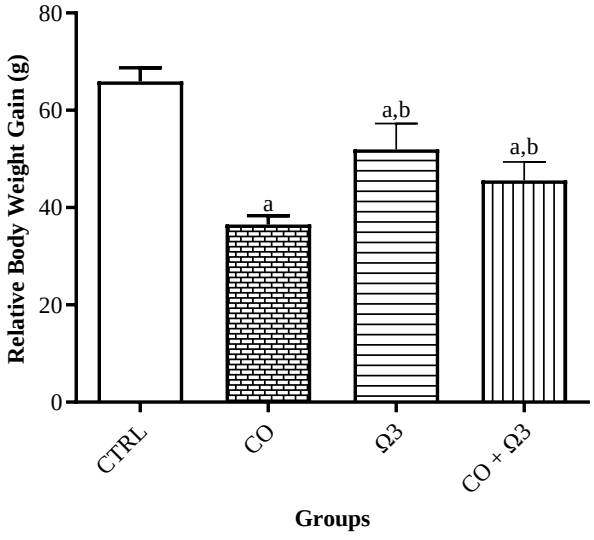

Figure 1: Effect of omega-3 fatty acid on the relative body weight of codeine exposed female rats *bars carrying a = significantly different from CTRL, b = significantly different from CO.*

The concentration of lactate in the kidney tissue homogenate samples was done using a ELISA assay kit according to the manufacturers guideline (Mornmed; Lot: MB-2866A). The concentration of pyruvate in the kidney tissue homogenate was measured using a colorimetric assay kit (Mornmed; Lot: MB-5759A).

Statistical analysis

Data was analysed using GraphPad Prism (Version 10). A one-way Analysis of variance (ANOVA) followed by Tukey's post hoc test for pairwise comparison was conducted. Data was expressed as Mean ± SD. A P value < 0.05 was considered statistically significant.

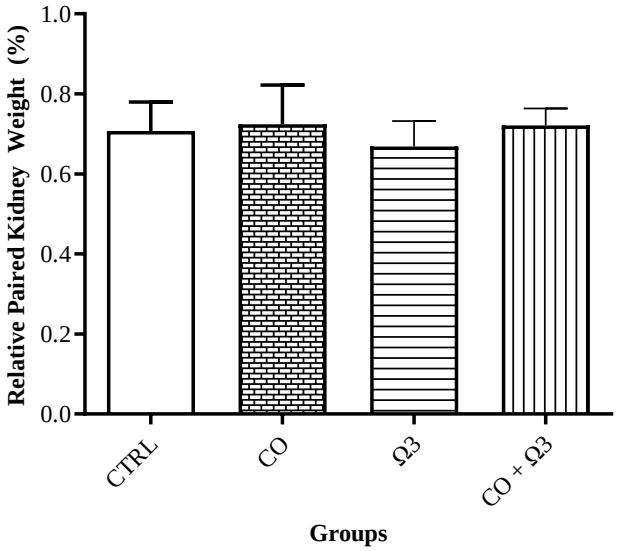

Figure 2: Effect of omega-3 fatty acids on the relative paired kidney weight of codeine exposed female rats.

Kidney Function Tests

Exposure of codeine in the rats caused a significant increase ($p < 0.0001$) in the concentration of urea and creatinine compared with the other treatment groups (Table 1). However, omega 3 fatty acid supplementation causes a significant decrease ($p < 0.05$) in serum creatinine and urea when compared to the codeine treated group (Table 1)

The serum electrolytes such as (Na^+, HCO_3^-) concentration of the codeine

treated rats are significantly ($p < 0.05$) increase compared to all other treatment groups while the result of (K^+, Cl^-) shows a significant ($p < 0.05$) decrease when compared to all other treatment groups (Table 1). However omega-3 fatty acid administration causes a significant ($p < 0.05$) decrease (Na^+,HCO_3^-) and increase (K^+, Cl^-) when compared to all other treated group (Table 1

Table 1: Effects of Omega-3 fatty acid on the Serum Urea, Creatinine and Electrolytes Concentration of Codeine Treated Rats

	CTRL	**CO**	**Ω3**	**CO + Ω3**
Urea (mg/dl)	39.71 ± 1.112	$53.56 \pm 1.550^{a,b}$	39.67 ± 0.899^{c}	39.62 ± 0.423^{c}
Creatinine (mg/dl)	0.790 ± 0.465	$4.890 \pm 0.109^{a,b}$	1.128 ± 0.489^{c}	0.620 ± 0.465^{c}
Na^+ (mEq/L)	149.5± 5.160	$205.6 \pm 5.477^{a,b}$	145.8 ± 9.471^{c}	149.0 ± 4.733^{c}
K^+ (mEq/L)	1.296 ± 0.070	1.070 ± 0.002^{ab}	1.330 ± 0.073^{c}	1.466 ± 0.090^{c}
Cl^- (mEq/L)	78.44 ± 7.821	$101.0 \pm 2.613^{a,b}$	71.08 ± 7.586^{c}	73.92 ± 4.017^{c}
HCO_3^- (mmol/L)	30.49 ± 1.323	$40.40 \pm 3.976^{a,b}$	31.05 ± 0.361^{c}	31.88 ± 1.112^{c}
Ca^{2+} (mmol/L)	2.415 ± 0.08	$2.692 \pm 0.043^{a,b}$	1.673 ± 0.144^{ac}	2.487 ± 0.02^{bcd}

Values are means of five replicates measurements ± SD; bars carrying: a = significantly (P < 0.05) when compared to CTRL; b = significantly (P < 0.05) when compared to CO; c = significantly (P < 0.05) when compared to Ω3.

Markers of Oxidative Stress

Codeine significantly ($p < 0.05$) increased the MDA and 8-OHdG in the kidney tissues when compared to all the other treatment groups in Figure 3 and 4 respectively. However, omega-3 fatty acid supplementation helps to reduce oxidative stress thereby causing repair of cell damages and lowering of the risk of kidney disease. Administration of Codeine only to female rats significantly ($p < 0.05$) reduced the activities of the kidney tissues superoxide-dismutase (SOD), catalase (CAT) and reduced glutathione when compared to the normal saline, Omega-3 fatty acid and the codeine and omega-3 fatty acid co-administered groups (Figure 5 ,6 and 7 respectively). The co-administration of Omega-3 with codeine significantly ($p < 0.05$) increased the activities of the kidney SOD when compared to the Codeine only treated rats (Figure 5).

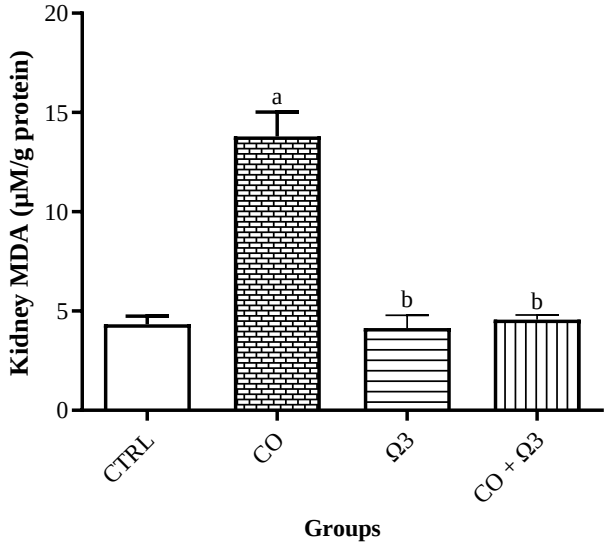

Figure 3: Effect Omega-3 fatty acid on the kidney tissues MDA concentration in codeine exposed female rats. *a = significantly (P < 0.05) when compared to CTRL; c = significantly (P < 0.05) when compared to CO.*

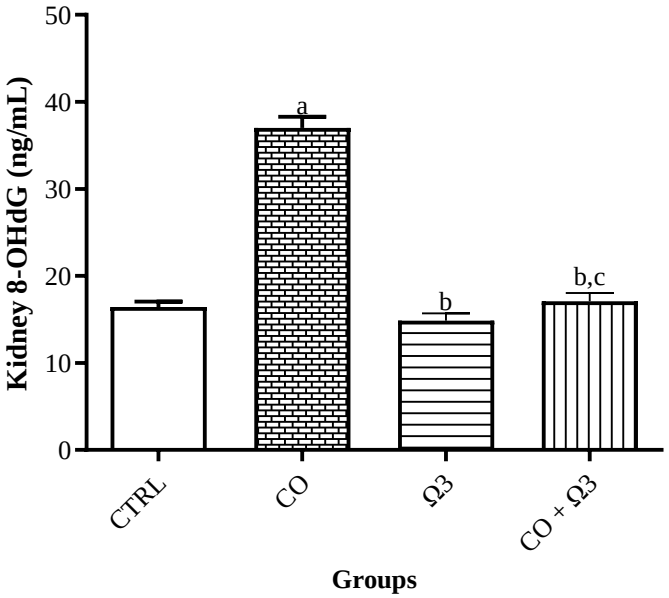

Figure 4: Effect Omega-3 fatty acid on the kidney tissues 8-OHdG level in codeine exposed female rats. *a = significantly (P < 0.05) when compared to CTRL; b = significantly (P < 0.05) when compared to CO; c = significantly (P < 0.05) when compared to Ω3.*

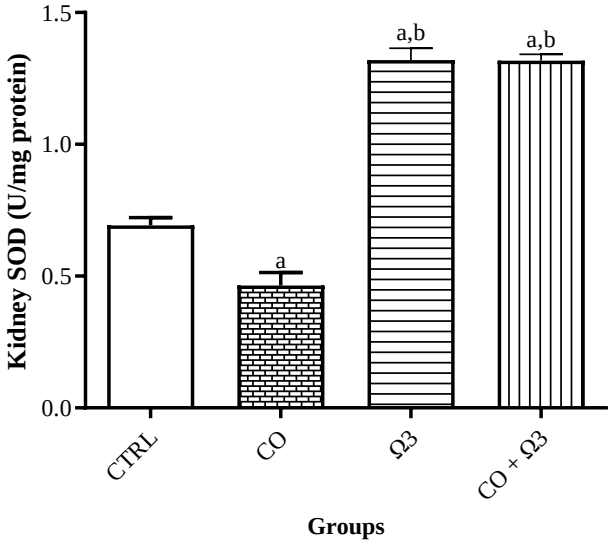

Figure 5: Effect of omega-3 fatty acid on the kidney tissues Super-Oxide Dismutase (SOD) activities in codeine exposed female rats.
Values are means of five replicates measurements ± SD; bars carrying: a = significantly (P < 0.05) when compared to CTRL; b = significantly (P < 0.05) when compared to CO.

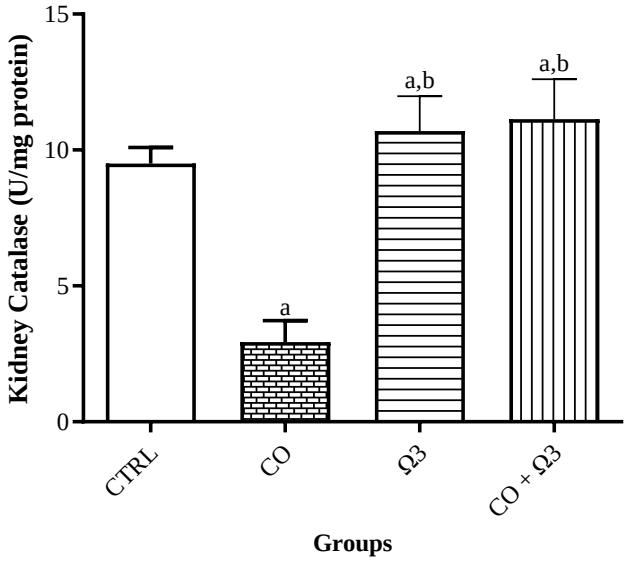

Figure 6: Effect of omega-3 fatty acid on the Kidney tissues Catalase (CAT) activities in codeine exposed female rats.

a = significantly (P < 0.05) when compared to CTRL; b = significantly (P < 0.05) when compared to CO.

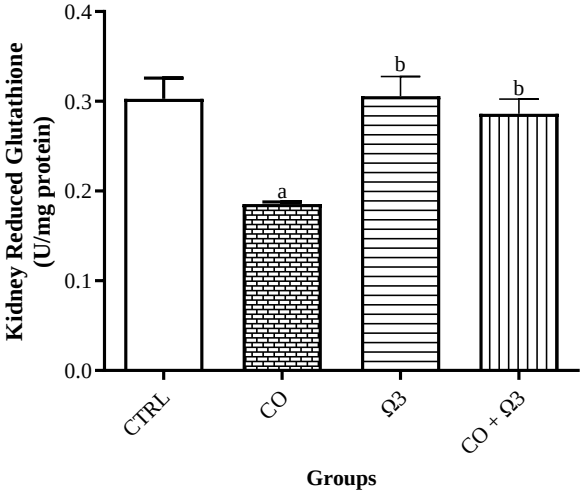

Figure 7: Effect of omega-3 fatty acid on the activities of the Reduced Glutathione (GSH) in the kidney tissues of codeine exposed female rats.
a = significantly (P < 0.05) when compared to CTRL; b = significantly (P < 0.05) when compared to CO.

Markers of Inflammation

The administration of codeine to the female rats significantly (P < 0.05) increased the activities of the kidney tissues myeloperoxidase enzymes when compared with all of the other treatment groups (Figure 8). Meanwhile omega-3 fatty acid helps in the reduction of MPO thereby reducing inflammation and improving renal function. Codeine administration to the female rats significantly (P < 0.05) increased the concentration of the kidney tissues nitric oxide (NO) when compared to the Normal saline, Omega-3 Treated groups (Figure 9). However, the treatment of Codeine exposed rats with Omega-3 did not significantly reduce the level of the Nitric oxide when compared to the codeine only treated rats (Figure 9).

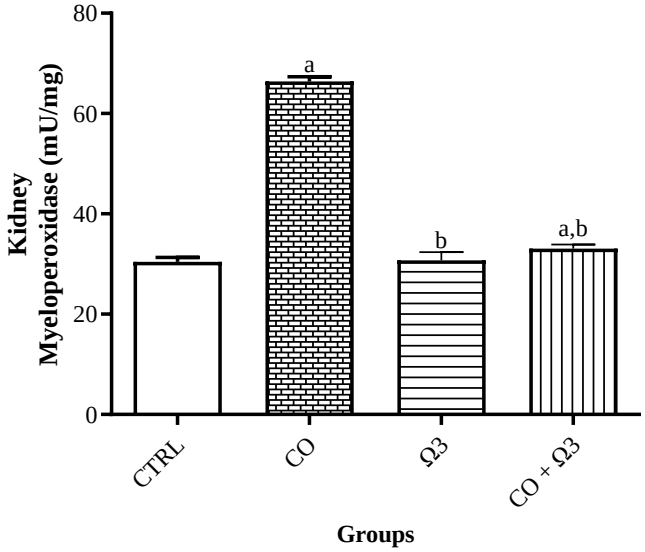

Figure 8: Effect of Omega-3 fatty acid on the myeloperoxidase (MPO) activities in the kidney tissues of codeine exposed female rats. *a = significantly (P < 0.05) when compared to CTRL; b = significantly (P < 0.05) when compared to CO.*

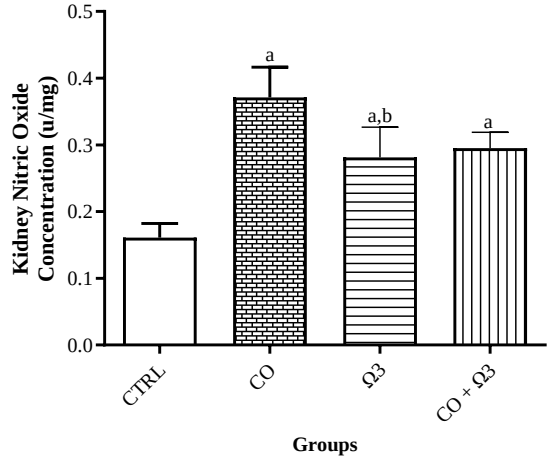

Figure 9: Effect of Omega-3 fatty acid on the Kidney Nitric Oxide Level in codeine exposed female rats. *a = significantly (P < 0.05) when compared to CTRL; b = significantly (P < 0.05) when compared to CO.*

Kidney Injury Markers and Damage Indices

Codeine administration significantly ($p < 0.05$) increased the kidney lactate, pyruvate, and the creatinine kinase concentration when compared to the normal saline, omega-3 fatty acid treated and the codeine + omega-3 fatty acid treated groups (Table 2).

Codeine treatment significantly ($p < 0.05$) reduced the ratio of the urea/creatinine when compared to all other treatment group (Table 2). The treatment of codeine exposed rats with omega-3 fatty acid significantly ($p < 0.05$) increases the urea/creatinine ratio when compared to the codeine only treated rats (Table 2). The kidney damage index of the codeine treated rats was significantly ($p < 0.05$) increased when compared to all other treatment groups (Table 2). However, the administration of Omega-3 fatty acid to the codeine exposed rats significantly ($p < 0.05$) reduced the kidney damage index when compared to the codeine treated only groups (Table 2). Meanwhile the renal risk score of the codeine exposed rats was significantly ($p < 0.05$) reduced when compared to the control and codeine + Omega-3 treated groups (Table 2).

Table 2: Effects of Omega-3 fatty acid on the Kidney Injury markers and Kidney Damage Indices of Codeine Treated Rats

	CTRL	CO	Ω3	CO + Ω3
Lactate ($mmol/L$)	1.52 ± 0.05	9.264 ± 0.33^{a}	1.429 ± 0.08^{b}	1.777 ± 0.02^{n}
Pyruvate ($\mu mol/mg$)	4.72 ± 0.53	16.46 ± 1.27^{a}	4.554 ± 0.53^{b}	6.166 ± 0.87^{b}
Lactate Dehydrogenase (u/l)	15.31 ± 0.80	28.51 ± 0.54^{a}	13.68 ± 0.761^{b}	13.89 ± 0.509^{b}
Creatinine Kinase (u/l)	4.48 ± 0.32	12.28 ± 0.31^{a}	4.118 ± 0.011^{b}	3.632 ± 0.455^{b}
Urea/Creatinine Ratio	34.42 ± 0.00	10.95 ± 0.24^{a}	29.83 ± 3.13^{ab}	35.15 ± 0.001^{bc}
Kidney Damage Index	3801 ± 111	4971 ± 373^{a}	2110 ± 144^{ab}	5898 ± 104^{bc}
Renal Risk Score	10.56 ± 0.20	9.752 ± 0.24^{a}	10.20 ± 0.523	10.59 ± 0.190^{b}

Values are means of five replicates measurements ± SD; bars carrying: a = significantly (P < 0.05) when compared to CTRL; b = significantly (P < 0.05) when compared to CO; c = significantly (P < 0.05) when compared to Ω3*.*

Apoptotic Marker
Caspase-3 Activities

The administration of codeine to the female rats significantly (P < 0.05) increased the caspase-3 in the kidney tissues when compared to all other treatment groups (Figure 10). Omega-3 fatty acid supplementation significantly (p < 0.05) reduced the caspase-3 activities in the kidney tissues when compared to the Normal saline, Codeine and Omega-3 fatty acid treated groups (Figure 10).

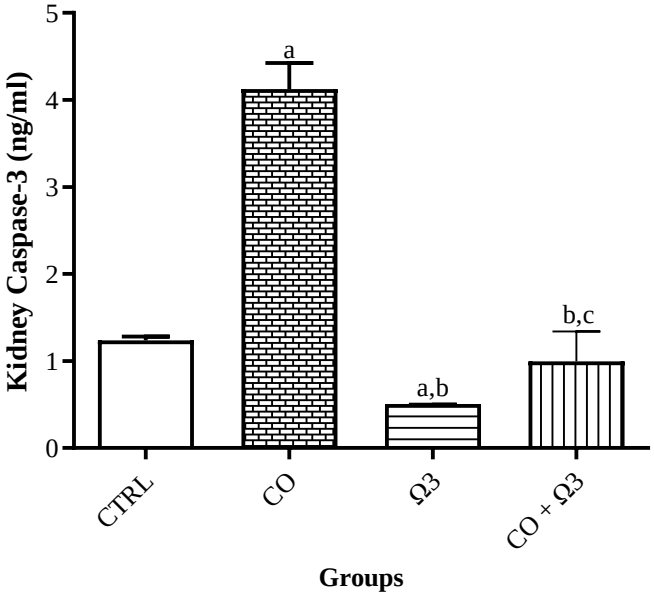

Figure 10: Effect of omega-3 fatty acid on the Kidney Caspase-3 activities in codeine exposed female rats. *a = significantly (P <*

0.05) when compared to CTRL; b = significantly (P < 0.05) when compared to CO; c = significantly (P < 0.05) when compared to Ω3.

Histopathological Findings

The histology of the section of the kidney tissue of the treated rats consisting of the renal corpuscle (arrow) and segments of the renal tubules. The corpuscle is made up of the glomerulus (G) containing podocytes and separated by a defined bowmans space (BS) (Figure 11). The renal tubules of CTRL and Omega-3 fatty acid are lined by low columnar-cubiodal epithelium and separated by interstitium that is free from congestions and collections, hence showing consistency with normal renal tissue while the renal tubules of codeine treated are lined by low columnar-cubiodal epithelium and separated by a congested interstitium (Star) containing branches of renal vessels showing features consistent with cellular reaction to injury (Figure 11). Meanwhile, the administration of omega-3 fatty acid to the codeine exposed rats shows a mild congestion of the interstitium (Star).

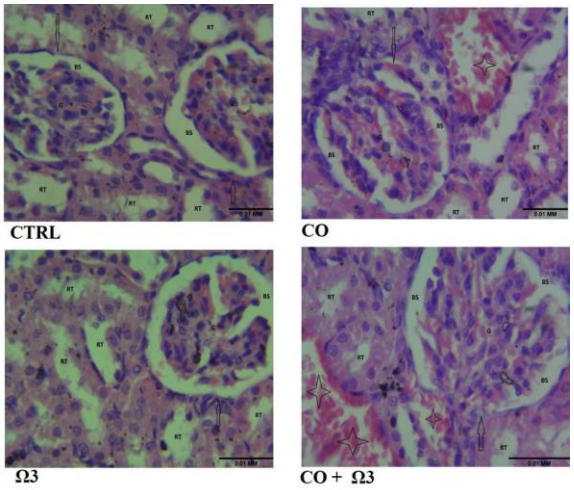

Figure 11 shows the histology of the kidney tissues (H & E Stain) × 400

RT: Renal Tubule, BS: Bowmans space, G: Glomerulus.

Discussion

Omega-3 fatty acids have been recognized for their potential health benefits, including anti-inflammatory and cardioprotective properties (11, 12). Several studies have reported the protective effects of omega-3 against various cardiovascular and renal conditions (13, 14). Omega-3 supplementation has been demonstrated to attenuate oxidative stress markers, reduce inflammation, improve endothelial function, regulate blood pressure, and enhance renal blood flow (15).

In this study, there is a decrease in the relative weight gain of female rats treated with codeine compared to other treated groups. This may be due to the ability of codeine to increase metabolic activities (28). However, Omega-3 administration may improve the metabolic activities of the codeine-exposed rats (29). This finding is consistent with previous studies. Regarding the relative paired kidney weight, there was no significant change in the treated weight after evaluation using statistical standards (30).

Kidney function markers such as urea, creatinine, and serum electrolytes (Na^+, K^+, Cl^-, and HCO_3^-) were assayed to estimate renal toxicity. According to McCann et al., urea assays in clinical studies are crucial for estimating amino acid metabolism (31), elimination via urinary excretion, and nephrotoxicity of xenobiotics. Plasma creatinine is used to measure glomerular filtration rate and renal function (32).

The present study recorded significantly increased urea and creatinine levels in the codeine-administered group compared to the other groups. The level of creatinine clearance, an index for glomerular filtration rate, decreased, resulting in a significant increase in plasma creatinine and urea concentrations in this group. The decrease in the glomerular filtration rate can be due to a

reduction in renal blood flow, an increase in the hydrostatic pressure within the Bowman space, back leak of glomerular filtration rate, or a combination of these factors, as reported by other studies (33). This indicates renal injury, which may lead to impaired kidney function. However, omega-3 fatty acids improve the glomerular filtration rate and renal clearance. This is evident from the decrease in serum creatinine and urea. Therefore, compared to the codeine group, previous studies suggest that omega-3 may improve the glomerular filtration rate (34).

The kidney is crucial in maintaining stable electrolyte concentrations in the blood irrespective of physiological body adjustment (31). The study recorded increase serum electrolytes such a (sodium (Na^+), bicarbonate (HCO_3^-)) and decrease in potassium (K^+), chloride (Cl^-), in the codeine administered rats compared to all other treatment groups. In the study of serum electrolytes, there is an increase in sodium and bicarbonate, while the result shows a decrease in in potassium and chloride, which suggest an electrolyte imbalance and can disrupt the acid-base balance in the kidneys and impair regulation of electrolyte levels in the blood. However, omega-3 fatty acid administration helps to decrease (sodium, bicarbonate) and increase (potassium, chloride) thereby ensuring electrolyte and acid-base balance and preventing cases like hypertension, metabolic alkalosis and acidosis, hypokalemia, hypercalcemia, edema, diabetes and many more as stated in previous studies (33).

In this study, codeine administration caused a rise in lactate, pyruvate, lactate dehydrogenase (LDH), and creatinine kinase concentrations compared to the normal saline, omega-3 fatty acid-treated, and codeine + omega-3 fatty acid-treated groups. Lactate, a byproduct of anaerobic metabolism, accumulates when there is insufficient oxygen to fully break down glucose into carbon dioxide and water. Pyruvate, a key intermediate in carbohydrate metabolism, could be converted into acetyl-CoA for the citric acid cycle or into lactate under anaerobic conditions. Elevated levels of lactate and pyruvate indicated mitochondrial dysfunction and were markers of cellular metabolism, often elevated in kidney injury (34). LDH, an enzyme that catalyzes the conversion of lactate to pyruvate and vice versa, played a critical role in maintaining the balance between aerobic and anaerobic metabolism. It was a marker of tissue damage and was commonly elevated in cases of kidney injury. Codeine exposure increased LDH levels due to cell damage and enzyme leakage into the bloodstream, leading to kidney injury (34).

In this study, codeine administration led to changes in creatine kinase and other injury markers, causing cellular damage and kidney dysfunction. Creatine kinase, an enzyme that catalyzes the conversion of creatine and ATP into phosphocreatine and ADP, is primarily found in muscle tissue and serves as a marker for muscle damage or injury. An increase in creatine kinase levels can impair kidney function and cause muscle damage due to its excess release into the bloodstream (35). The findings showed that codeine exposure altered the activities of these markers, indicating potential cellular damage and dysfunctions in the kidneys. However, omega-3 fatty acid supplementation reduced the abundance of these enzymes, improved mitochondrial function, repaired muscle damage, and ameliorated kidney injury, in line with previous studies (36). Additionally, the study found that codeine treatment significantly reduced the urea/creatinine ratio compared to all other treatment groups, whereas omega-3 supplementation in codeine-exposed rats significantly increased this ratio, indicating an improvement in kidney function.

Also, codeine administration significantly increased the levels of malondialdehyde (MDA) and 8-hydroxy-2-deoxyguanosine (8-OHdG) in kidney tissues compared to all other treatment groups. MDA, a byproduct of lipid peroxidation, is a biomarker for oxidative stress, and its excessive production indicates increased oxidative stress caused by codeine exposure in female rats, leading to DNA, protein, and cellular damage (37). Similarly, 8-OHdG, a reliable marker of oxidative DNA damage formed by the oxidation of guanine in DNA, was elevated, suggesting kidney dysfunction due to codeine exposure (38). The administration of codeine also significantly reduced the activities of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and glutathione in kidney tissues, indicating impaired oxidative stress defense mechanisms. Conversely, omega-3 fatty acid supplementation reduced oxidative stress, improved mitochondrial function, repaired muscle damage, and ameliorated kidney injury by increasing the levels of these antioxidant enzymes (33).

Furthermore, codeine administration significantly increased the activities of myeloperoxidase (MPO) and the concentration of nitric oxide (NO) in kidney tissues, indicating heightened inflammatory responses and oxidative stress (39). MPO, an enzyme involved in generating reactive oxygen species (ROS) and regulating immune responses, was elevated, which is associated with kidney dysfunction and other diseases. Although omega-3 supplementation did not significantly reduce NO levels compared to the codeine-only group, it helped regulate MPO activity and reduce inflammation, enhancing kidney function (40). Additionally, codeine significantly increased caspase-3 activity, a key enzyme in apoptosis, indicating potential kidney damage and cell apoptosis. Omega-3 fatty acids significantly reduced caspase-3 activity, suggesting a protective effect against codeine-induced kidney cell apoptosis and damage (5, 41).

The histopathological study of the kidney showed that the renal tubules of Normal saline, Omega-3 are lined by low columnar-cubiodal epithelium and separated by interstitium that is free from congestions and collections, hence showing consistency with normal renal tissue while the renal tubules of codeine treated are lined by low columnar-cubiodal epithelium and separated by a congested interstitium (Star) containing branches of renal vessels showing features consistent with cellular reaction to injury .Meanwhile, the administration of omega-3 fatty acid to the codeine exposed rats shows a mild congestion of the interstitium (Star) . According to Monir et al. (42), reactive oxygen species have been reported as the hallmark mechanism for the development of kidney injury/ damage via increased kidney biomarkers. Natural compounds that possess high antioxidant and anti-inflammatory effects are expected to possess a renal protective effect (43). Several studies have reported the antioxidant effect of omega 3 against kidney injury/damage.

Findings from studies that Omega-3 fatty acids have been shown to have potential benefits on kidney health. Studies suggest that omega-3 fatty acids helps to reduce inflammation and oxidative stress, improve blood flow to the kidneys, improves vascular function and decrease proteinuria (excretion of excess protein in the urine) (44).

Omega-3 fatty acids also helps to lower blood urea nitrogen (BUN) levels by improving kidney function and reducing the production of urea. There is some evidence to suggest that omega-3 fatty acids may have a protective effect on kidney function and help lower creatinine levels in patients with chronic kidney disease. Omega-3 fatty acids have anti-inflammatory properties and may help reduce inflammation in the kidneys, leading to improved kidney function and lower

creatinine levels (45). Studies have suggested that omega-3 fatty acids help to lower blood pressure, reduce inflammation, and improve blood vessel function. These effects has potentially lead to changes in the levels of sodium (Na^+), potassium (K^+), chloride (Cl^-), and bicarbonate (HCO_3^-) in the body, as these electrolytes play a crucial role in maintaining fluid balance and blood pressure regulation.Omega-3 fatty acids also helps reduce sodium (Na^+) retention, which leads to lower levels of sodium (Na^+) in the body. Additionally, omega-3 fatty acids have been shown to increase potassium (K^+) excretion, which result in higher levels of potassium. The effects on chloride (Cl^-) and bicarbonate (HCO_3^-) levels are less clear and depend on individual factors and the specific form of omega-3 fatty acids consumed (46).

Omega-3 fatty acids has been shown to have a potential protective effect on the kidneys by improving lipid metabolism. Lactate and Pyruvate: Omega-3 fatty acids may help to improve energy metabolism and mitochondrial function, which has potentially led to a reduction in lactate and pyruvate levels. Lactate dehydrogenase (LDH): Omega-3 fatty acids has a protective effect on tissues and cells, which has potentially reduce the release of LDH into the bloodstream (47-51).

CONCLUSION

This present study demonstrated that omega 3 fatty acids supplementation to codeine treated female rats improved the kidney injury by increasing kidney redox status, improves kidney function and causes reduction in oxidative stress thereby enhancing antioxidant properties and reducing kidney inflammation The therapeutic effect of omega 3 fatty acids is associated with the inhibition of activation of caspase-3 and other apoptotic pathways, thus exerting a protective effect against cell death and also causing reduction in DNA damage and fragmentation, which may be related to their anti-apoptotic effects. Therefore, This research suggest that omega 3 fatty acids supplementation is therapeutic enough to improve codeine induced kidney damage by reducing oxidative stress and inflammation while enhancing antioxidant activities thereby decreasing kidney injury and inhibiting kidney apoptosis.

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