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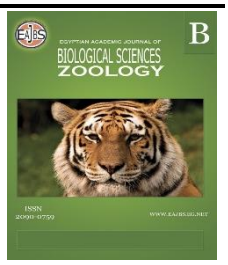

Prophylactic Effect of Cod Liver Oil on Stone Formation in Rat Kidney: A Novel Approach

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ABSTRACT

The incidence of kidney stones has increased worldwide in association with economic development; however, treatment modalities are not satisfactory. This study aimed to explore the effect of cod liver oil on the prevention of kidney stone formation in male rats. Renal stones were induced by oral administration of 0.75 % ethylene glycol (EG) with 1% ammonium chloride for 10 days. Twenty-four male rats were separated into 4 groups. Group 1 served as control; group 2 received EG plus NH4CL; group 3 was pre-treated with CLO (480 mg/kg body weight) for 2 weeks, followed by EG plus NH4Cl administration; group 4: was post-treated with CLO plus EG plus NH4Cl. At the end of the experiment, histopathological analysis and the degree of calcification in the renal tissue were analyzed. Blood levels of triglycerides, cholesterol, urea, creatinine, calcium, and phosphorus were determined. Lipid markers, antioxidant markers, and oxidative stress indicators were assessed in renal tissues. EG caused increases in kidney weight, malondialdehyde levels, and the reduction of glutathione, catalase, and SOD in kidney tissues. The results showed a protective effect of CLO against EG-induced nephrolithiasis concomitant with a reduction effect on lipid parameters, amelioration of kidney function markers, and a restoration of antioxidant status. No stone formation was detected in both kidneys, and the amount of calcification was significantly reduced in CLO groups in comparison to the EG group. In conclusion, CLO could be useful in preventing stone formation in rats through modulating lipid profiles and renal membrane lipids and enhancing antioxidant status.

INTRODUCTION

The prevalence of kidney stones has globally been on the rise over the last few decades in industrialized countries (Sorokin *et al.*, 2017). Furthermore, the high recurrence rate of nephrolithiasis presents an increased economic concern. This high incidence of kidney stone disease necessitates different intervention approaches (Rukin *et al.*, 2017). Although advancements steadily occur in the medical treatment of kidney stones, there is no perfect drug or medication to fully and effectively treat kidney stones (Laroubi *et al.*, 2007). Urinary tract disorders are influenced by a wide range of risk factors, including diet habits, obesity, metabolic syndrome; stress, inadequate fluid intake and climate.

Additionally, they are associated with other diseases (diabetes, hypertension) (Sakhaee *et al.*, 2012; Sofia *et al.*, 2016). According to previous studies, hyperoxaluria is positively correlated with body mass index (Prochaska *et al.*, 2018; Taylor and Curhan, 2008). The most prevalent sort of kidney calculi is calcium oxalate (CaOx) (Daudon *et al.*, 1993). Extracorporeal shockwave lithotripsy modality nearly become the standard process for eliminating kidney stones (Lawler *et al.*, 2017). Nevertheless, this invasive therapeutic strategy may cause trauma, residual stone deposition, and renal infection, and may lead to a renal impairment (Atmani *et al.*, 2003). It has been suggested that these small nascent crystals cannot be retained in the kidney unless they adhere to altered renal surfaces (Khan, 2006). Such abnormal renal surfaces may result from insult-provoking physical breaches that are repaired through membrane remodeling (Ammendolia *et al.*, 2021). In this context, it has been documented that oxalate reacts with polyunsaturated fatty acids (PUFAs) in cell membranes to trigger lipid peroxidation and produce kidney injury (Ernester and Nordenbrand, 1967). On the other hand, previous studies have found that rats fed a diet that promotes kidney stone formation have decreased antioxidant levels and increased lipid peroxidation within the kidneys (Sumathi *et al.*, 1993; Saravanan *et al.*, 1995). Recent studies revealed a protective effect of CLO on membrane integrity and structures (Casares *et al.* 2019; Ammendolia *et al.*, 2021). The source of cod liver oil (CLO) is cod fish which belong to the genus Gadus and the family Gadidae. CLO, extracted from the livers of cod fish, is considered a functional food oil in the pharmaceutical market, either in liquid or capsule form. Cod liver oil, like many other fish oils, has high active content of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (defined as omega-3 fatty acids), in addition to vitamin A and vitamin D (Trofimiuk *et al.*, 2011). Moreover, CLO has antioxidant properties (Mohamad *et al.*, 2015). Furthermore, omega-3 fatty acids proved to reduce inflammation (Calder, 2017). Previously, the supplementation of cod liver oil has been proposed to assist in healing stomach ulcers (Narkhede *et al.*, 2019), ameliorate cognitive impairment resulting from stressful situations (Trofimiuk and Braszko, 2011), have anticancer effects (Dyck *et al.*, 2011), alleviate oxidative stress, and protect against heart diseases (Y Abeywardena *et al.*, 2011). Recently, CLO supplementation demonstrated a lipid-lowering effect on TG, LDL cholesterol, and increased HDL cholesterol levels in human serum (Illingworth and Ullmann, 2020; Fatima *et al.*, 2021). Thus, the current study aimed to assess the antioxidant and antilipidemic roles of cod-liver oil in preventing the development of kidney stone nuclei within renal tubules. We endeavored here to validate a novel aspect of protection using CLO against stone formation based on biochemical and morphological markers evaluation, and collating research works to extract and analyze relevant evidence.

MATERIALS AND METHODS

Chemicals:

All chemicals were obtained from Sigma-Aldrich (St. Louis, MO). Cod liver oil was provided from the pharmacy as capsules provided by Schwalbach am Taunus, Germany.

Experimental Design:

Twenty-four male rats (180 -200 g) were retrieved from the animal house, National Cancer Institute, Cairo University and kept under typical circumstances of humidity, temperature (24°C) and 12 h light/dark cycle at 24 ℃, with free access to standard diet and water (ad libitum). Renal calcium oxalate deposition was induced by adding 0.75% v/v ethylene glycol (EG) and 1% ammonium chloride (NH4Cl) (to accelerate the formation of kidney stones) to the drinking water (Gokhale *et al.*, 1996; Grases *et al.*, 1998) for 10 consecutive days. They were divided into four groups: the first served as a control and received no treatment. The second group was given EG and NH4Cl. The third group was

orally (by gavage) pre-treated with CLO (480mg/kg body weight/ day) for 2 weeks, followed by the administration of EG for the next 10 days. The fourth group was co-administered CLO (480 mg/kg body weight), and EG and NH4Cl, and served as a post-treatment group. All groups were fed the standard diet. The experiment was conducted following internationally standard guidelines for the Care and Use of Laboratory Animals. The study protocol was approved by the ethical committee of October 6 University under number 20230722.

At the end of treatment, all animals were weighed and anesthetized by an intraperitoneal injection of urethane. Blood was taken via the retro-orbital venous plexus and serum levels of, creatinine, urea, triglycerides, cholesterol and calcium, phosphorus were measured using an automatic analyzer, AU 680 NCI, Cairo, Egypt. After removing both kidneys, they were cut longitudinally and checked under a light microscope for potential stone development. Following the fixation of kidney tissue with 10% formaldehyde, a thin section was prepared for the subsequent analysis. The renal papillae were examined macroscopically to determine the presence of calcification. Another part of both kidneys was wrapped in foil and kept in a deep freezer (-20 ^{o} C) until further analysis of oxidants, antioxidants, triglycerides, cholesterol and phospholipids.

Assessment of Lipid Peroxidation:

Kidney tissue malondialdehyde (MDA) concentrations, an indicator of lipid peroxidation, were determined employing thiobarbituric acid, as described formerly by Nielsen *et al.* (1997).

Determination of Superoxide Dismutase (SOD) Activity:

Renal SOD activities were measured using the kinetic procedure according to Nishikimi *et al.* (1972) employing Bio-diagnostic kits, in Giza, Egypt.

Determination of Catalase (CAT) Activity:

Catalase was evaluated using the method of Aebi (1984), and its renal tissue activity was established as the amount of H_2O_2 employing Bio-diagnostic kits (Giza, Egypt).

Determination of Reduced Glutathione Concentrations (GSH):

Using the 2-nitrobenzoic acid (DTNB) technique, GSH was estimated (Beutler *et al.*, 1963)

Determination of Kidney Tissue Lipids:

For the determination of TG, cholesterol, and phospholipids, Kidney tissues' total lipids were extracted as described by Bligh and Dyer (1959) followed by enzymatic determination employing Bio-diagnostic kits (Giza, Egypt).

Statistical Analysis:

One-way ANOVA was conducted to determine the significant variation in measured indices among experimental groups. A paired t-test was used for measuring the mean and standard error of the data and was statistically analyzed using SPSS software (version 18). The statistical significance was determined using a criterion of $p < 0.05$ for the biochemical data.

RESULTS

Effect of Cod Liver Oil on Body Weight and Kidney Weight:

This study revealed a significant reduction in body weight in the EG group in comparison to the control and CLO pretreated group; On the other hand, a significant increase in kidney weight/body weight ratio was noticed in the EG group compared to the normal or CLO pre-treated group (Table 1). In the CLO post-treated group, body weight was slightly insignificantly different from the control and CLO (pre) group; however, the kidney-to-body weight ratio was significantly different from the control and CLO pre-treated groups.

Table 1. Effect of EG and CLO treatment on body weight and kidney weight.

	Kidney weight (g)	Body weight (g)	kidney weight / Body weight ratio
Control	1.5 ± 0.14[a]	210±1.4[a]	0.0071±0.0005 [a]
EG + NH_4Cl	2.9 ± 0.22	189±1.6[b]	0.015±0.0009[b]
CLO (pre)	1.7± 0.26[a]	215±2.4[a]	0.0079±0.0008[a]
CLO (post)	2.1± 0.38[c]	200 ± 2.9[a]	0.0105 ± 0.0008[b]

Values illustrate the mean ± SE of 6 rats. The values inside the row labeled with similar letters did not show any significant differences at $P < 0.05$. A significant difference is observed at $p < 0.05$ between values within a row that do not share the same superscripted letters.

Effect of Cod Liver Oil on Renal Functions:

Table (2) showed that EG administration significantly ($P<0.05$) increased serum urea and creatinine compared to the control and CLO (pre)groups. In post-treatment with CLO, a significant increase in kidney function tests was observed but still significantly lower than the EG group demonstrating that post-treatment partially protected kidney tissue against the damaging effect of EG. The serum calcium levels did not differ significantly between the treated group and the untreated group ($p > 0.05$). However, the post-treated group with CLO showed a significant difference in phosphorus levels in comparison with other groups reflecting some disturbances in kidney functions (Table 2).

Effect of Cod Liver Oil on Lipids Biomarkers:

Serum cholesterol and triacylglycerol concentrations noticeably increased in the EG rats group compared to the control and CLO groups. CLO treatment significantly prevented these abnormalities in serum triacylglycerol (TG) and cholesterol concentrations, while in the CLO post-treated group TG was similar to control and CLO (pre) groups, while cholesterol levels were significantly higher than control and CLO-pre-treated groups ($p < 0.05$) (Table 2).

Table 2. Effect of CLO on serum biochemical parameters.

	Control	EG + NH_4Cl	CLO (pre)	CLO (post)
Triglycerides (mg/dl)	68 ± 6.12[a]	88 ± 5.5 [b]	60 ± 4.3 [c]	67 ± 6.11[a]
Cholesterol mg/dl)	86 ± 5.8[a]	180 ± 6.2 [b]	75 ± 4.7 [c]	171 ± 9.89[d]
Creatinine (mg/dl)	0.52 ± 0.03[a]	6.2 ± 1.5 [b]	0.48 ± 0.06 [a]	2.26 ± 0.9[c]
Urea (mg/dl)	45 ± 5.1[a]	470 ± 15.3 [b]	49 ± 1.3 [a]	243 ± 14.12[c]
Calcium (mg/dl)	10.3 ± 0.4[a]	11.64 ± 1.5 [b]	10.8 ±0.9 [a]	10.91 ± 0.80[a]
Phosphorus (mg/dl)	5.62 ± 1.2[a]	23.27 ± 1.7[b]	6.4 ± 2.1[a]	20.12 ± 3.15[c]

Values illustrate the mean ± SE of 6 rats. The values inside the row labeled with similar letters did not show any significant differences at $P < 0.05$. A significant difference is observed at $P < 0.05$ between values within a row that do not share the same superscripted letters.

Effect of CLO on Renal Lipids Parameters:

Both EG and CLO post-treated groups demonstrated significantly higher levels of TG compared to the control and CLO (pre-treated), however, the post-treated one was significantly different from the group receiving EG. Phospholipid level was significantly decreased in EG groups, while in CLO pre-treated groups, it significantly normalized to the control group level. On the other hand, it was slightly but significantly affected by post-treatment with CLO (Table 3).

Table 3 Effect of CLO on renal tissue lipid

	Control	**EG +** NH_4Cl	**CLO (pre)**	**CLO (post)**
Triglycerides (mg/g tissue)	4.7 ± 0.38^a	7.36 ± 1.1^b	4.26 ± 0.7^a	6.26 ± 3.1^c
Cholesterol (mg/g tissue)	1.6 ± 0.05^a	1.9 ± 0.06^b	1.3 ± 0.07^c	1.86 ± 0.1^d
Phospholipid (mg/g tissue)	14.2 ± 0.6^a	12.7 ± 0.7^b	14.5 ± 0.51^a	13.7 ± 0.7^c

Values illustrate the mean ± SE of 6 rats. The values inside the row labeled with similar letters did not show any significant differences at $P < 0.05$. A significant difference is observed at $P < 0.05$ between values within a row that do not share the same superscripted letters.

Effect of Cod Liver Oil on Oxidants and Antioxidant/Oxidant Parameters:

The effect of CLO on antioxidant/oxidant markers in normal rat tissues is shown in Table 4. The renal tissues of the EG groups exhibited a significant decrease in GSH, SOD, and CAT levels, while the MDA level experienced a considerable rise, in contrast to both the control and CLO (pre) groups. Meanwhile, rats co-treated with CLO (post) exhibited a significant increase in GSH, SOD, and CAT levels along with a significant decrease in the MDA level in the renal tissues in comparison to EG groups. Additionally, in the CLO (post) group, a significant difference is noticed when compared to the control or CLO (pre) group. The pre-treatment of rats with CLO restored the normal control ranges of antioxidant parameters and MDA.

Table 4. Effect of CLO on GSH, MDA levels, CA and SOD activities in the kidney of rats.

Groups	**MDA (nmol/g tissue)**	**GSH (mg/g tissue)**	**Cat (U/g tissue)**	**SOD (U/g tissue)**
Control	0.16 ± 0.005^a	0.58 ± 0.006^a	0.31 ± 0.006^a	0.68 ± 0.003^a
EG + NH_4Cl	0.82 ± 0.01^b	0.15 ± 0.001^b	0.15 ± 0.007	0.11 ± 0.01^b
CLO (pre)	0.12 ± 0.006^a	0.48 ± 0.008^a	0.36 ± 0.008^a	0.65 ± 0.006^a
CLO (post)	0.41 ± 0.008^c	0.36 ± 0.009^c	0.31 ± 0.007^c	0.29 ± 0.006^c

Values illustrate the mean ± SE of 6 rats. The values inside the row labeled with similar letters did not show any significant differences at $P < 0.05$. A substantial difference is observed at $P < 0.05$ between values within a row that do not share the same superscripted letters.

Effect of CLO on Kidney Tissue Morphology:

Histopathological examination of kidney tissue sections from mice treated with ethylene glycol (EG) (Figs. 2 and 3) showed a significant increase in the deposition of salt crystals, progressing to calcified stones in the cortical and medullary tubules, collecting ducts, and the calyceal system of the renal tissue, compared to normal control rat (Fig. 1). This was accompanied by tubular epithelial damage. In contrast, groups of mice receiving CLO as a protective therapy prior to EG administration exhibited no or minimal crystal deposition, with only minor or no tubular epithelial injury (Fig. 4). However, administering CLO as a therapeutic drug after EG administration was less effective in preventing renal stone formation. as histopathological examination of kidney tissue sections in this group showed a few non-calcified crystals in the tubules (Fig. 5).

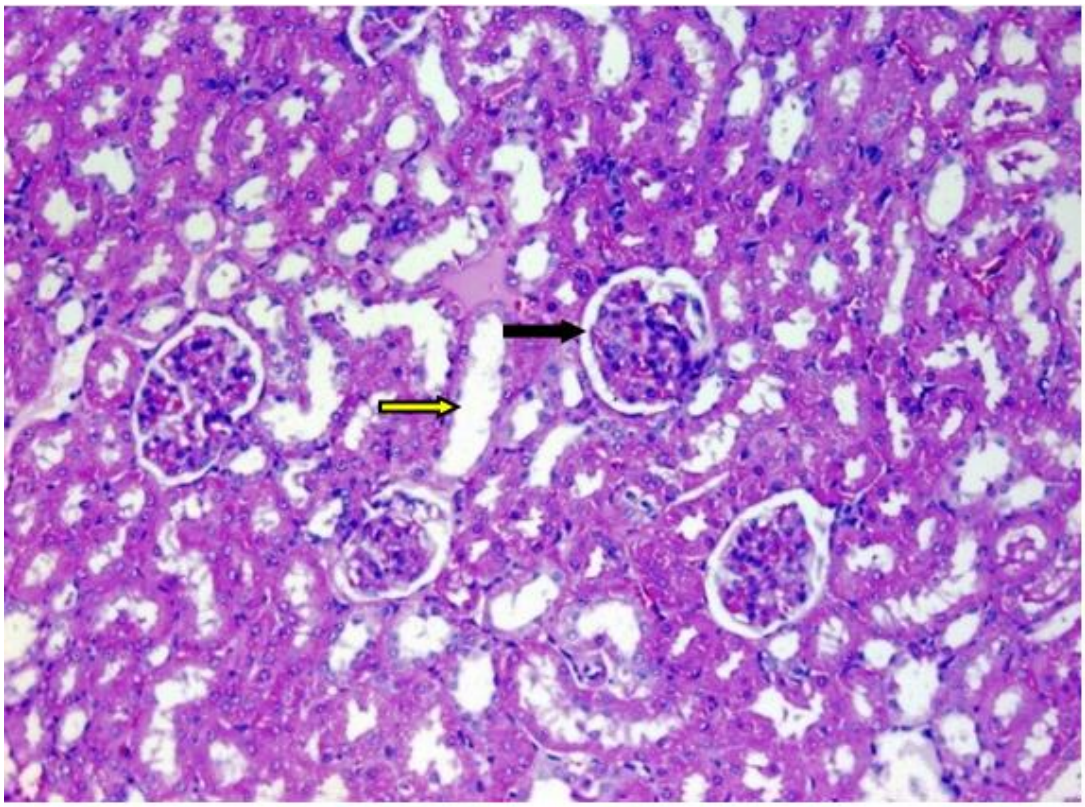

Fig. 1. Section in the kidney of a control rat showing normal glomerular (black arrow) and tubular (yellow arrow) structures (Hematoxylin and eosin stain, X200).

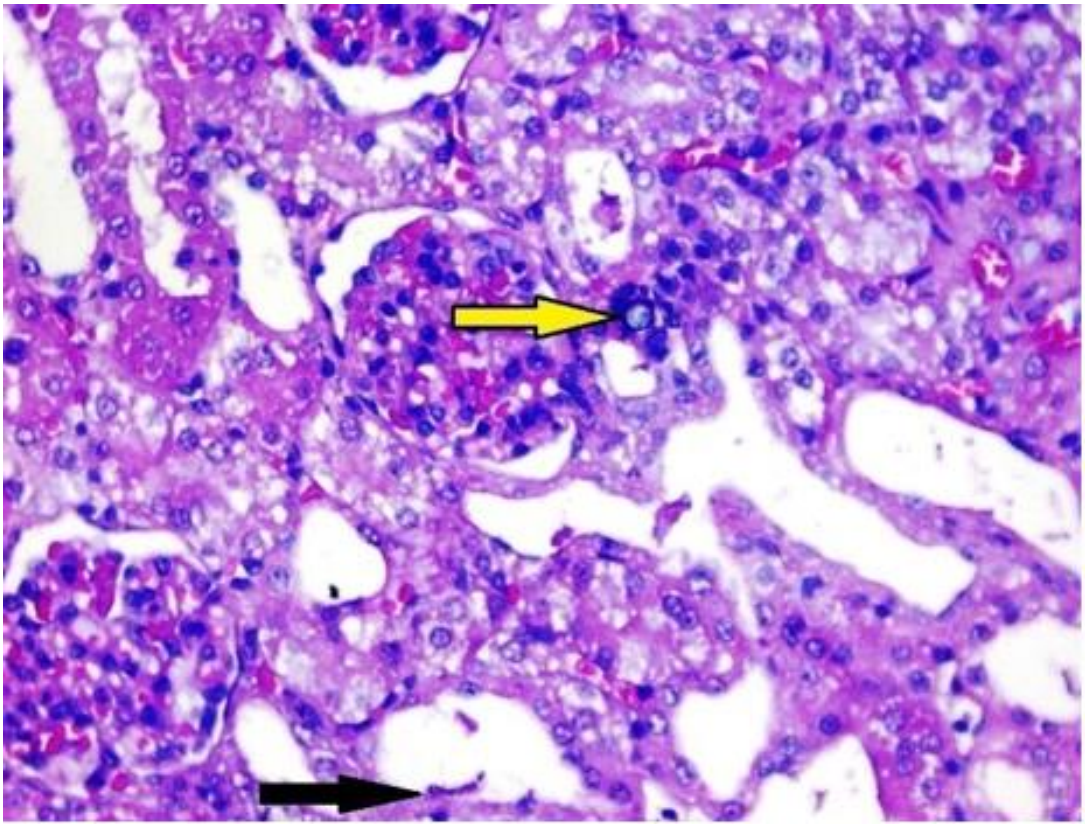

Fig. 2. Section in the kidney of rat given EG showing focal tubular epithelial degeneration (black arrow) and intra-tubular crystal deposition (yellow arrow) (Hematoxylin and eosin stain, X400)

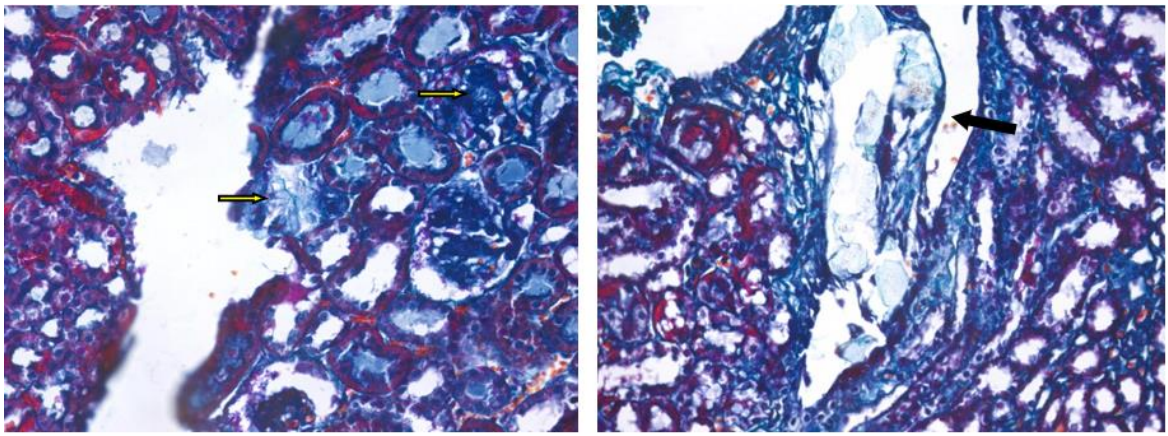

Fig. 3. Sections in the kidney of a rat receiving EG showing focal intra-tubular (yellow arrows) and intra-calyceal crystal deposition (black arrow) (Masson Trichrome stain, X400)

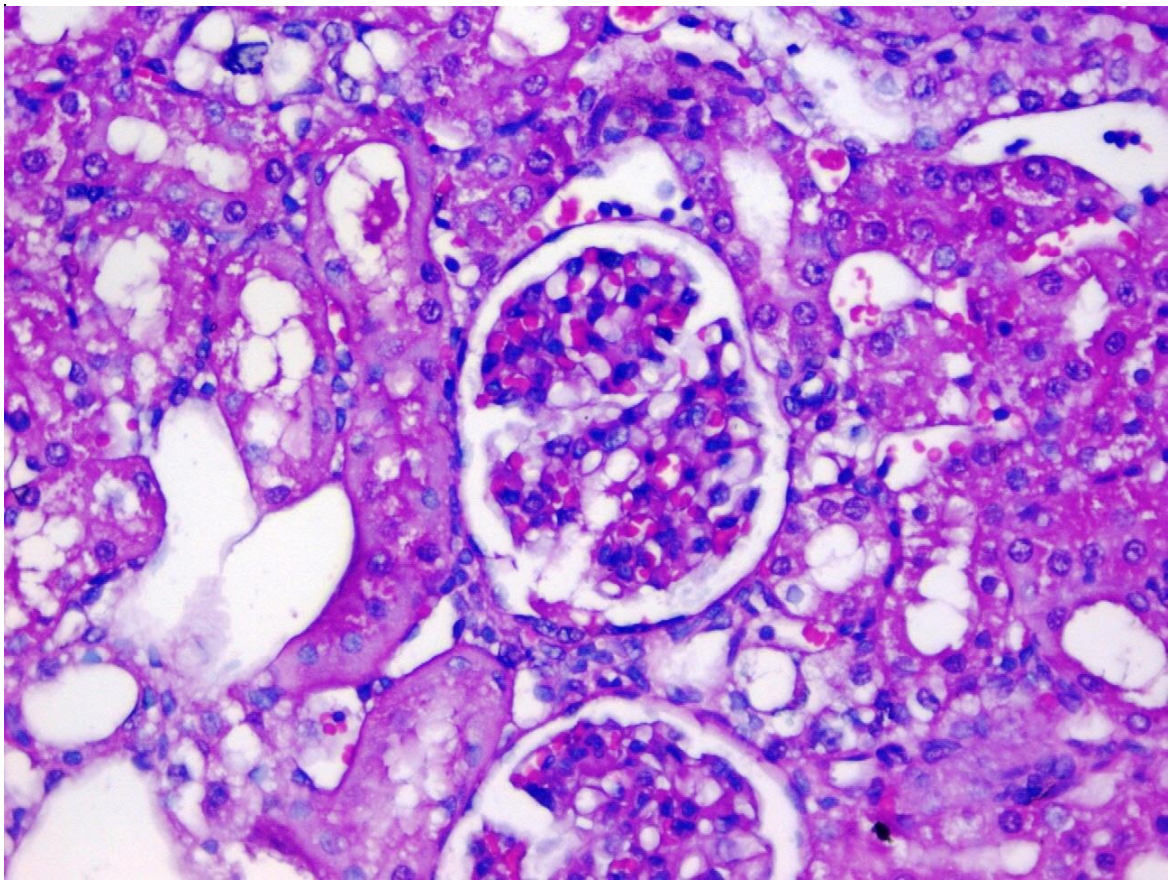

Fig. 4. Section in the kidney of pre-treated rat with cod liver oil showing normal glomerular and tubular structures (Hematoxylin and eosin stain, X400)

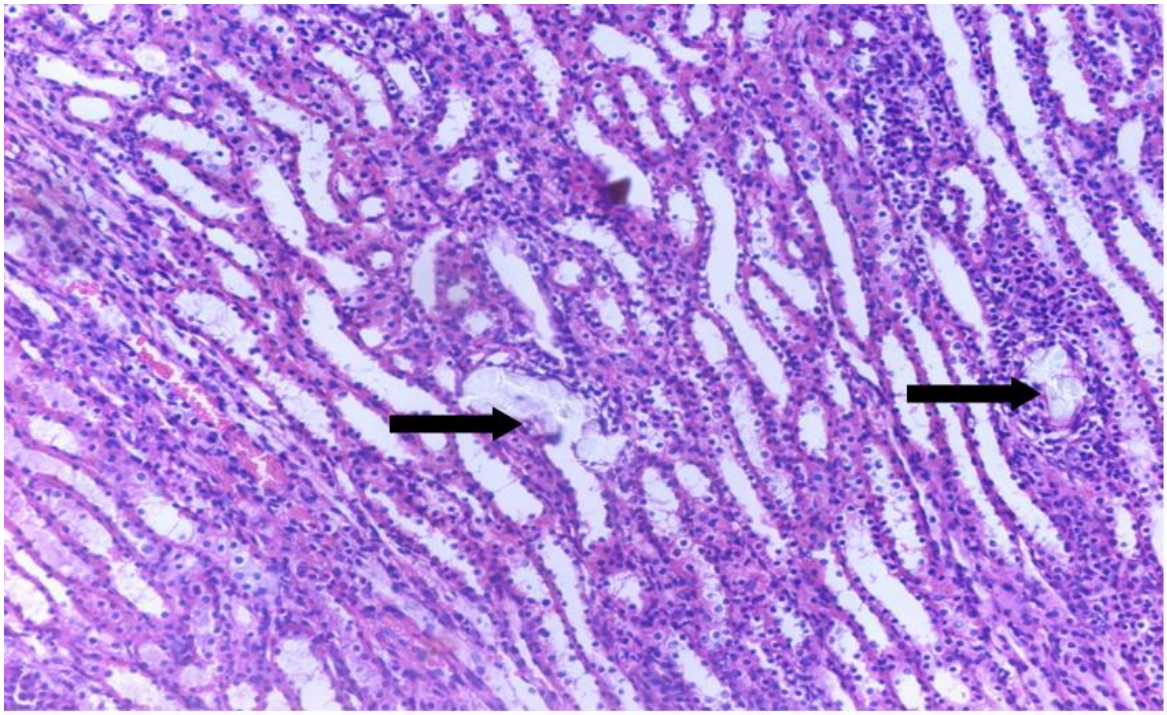

Fig. 5 Section in the kidney of post-treated rats by cod liver oil after stone induction, showing few crystals' depositions in the renal tubules (black arrows) (Hematoxylin and eosin stain, X200)

DISCUSSION

Kidney stone incidence has been steadily increasing globally in the previous few decades in association with economic development and stressful life. A complicated interaction between dietary consumption, environmental conditions, and genetic predisposition may predispose to stone development (Lieske, 2014; Wong *et al.,* 2015; Sofia *et al.,* 2016; Taguchi, *et al.,* 2017; Ferraro *et al.,* 2020). Despite considerable advancement in medication, there is still no ideal therapy for renal stones. Up to 80% of analyzed stones in the urinary system are calcium oxalate (CaOx) (Dauden *et al.,* 1993). The current study aimed to investigate a safe natural product like cod liver oil as a protective and/or therapeutic agent against kidney stone formation. EG model of inducing nephrolithiasis was adopted in this study and caused stone deposition in the kidney after 10 days of the experiment as demonstrated by a significant increase in kidney weight in relation to body weight, abnormal kidney morphology, altered biochemical markers and imbalanced antioxidants/oxidant status. Our results are similar to those of Schladt *et al.* (1998) who reported a significant increase in kidney weights in rats given EG, and ascribed these abnormal weight changes to stone deposition, inflammation and necrosis in renal tubules. Furthermore, the histopathological results confirmed abnormal renal structures indicative of crystal deposition.

The beneficial effect of CLO on stone formation has not been previously studied. For the first time, CLO administration inhibited effectively the deposition of CaOx crystals in EG-given rats. Our results shed light on the multiple beneficial effects of CLO. Regarding the role of CLO in lipid profile modulation and its effects on kidney function markers, this study showed restorative impacts of CLO on lipid profile (decreased serum TG and cholesterol), kidney function, and Ca and phosphorus in the blood. A decrease in TG levels has been noted in most fish oil trials in humans. Changes in total cholesterol levels are not consistently noted (Gudbjarnason *et al.,* 1977; Skuladottir *et al.,* 1990; Brox *et al.,*

2001; Fatima *et al.,* 2021); but generally speaking they showed a reduction trend.

Consistent with the biochemical results, histopathological outcomes revealed a significant abolishment of renal stones deposition, as well as a substantial reduction of the amount of calcification in the kidneys of rats treated with cod liver oil in comparison to the EG-treated group. The pre-treated group with cod liver oils showed no kidney stone and significant calcification, while the CLO post-treated group showed scattered stone deposition. The short treatment interval adopted in this study may have led to a CLO partial effect on the post-treatment group. To explain why the post-treated group displayed some stone development in the kidneys. We assumed that CLO would not immediately eradicate the stone nucleus once adhered to tissues as its potential to adjust lipid composition, and hence its modulatory effect on kidney tissue membranes would demand prolonged treatment and/or an extensive dose of CLO. This could imply that stone deposition vulnerability would develop in the long term, reflecting an accumulative pattern of membrane lipids perturbation.

In this study, phospholipid levels were significantly reduced by the EG supplement. CLO pre-treatment succeeded in restoring its level to near the control group level, while CLO post-treatment could partially alleviate the EG effect on its concentration. Phospholipids structural components play a crucial role in determining the unsaturated fatty acid composition of the membranes. The proportion of docosahexaenoic acid (n-3) (DHA) and eicosapentaenoic acid (n-3) (EPA) in plasma membrane lipids is significantly increased by fish oil intake. Additionally, the concentration of DHA in plasma phospholipids increases in a dose-dependent and saturable way in response to dietary intake of DHA (Arterburn *et al.,* 2006). The findings of this work align with numerous studies founding that groups receiving cod liver oil had significantly greater reductions in total cholesterol and TG levels than the control group (Noels *et al.,* 2021; Pan *et al.,* 2022; Fatima *et al.,* 2021). Abnormal lipid metabolism has been noted in patients with urinary stones (Taylor *et al.,* 2005). Recently, kidney diseases have been linked to lipotoxicity in various ways (Noels *et al.,* 2021; Pan *et al.,* 2022). A strong association between atherosclerosis and stone formation was suggested that may be ascribed to common etiological factors like dyslipidemia (Yasui *et al.,* 2007; Kohri *et al.,* 2012; Huang *et al.,* 2020). Alternatively, obesity has recently been associated with kidney stone diseases (Carbone et al., 2018). As obesity and dyslipidemia are caveats in many diseases such as urolithiasis, atherosclerosis (Henning, 2021) and gall bladder stones (Parra-Landazury *et al.,* 2021), it could be said that all these abnormal conditions would respond and presumably be improved by the same lipid modulatory agents like uch as CLO. In this context, CLO has been demonstrated to protect against dyslipidemia (Ceylan-Isık *et al.,* 2007).

In this work, CLO succeeded in restoring kidney antioxidant status and abolishing lipid peroxidation. The antioxidant effect of CLO is covered in a wealth of literature (Hünkar *et al.,* 2002; Mohamed *et al.,* 2015). Moreover, this work showed a mitigation impact of CLO on oxidative stress in the kidney. It has been shown previously that the levels of lipid peroxidation have been linked to the pathological complications of EG-induced nephrolithiasis (Thamilselvan *et al.,* 1997). CLO effect could be mediated through its antioxidants (Mohamed *et al.,* 2015), and anti-inflammatory role (Sherif and Al-Gayyar, 2015; Hansen *et al.,* 2021), as well as its modulatory effects on PUFAs and other lipid components in kidney tissue membranes (Gudbjarnason *et al.,* 1977; Skuladottir *et al.,* 1990; Fatima *et al.,* 2021). Numerous studies have reported an association between CLO supplementation and membrane structure (Cao *et al.,* 2006; Fuentes *et al.,* 2018; Ayee *et al.,* 2020). The lipid bilayer membrane becomes increasingly fluidized with the supplementation of omega-3 PUFAs due to their incorporation into the membrane (Ayee *et al.,* 2020). Additionally, the lipid composition and structure of cell membranes are crucial determinants of the key pathophysiological characteristics of cells (Casares *et al.,* 2019). In

epithelial tissues, aberrant FA synthesis is associated with the loss of cell polarity, a physiological modulator of cell function (Baenke *et al.*, 2013). Furthermore, the anti-inflammatory effects of the omega-3 fatty acids EPA and DHA are partially mediated through the modification of phospholipids composition in cell membranes, as well as the disruption of lipid raft domains within those membranes (Calder, 2017). Thus, PUFA's modulatory effect on the lipid bilayer would help preserve the integrity of the kidney cell membrane. Tubulas *et al.* (2015) showed a protective effect of omega 3 (W3), through antioxidant mechanisms on kidneys. Collectively, all these results pinpoint the multi-dimensionality of CLO's impact on various organs including the kidney, through its antioxidant, antilipidemic, anti-inflammatory as well as through a modulatory role on membrane lipids; and thereby it may assist in preventing stone formation and adherence to kidney tubules.

To elaborate further on the mechanism involved in CLO modulation of tissue membranes and how it could prevent kidney stone deposition, we hypothesized that people in stressful conditions are highly prone to health problems and may have the highest nutrient demands regarding PUFAs, especially omega-3 FA. As the brain needs higher unsaturated fatty acids than other organs (Bourre,2004), its PUFA concentration is most significantly influenced by fish oils (Trofimiuk and Braszko, 2011; Shet *et al.*, 2023). This high demand, if not adequately supplied from food sources, would be recruited from the body PUFAs pool at the expense of its provision to other organ membranes, which culminate, over time, due to a disseminated sub-manifestation of PUFAs deficiency status, in structural changes of epithelial cell membranes, in various organs including the kidney, This may interpret why some organs are highly vulnerable to concomitant lipotoxicity, oxidative stress and stone formation. As such lipotoxicity conditions could affect the integrity, fluidity and smoothness of renal membranes; consequently we suggest that this dysregulation may enhance the anchoring of minute stones on this abnormal surface membrane of renal epithelial tissues depending on the degree and types of lipids anomaly manifestation, in different sites whether papilla, tubular lumen, ureter or bladder. Thus, the putative ongoing process culminates into aggregate accretion. While supersaturation of urine happens in both normal and stone-forming individuals, nucleation of calcium oxalate seldom happens, and when it does, the resulting crystals do not develop to a big size and/or maintained in the kidney due to an inhibitory process (Ratkalkar and Kleinman, 2011). Inhibitors of stone accretion affect tubular fluid constituents, the crystal surface, and the epithelial surface lining of kidney tubules (Lieske *et al.*, 1995; Ratkalkar and Kleinman, 2011; Evan *et al.*, 2007; Kohri *et al.*,2012); however possible inhibitory factors of stone deposition on the surface lining of tubules are not tackled. Previous studies suggested that the first nucleus aggregates of stones, cannot develop and be retained in the kidney unless they adhere to particular renal surfaces (Kok and Khan,1994; Ratkalkar and Kleinman, 2011). Besides, the formation of a large stone has been described by its attachment to the papillary surface membranes (Coe *et al.*, 2010). This primary process may be further exacerbated by high calcium oxalate levels, and low urine volume in kidney tubular lumen (Lieske, 2014; Bird and Khan, 2017). Although membrane fluidity and adhesion properties of epithelial membranes are described as physical features, they have their roots in the chemical constituents of membranes, particularly PUFAs which form basic components of membrane phospholipids (Harayama and Riezman, 2018; Casares *et al.*, 2019). A study on the lipid composition of kidney and ureter membranes reveals an association between molecular composition and morphological patterns reflecting physicochemical characteristics of the epithelial membrane (Popp-Snijders *et al.*, 1984; Heras-Sandoval *et al.*, 2016; Steinkühler *et al.*, 2019), which presumably could enhance stone formation. Noticeably, based not only on the antioxidants, anti-inflammatory and antilipidemic effects that CLO could protect against stone formation, but also on such biomechanical function affecting fluidity and

smoothness of membranes, and thereby, preventing the adherence of the small crystals of stones on the internal structures of the kidney. Additionally, the steep calcium gradient between the extra- and intra-cellular environment ensures calcium uptake by damaged membrane (Cheng *et al.*, 2015). As Ca is entangled in Ca oxalate, it could anchor itself to the renal epithelial surface and not complete its path into the cell. Taken together, all these factors may enhance the first deposition of the minute stone nucleus. This proposed scenario might help clarify how a crystal attaches itself to a particular cell membrane. Consequently, the beneficial effect of CLO on the physicochemical properties of renal membranes could assist in preventing stone deposition even in the presence of other precipitating factors of nephrolithiasis.

Conclusion

Pre-treatment with CLO succeeded in lowering lipid marker levels and modulating tissue lipids as well in reducing MDA, an indicator of lipid peroxidation, and restoring antioxidant status. Pre-treatment with an adequate concentration of CLO was effective in preventing stone formation; while post-treatment with CLO was partially effective in abolishing stone formation. Extensive dose of CLO is anticipated to be effective in treating already deposited stone, however, a detailed assessment of the molecular mechanism of PUFAs involvement in kidney tissue function is still needed. This study opens new avenues for the prevention and treatment of renal stone deposition and recurrence by using a dietary approach based on CLO supplements.

Recommendation

Intake of cod liver oil is advised at least 3 times a week, especially for those vulnerable to stone formation or suffered previously from renal stones. It is also recommended for all adults as a preventive supplement for all diseases with membrane involvement.

List of abbreviations

Cod liver oil	CLO
Ethylene Glycol	EG
Superoxide dismutase	SOD
Malondialdehyde	MDA
Catalase	CAT
Glutathione	GSH
Polyunsaturated Fatty acids	PUFAs
docosahexaenoic acid	DHA
eicosapentaenoic acid	EPA

Declarations:

Ethical Approval: The October 6 University approved all animal treatments, which followed the Canadian Committee for Care's guidelines.

October 6 University has granted the following ethical approval number for the experimental study: 20230722.

Competing interests: The authors declare there were no competing interests.

Authors Contributions: SK contributed to the study conception and has written the first draft of the manuscript. SK and NH have done the biochemical and antioxidant status parameters. All authors participated in the design of the study, material preparation, animal experiments, data collection, and analysis. SK and TA have done histopathological analysis. Every author has offered feedback on the work. The final manuscript has been read and accepted by all authors.

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