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THE PROTECTIVE EFFECT OF SESAME OIL AGAINST RENAL TOXICITY INDUCED BY CCL_4 IN EXPERIMENTAL MODEL

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ABSTRACT

Renal toxicity is one of the most common kidney problems that occur when the body is subjected to drugs or chemical reagents as carbon tetrachloride (CCl_4). The aim of present study was to investigate the protective effect of a daily oral dose of sesame oil on oxidative stress, lipid peroxidation and DNA damage associated renal injury induced by CCl_4 injection. Renal injury was induced in rats by interaperitoneal injection of 10% CCl_4 dissolved in olive oil twice a week in a dose of 1ml/kg, another group of rats simultaneously received sesame oil orally at a dose of 1ml/kg for six weeks. At the end of the experimental period, the protective effect of sesame oil was measured on kidney injury molecule (KIM-1), oxidative stress (malondialdehyde (MDA)), total antioxidant capacity (TAC) and DNA damage. The present study found that sesame oil inhibited CCl_4-induced renal injury, lipid peroxidation, DNA damage and increased antioxidant capacity. It was found that co-administration of sesame oil along with CCl_4 ameliorated the toxic effect of CCl_4 which led to a significant decrease of urea, creatinine, MDA and significant increase in glutathione (GSH) levels, also decrease DNA fragmentation by reduced olive tail moment and the % of tail DNA and decrease KIM1 expression. Co-administration of sesame oil along with CCL_4 increased antioxidant capacity by increasing Nuclear factor-erythroid-2-related factor (Nrf2) and TAC levels. We hypothesize that a daily sesame oil supplement attenuates oxidative stress associated renal injury by reducing oxygen free radicals and lipid peroxidation in CCl_4 treated rats and increased antioxidant capacity.

Keywords: *Glomerular filtration rate, glomerular hyperfiltration, renal functional reserve, renal plasma flow, chronic kidney disease*

INTRODUCTION

Nowadays, natural products are gaining attention for their beneficial biological functions. Due to their antioxidative qualities, safe application, and potential involvement in intracellular and extracellular defense against lipid peroxides and oxygen radicals in response to oxidative stress One of these natural products that we choose to focus on in our inquiry is sesame oil (SO).[(1)] Sesamum indicum L. seeds, a member of the Pedaliaceae family, are used to make sesame oil (SO). The percentages of lipids, carbohydrates, moisture, and proteins are respectively 50%, 15%, and 15%. Sesamol, sesamin, sesamolin, and tocopherol are lignin constituents that provide sesame oil its oxidative stability. It also provides the best defense against lipid peroxidation by boosting both nonenzymatic and enzymatic antioxidants [(2,3).] Sesame oil is also a good source of dietary fiber, vitamin B1, calcium, magnesium, phosphorus, manganese, zinc, and copper. [(4)]

When the body is exposed to chemical agents or medications, it might develop a dangerous kidney condition called renal toxicity. The rise of harmful substances and hazardous wastes in our environment has emerged as the most pressing environmental pollution issue facing humans.[(5,6)] As a cleaning agent, carbon tetrachloride (CCl_4) was frequently employed in the chemical industry. [(7)] CCl_4, a non-polar molecule and one of the environmental poisons, may easily dissolve in non-polar substances including fats, oils, and iodine. [(8)] CCl_4 is a odorless, transparent and non-flammable material. The kidney and liver suffer severe harm from chronic exposure to CCl_4, which also raises the risk of cancer.[(9)] Research has shown that CCl_4

poisoning can cause the creation of free radicals in the majority of body tissues, including the kidney, liver, testicles, lungs, heart, brain, and blood. The cytochrome oxidase enzyme complex breaks down CCl^4 into the radicals trichloromethyl (CCl_3) and trichloromethyl peroxyl (Cl_3COO). [10,-12] CCl_4 is a nonthreshold multitargeted toxin that alters various human organs, including the kidneys[13,14], the heart[15], the blood vessels, and the hematotoxicity. [16]

It has been discovered that antioxidant response element (ARE) in the region where the genes that encode antioxidants are located is bound by Nrf2 in a variety of cell types and tissues.[17] In order to combat oxidative stress, the Nrf2-mediated modulation of cellular antioxidant and anti-fibrosis machinery is crucial.[18] In the cytoplasm, Nrf2 is kept inactive by a repressor molecule called Kelch-like ECH-associated protein1 (Keap1). Due to oxidative stress, Nrf2 and Keap1 are released from their bound state in the cytoplasm. Nrf2 moves into the nucleus and attaches to the ARE site.[19] Consequently, it has been determined that activating Nrf2 is one of the most important and promising molecular targets for protecting cells from oxidative stress and inflammatory shock. [20]
In both humans and animals, KIM-1 plays crucial functions in kidney function. [21] When it was discovered that KIM-1, also known as mucin-domain-containing molecule-1 (TIM-1) and T-cell immunoglobulin, was noticeably elevated in the proximal tubular cells of rats after ischemic injury, it was first postulated that it might play a role in restoration after a kidney injury.[22] It was shown that transgenic mice with continuous KIM-1 expression in their renal epithelial cells developed tubular interstitial fibrosis and inflammation, whereas mice with a shortened form of KIM-1 were resistant to fibrosis. [23]
Our study aimed to evaluate the protective effect of a daily oral administration of sesame oil against oxidative stress, lipid peroxidation and DNA damage associated with renal injury induced by CCl_4 injection and study the ameliorated effect of sesame oil on antioxidant capacity.

MATERIAL AND METHODS:

Experimental animals:

We utilised forty male Wistar rats weighing 180±20 g. After being randomly allocated to different groups, the rats were acclimated for 7 days under conventional conditions, including room temperature (25±3 °C) and 12 hours of light and dark cycles. All the animals were housed in strict sanitary circumstances, fed a diet of rodent pellets, and allowed unrestricted access to water. The work was authorised by the ethical committee for animal studies (Approval number: AU012-19-03-19-3-4). The investigation was conducted in accordance with the Medical Research Institute's Guide for the Care and Use of Laboratory Animals.

Experimental design:

The rats were divided up into four groups, each with ten rats. ***Group 1:*** functioned as the control group and was provided the regular food. ***Group 2:*** rats received 1 ml/kg of body weight of sesame oil orally twice a week for six week.. [24] ***Group 3***: rats were injected intraperitonealy with 1 ml/kg of body weight of 10% CCl_4 dissolved in olive oil twice a week for six weeks. [25] ***Group 4***: rats were injected with CCl_4 combined with an oral administration of sesame oil twice a week for six weeks.

Biochemical investigation:

Renal function tests

All rats were sacrificed by decapitation under anesthesia using ketamine 10% 75 mg/kg of body weight and xylazine 10 mg/kg of body weight. [26] Collected the blood and centrifuged at 3000 rpm for 10 min to obtain serum. Serum urea and creatinine were assessed calorimetrically using commercial diagnostic kits (Spectrum, Egypt).

Kidney homogenates

Upon decapitation, the kidney tissues of the rats were removed and thoroughly washed with ice-cold normal saline to get rid of all the blood cells. After being divided into small pieces, samples were then homogenized with a Heidolph (Silent Crusher) homogenizer to produce 10% homogenates by placing one piece in 50 mM Tris solution (pH 7.4). After centrifuging the homogenate for fifteen minutes at 3000 rpm, the supernatant was taken out, placed in an eppendorf tube, and centrifuged for twenty minutes at 12000 rpm. MDA; a marker of lipid peroxidation was measured in the whole homogenate, and TAC and glutathione-S-transferase (GST) activity were measured in the supernatant.

Determination of MDA level:

According to Draper and Hadley, lipid peroxidation in kidney homogenates was measured by measuring MDA levels spectrophotometrically in kidney homogenates. Lipid peroxidation was then represented in terms of thiobarbituric acid reactive compounds (TBARS). [27] 0.1 milliliters of the sample were combined with 0.75 milliliters of acetic acid, 0.75 milliliters of thiobarbituric acid (TBA), and 0.3 milliliters of distilled water before being cooked in a boiling water bath for an hour. Each tube first received an aliquot of 0.5 ml of distilled water, which was followed by 2.5 ml of butanol. The pink chromogen created when TBA and MDA reacted was extracted using n-butanol and detected at 532 nm. MDA concentrations were given as nmol/gm tissue.

Determination of GST activity:

GST initiates the conjugation process with glutathione n the initial phase in the production of mercapturic acid. The modified Carmagnol et al. approach was used to calculate GST activity. [28] Spectrophotometrically, the conjugation of GSH with 1 chloro 2, 4-dinitrobenzene (CDNB), which was catalyzed by GST, was determined at 340 nm. The amount of enzyme required to catalyze the synthesis of 1 mol of S conjugate per minute under the assay conditions is referred to as one unit of enzyme activity. [28]

Determination of TAC:

The TAC in the kidney was measured using an assay kit that is readily available in the market (Cat No. A015-1; Nanjing Jiancheng Bio Co., Nanjing, China). The analysis kit's methodology relies on iron (III)-mediated intracellular antioxidant oxidation in an acidic medium. The TAC of the samples was determined in compliance with the guidelines provided by the manufacturer. One unit of TAC was defined as the capacity to raise the optical density at 520 nm by 0.01 per mg protein per minute at 37 °C.

Determination of total protein concentration; by the method of Lowry *et al.* [29]

Determination of DNA damage by the comet assay:

The comet assay, which involved transferring 0.5 g of crushed materials to 1 ml of ice-cold PBS, was used to evaluate DNA damage. This suspension was filtered after 5 minutes of stirring. A total of 600 l of low melting agarose (0.8% in PBS) were combined with 100 l of the cell suspension before being dispersed over a microscope slide that had been previously coated with agarose with a normal melting point of 0.5%. After coating the slides, they were incubated for fifteen minutes in lyses buffer (0.045mol/l Tris/Borate/EDTA (TBE), pH 8.4, 2.5% Sodium dodecyl sulphate (SDS)). The slides were then placed in an electrophoresis chamber without SDS but with the same TBE buffer. The parameters used for the electrophoresis were 100mA and 2 V/cm for two minutes. After electrophoresis, the slides were washed in a neutralising solution (0.4 M Tris hydrochloride, pH 7.5). Ethidium bromide 20 g/ml was then used to stain the slides at 4°C. Observations of ethidium bromide-strained DNA were done using a 40 objective on a fluorescence microscope [with an excitation filter 420-490 nm connected to an Olympus camera] to visualize DNA damage. Using a computer-based image analysis system, DNA damage was measured using the Olive tail moment and the percentage of DNA in the tail (%Tail DNA) from 50 cells in each sample. [30]

One-step quantitative real-time polymerase chain reaction (qRT-PCR):

Total RNA was extracted from kidney tissues using the GF-1 Total RNA Extraction Kit (Vivantis, Malaysia). On Rotor-Gene Q, (Qiagen®, Valencia, CA, USA), qRT-PCR tests were carried out using the Rotor-Gene SYBR Green RT-PCR Kit. The initial reverse transcription step for cDNA synthesis took place for 10 min at 55 °C, and the resulting cDNA was then amplified by 40 cycles of PCR using the following temperatures: denaturation at 95 °C for 5 s, annealing at 55 °C for 15 s, and extension at 60 °C for 15 s. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH), a common gene, served as the reference gene for standardization. For rat genes, the following primers were used:
Nrf2:F:5′-CATTTGTAGATGACCATGAGTCGC-3′, R:5′CGGTGGGTCTCCGTAAATGG-3′,[31]
KIM-1: F: 5′-CGCAGAGAAACCCGACTAAG-3′, R: 5′-CAAAGCTCAGAGAGCCCATC-3′. [32]
GAPDH: F: 5′-CAACTCCCTCAAGATTGTCAGCAA-3′, R: 5′-GGCATGGACTGTGGTCATGA-3′. [33]

To determine the threshold cycle (Ct) values, Qiagen®, Valencia, CA, USA, provided Rotor-Gene Q-Pure Detection version 2.1.0 (build 9). The **2−ΔΔCt** technique was utilised to assess the relative change in mRNA in samples for each gene, and it was then normalised to the housekeeping gene GAPDH.

STATISTICAL ANALYSIS:

Version 18.0 of the SPSS software package (SPSS, Chicago, IL, USA) was used to analyse the data. The information was presented as mean ± SD. One way ANOVA was used to compare data between different groups.

RESULTS:

Effect of CCl_4 and SO on renal toxicity and oxidative stress:
Our result showed that CCl_4 cause renal toxicity indicated by significant higher urea and creatinine levels and oxidative stress indicated by significant higher MDA level and significant lower GSH level compared to control group. The co-administration of SO with CCl_4 ameliorated the toxic effect of CCl_4 which led to significant lower urea and creatinine levels compared to the rats administered with CCl_4. In comparison to the control, SO demonstrated a significant rise in GSH and a considerable decrease in MDA levels. **(Table 1)**

Effect of CCl_4 and SO on Nrf2 and TAC:
The current findings showed that the Nrf2 gene expression was significantly higher in the SO-treated group than in the control group. Conversely, rats treated with CCl_4 showed a 60% decrease in this gene's expression when compared to the control group; however, co-administration of SO with CCl_4 attenuated this impact, as demonstrated by a substantial rise in Nrf2 gene expression **(Figure 1).** According to the current findings, rats given SO had significantly higher TAC levels than the control group. Conversely, rats treated with CCl_4 had a substantially reduced TAC level than the control group; however, co-administration of sesame oil with CCl_4 attenuated this effect, as evidenced by a significantly higher TAC level **(Figure 2).**

Effect of CCl_4 and SO on DNA damage
Rats given CCl_4 showed evidence of DNA damage, as shown by the comet assay, which causes significant increase in olive tail moment and the percentage (%) of tail DNA while co-administration of sesame oil and CCl_4causes significant decrease in olive tail moment and the % of tail DNA compared to group treated with CCl_4 **(Figure 3).**

Effect of CCl_4 and SO on KIM-1 gene expression
In comparison to the control group, our investigation revealed a three-fold increase in KIM-1 gene expression in rats treated with CCl_4 and a substantial decrease in this gene expression in rats treated with SO. In contrast, the co-administration of sesame oil and CCl_4 significantly reduced the expression of the KIM-1 gene **(Figure 4).**

Table 1: Serum urea, creatinine and MDA levels and activity of GST in kidney tissue in control rats and rats treated with sesame oil, CCl_4 and rats co-administered with CCl_4 and sesame oil

Parameters	Control (n=10)	Sesame oil (n=10)	CCl_4 (n=10)	CCl_4+ Sesame oil(n=10)
Urea (mg/dl)	1.21±24.3	1.1± 23.1	42.5± 2.8 ab	32± 1.7 abc
Creatinine (mg/dl)	0.081±0.81	0.05 ±0.86	1.4± 0.16 ab	1.1± 0.02 abc
MDA (nmol/gm tissue)	2.65± 0.3	3.8± 0.3 a	7.2± 2.4 ab	4.5± 0.6 abc
GST (IU/l)	2.9± 57.1	65.8± 4.1 a	30.3± 3.5 ab	42.6 ±2.6 abc

Data illustrated as Mean ± SD (n=10); comparison was done using ANOVA at $p<0.05$
a: significant with control.
b: significant with Sesame oil group.
c: significant with CCl_4 and sesame oil.

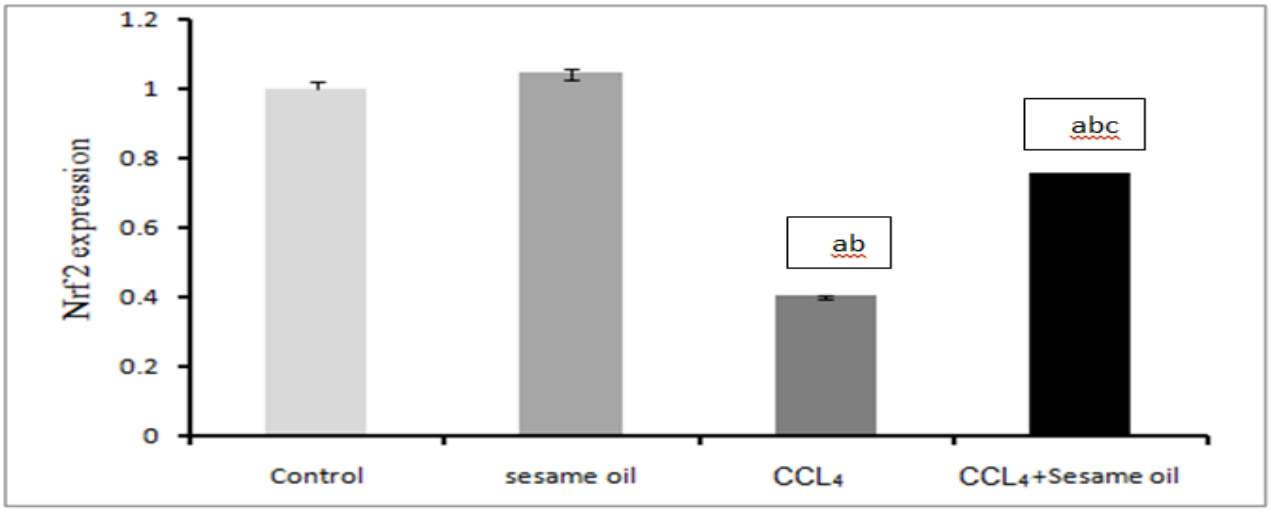

Fig. (1): Nrf2 expression in control and rats treated with sesame oil, CCl_4 and co-administrated with CCl_4 and sesame oil
a: significant with control.
b: significantly with Sesame oil group.
c: significant with CCl_4 and sesame oil

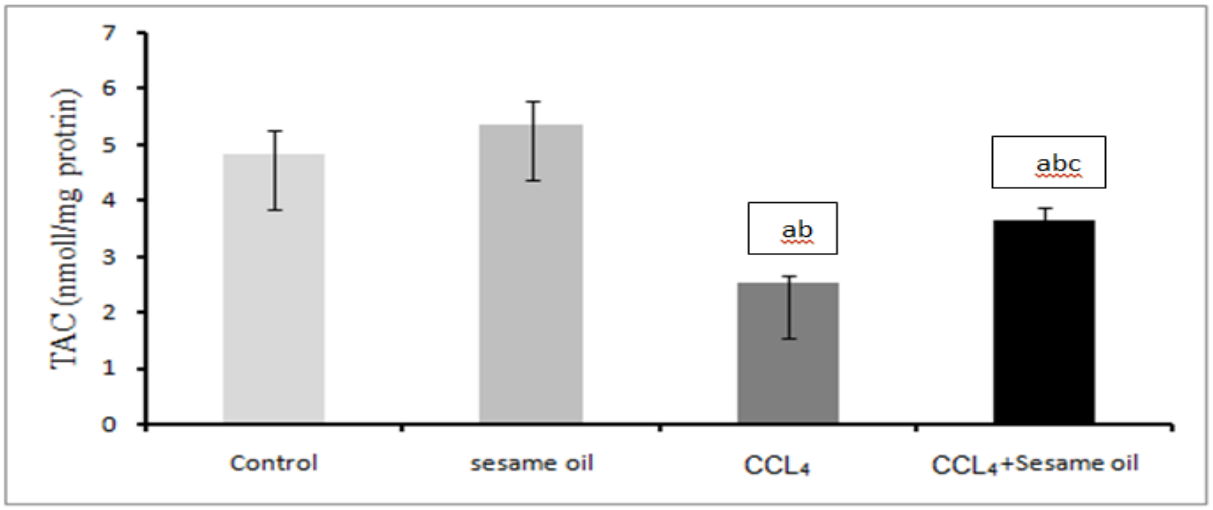

Fig. (2); TAC content in control and rats treated with sesame oil, CCl_4 and co-administrated with CCl_4 and sesame oil

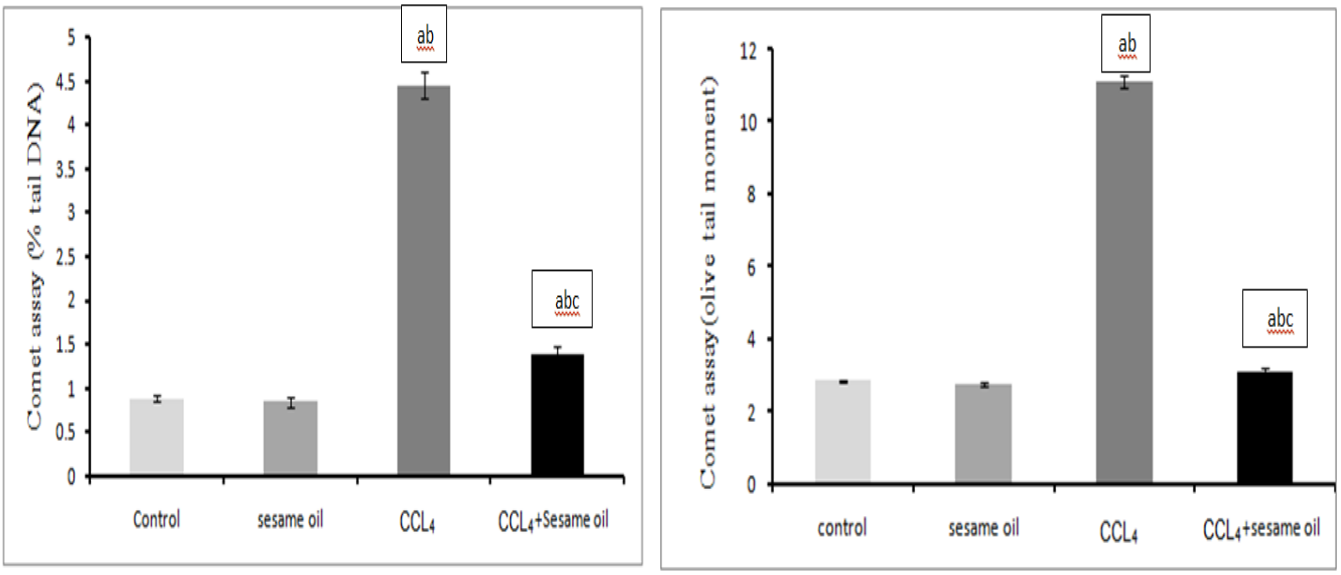

Fig. (3): Tail intensity (% of total genomic DNA found in the tail of the comet) and tail moment measured with comet assay in kidney cells in control rats and rats treated with sesame oil, CCl_4 and co-administrated CCl_4 and sesame oil

a: significant with control.
b: significant with Sesame oil group.
c: significant with CCl_4 and sesame oil.

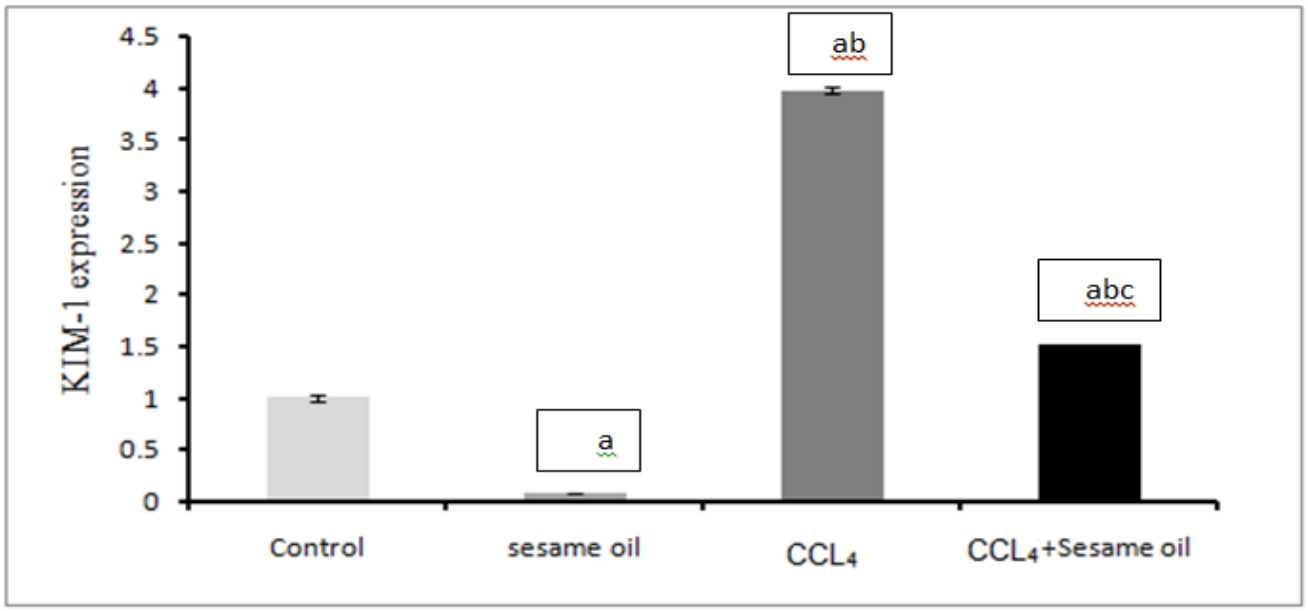

Fig. (4); KIM-1 expression in control and rats treated with sesame oil, CCl_4 and co-administrated with CCl_4 and sesame oil

a: significant with control.
b: significant with Sesame oil group.
c: significant with CCl_4 and sesame oil.

DISCUSSION:

Antioxidants are crucial components that help shield the body from damage brought on by free radicals. Chemicals' ability to act as antioxidants may help researchers create new treatments for diseases with a degenerative nature. Antioxidants are viewed as a strategy to prevent or delay the course of illnesses associated with oxidative stress due to the growing interest in free radical biology and the effectiveness of antioxidants in preventing oxidative stress. [34] It has been demonstrated that a significant number of herbal formulations have therapeutic effects against a variety of fatal diseases. [35]

Renal toxicity brought on by medications and chemicals is a leading cause of death globally. According to previously published research investigations, CCl_4 exposure damages the kidney by increasing the generation of reactive oxygen species (ROS). [36] It has been shown that a number of natural items have antioxidant capabilities and can control the production of free radicals to prevent acute kidney injury. [37] The results of the current studies demonstrated that giving rats CCl_4 greatly raised their

levels of creatinine and urea, but giving them sesame oil concurrently significantly decreased those levels.
Many investigations have demonstrated that the main source of free radical production in a number of organs, including the liver, kidney, lungs, brain, and blood, is CCl_4 poisoning. [38,39] Given that the kidney has a greater affinity for CCl4, it has also been demonstrated that, in rats treated with CCl_4, the kidney has a higher concentration of the drug than the liver. [39]Trichloromethyl radicals ($CCl_3^{\bullet}$) and trichloromethyl peroxyl radicals ($CCl_3O_2^{\bullet}$) are the most prevalent free radicals derived from CCl4. These free radicals attach to DNA, cell membrane lipids, and intracellular proteins, causing oxidative DNA damage, protein denaturation, and cell death. One of the key indicators of oxidative stress is the peroxidation of lipids.[40] After the treatment of CCl4, it was discovered that the level of MDA had dramatically increased in the renal tissue. Sesame oil co-administration has resulted in a significantly lower MDA level. This might be because sesame oil has antioxidant capabilities that scavenge free radicals and prevent lipid peroxidation.

An antioxidant called glutathione (GSH) is crucial in stopping any cellular damage brought on by free radicals and peroxides. [41] Our findings revealed a considerable decrease in GSH levels in CCl_4-treated rats, which can be attributed to CCl_4's involvement in inhibiting H_2O_2 clearance and encouraging the production of hydroxyl radicals ($^{\bullet}OH$), which cause oxidative stress. [42] There is growing proof that inflammation and oxidative stress work together to cause kidney injury. Recent research has identified the function of Nrf2 signaling in the regulation of inflammatory response and kidney protection against oxidative damage. Therefore, human studies have shown that plant-derived Nrf2 activators like curcumin are both safe and beneficial to health. As a result, Nrf2 activators have antioxidant and anti-inflammatory effects on kidney damage, which makes them potentially effective in treating or delaying renal disease. [43] After CCl4 administration, Nrf2 level drastically fell; however, co-administration of sesame oil restored the lowered level of Nrf2.
Contrarily, TAC assays detect low molecular weight, chain-breaking antioxidants without the contribution of metal binding proteins or antioxidant enzymes, rather than total antioxidant capacity. Numerous substances with chain-breaking antioxidant activity can be found in biological fluids, including thiol, ascorbate, bilirubin, and urates in the aqueous phase and -tocopherol, flavonoids, and carotenoids in the lipid phase. [44] Our study showed that TAC levels were significantly decreased in rats treated with CCl_4 while co-administration of sesame oil showed significant increase in TAC levels.

DNA laddering (DNA fragmentation) was undetectable in the kidney of control rats using agarose gel electrophoresis. There doesn't appear to be any DNA smearing or fragmentation in the DNA intact band, which is condensed close to the application spot. [45] Contrarily, CCl4 treatment led to significant DNA fragmentation and the production of a DNA smear on an agarose gel, a defining characteristic of necrosis without ladder formation, which suggests CCl4-induced renal cell injury. It was discovered that sesame oil worked well to stop the CCl4-induced smear development.
A emerging diagnostic for the early identification of acute kidney injury 17 (AKI17) is the transmembrane protein known as KIM-1. It is little expressed in healthy kidney tissue, but it is moderately to strongly expressed in the proximal convoluted tubule endothelial cells in the early stages of nephrotoxic damage or renal ischemia. It also has a relationship to how severe renal injury is. Furthermore, it seldom expresses in other organs and has a strong selectivity, particularly for ischemia or nephrotoxic acute kidney damage (AKI). [1] Our study showed that administration of sesame oil in normal rats showed a marked decrease in KIM-1 gene expression, on the other hand, CCl_4 showed a significant increase in its gene expression, whereas co-administration markedly decreased its expression. [46]
Diet and nutrition are crucial for managing symptoms and preventing the progression of CKD. SO is a non-toxic dietary oil that is used in most nations' diets that works well against a variety of illness conditions. It acts as a disease preventive and symptom management agent, preventing multiorgan failure. SO includes glycerol, vitamins, liganans and esters of fatty acids such sesamol, a strong antioxidant, which makes it preferable to chemical clinical care of CKD. [20] Sesamol's antioxidant property guards against organ damage brought on by iron, hepatotoxicity brought on by cyclophosphamide, and mucosal illness brought on by stress.

CONCLUSION

Findings arising from the present study suggest that SO attenuates CCl_4 induced CKD by activating Nrf2 and increases antioxidants thereby reduced oxidative stress and attenuating KIM-1 expression in rats. The positive effect of sesame oil further substantiates previous studies and jointly postulate therapeutic efficacy of SO in clinical conditions associated with CKD.

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CONFLICT OF INTEREST
There is no declared conflict of interest.

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