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The Study On Antioxidant And Anti-Inflammatory Effects Of Sodium Copper Chlorophyllin In Male Rats Receiving Indomethacin

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Abstract

Sodium copper chlorophyllin (SC-C) is widely used for its biological properties and color. This study evaluated SC-C's antioxidative and anti-inflammatory effects in male rats treated with indomethacin. Fifty rats were divided into five groups: control, indomethacin, rabeprazole, and two SC-C dosages (35 mg/kg and 70 mg/kg). Indomethacin reduced serum GPx levels, but rabeprazole and SC-C treatments restored them. MDA levels increased with indomethacin but normalized with other treatments. Indomethacin also lowered IL-10, IL-4, and TNF, which returned to normal in treated groups. Histopathology showed SC-C reversed liver damage from indomethacin. SC-C demonstrates strong antioxidant and anti-inflammatory activities, suggesting its potential as a therapeutic agent.

Highlight:

SC-C significantly restores serum GPx levels reduced by indomethacin.
SC-C normalizes MDA, IL-10, IL-4, and TNF levels, countering oxidative stress.
Histopathology shows SC-C reverses liver damage induced by indomethacin.

Key words: Sodium copper chlorophyllin, antioxidant, anti-inflammatory, indomethacin, oxidative stress

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Introduction

Naturally occurring pigments found in food, chlorophylls are becoming increasingly and more important, especially in light of consumers' growing inclination toward healthier lifestyles. Dietary chlorophyll is present in fresh vegetables and fruits as chlorophyll a and b, and in thermally processed produce such as metal-free pheophytins and pyropheophytins. Supplements, liquid solutions, extracts, pills, and insufficiently utilized greens in fresh vegetables can all be good sources of chlorophyll for a nutritious and healthful diet (Park, Jeon, Kim, & Kim, 2023; Tagaov et al., 2023). Plants have both chlorophyll a and chlorophyll b, but chlorophyllin is a water-soluble, semi-synthetic combination of sodium copper ions that is generated from chlorophyll. Through a variety of processes, it can provide protection against conditions like oxidative stress, neurological illnesses, cataracts, osteoporosis, atherosclerosis, and some forms of cancer. Chlorophyll-a accounting for about 75% of all plant-found green pigments.

Sodium copper chlorophyllin, or SC-C, is a naturally occurring chlorophyll derivative that is being utilized more and more as a food supplement and colorant. Without any noticeable negative effects, SC-C has been linked to a wide range of health advantages, including erythropoietic, deodorizing, and anti-inflammatory properties. Additionally, SC-C derivatives demonstrated the capacity to activate phase II enzymes such as quinone reductase. SC-C has attracted a lot of attention as a possible cure and preventative measure for chronic illnesses as a consequence of previous studies. Growing evidence suggests that Lv Wei Capsule, which mostly comprises of SC-C, is a potentially useful drug for enhancing immunity and treating stomach ulcers (Lv, Lin et al. 2019). Based on our previous research, we hypothesize that SC-C may reduce indomethacin-induced stomach ulcers by decreasing oxidative stress and reducing inflammation.

Reactive nitrogen species (RNS) and highly reactive oxygen species (ROS) are produced when there is an imbalance in the homeostasis of pro-oxidant, and these species can play a crucial role in the breakdown of lipids and DNA in cells. One important aspect of health is oxidative damage. Low to moderate concentrations of ROS/RNS produce molecular signals that control a number of physiological processes, including breathing regulation, vascular tone preservation, and redox-homeostasis support. The body's enzymatic and non-enzymatic antioxidant systems, such as water-soluble vitamin C, lipid-soluble vitamin E, carotenes, glutathione peroxidase (GPX), catalase (CAT), and superoxide dismutase (SOD), control the ratio of reactive oxygen species (ROS) to antioxidants. However, dietary antioxidants; which are typically found in fruits and vegetables have also been linked to a healthy balance between antioxidant status and free radicals, which lowers the risk of oxidative stress and, consequently, cancer, cardiovascular disease, and aging. (Salehi et al., 2018)

Two significant redox antioxidant systems are the glutathione and thioredoxin systems (Minich 2022). The most important of these selenoproteins are iodothyronine deiodinase (DIO), selenoproteins W, N, and P, as well as glutathione peroxidase (GPXs) and thioredoxin reductase (TRXRs) (Juszczuk-Kubiak et al. 2016). Five of the eight glutathione peroxidases (GPXs) found in mammals (GPX1-4, GPX6) are selenoproteins. As reductants, they mostly use glutathione or thiol oxidoreductases. They play a number of functions, including ferroptosis regulation, hydroperoxide detoxification, and hydrogen peroxide signaling (Tsuji et al. 2022). Thioredoxin reductases are a class of selenoproteins whose primary activity is to decrease thioredoxins. Because of their broad selectivity, they can also reduce other endogenous and exogenous substrates (Martinez et al. 2022). Lipid peroxidation produces malondialdehyde (MDA), a naturally occurring chemical. MDA is mutagenic and carcinogenic because lipid peroxidation is a well-established mechanism of cellular harm and functions as a trustworthy biomarker of oxidation (N. Neamah, Naaem Khudair, & Al-Jadaan, 2020).

The immune system's initial biological reaction to an infection, wound, or irritant is inflammation. There is evidence to suggest that the modulation of many inflammatory cytokines, including prostaglandin E₂, interleukins, tumor necrosis factor alpha- α , and interferon gamma- γ , as well as nitric oxide, is responsible for the anti-inflammatory impact. High concentrations of phytochemicals found in fruits, vegetables, and dietary legumes have been shown to have anti-inflammatory effects; however, their exact mechanisms of action are yet unknown (Zhu, Du, & Xu, 2018).

Interleukins (ILs) are a class of cytokines that were formerly thought to be expressed only by leukocytes. Later findings, however, have shown that several other cells throughout the body also produce them. These chemicals are essential for immune cell activation and differentiation, as well as for processes like adhesion, migration, maturation, and proliferation. ILs have anti-inflammatory as well as pro-inflammatory characteristics. Inflammatory and immunological responses involve growth, differentiation, and activation, which are all fundamentally regulated by ILs. IFNs (immune ligands) are a complex class of proteins that bind to cell surfaces' high-affinity receptors to induce a variety of cellular and tissue responses. They are useful for both autocrine and paracrine processes. Moreover, ILs are used in animal research to investigate elements of clinical medicine (Rafaqat, Gluscevic, Mercantepe, Rafaqat, & Klisic, 2024). The absence of a clearly defined role for IL-4 may be due to its wide range of functions. As a result, the variation in IL-4 actions may account for discrepancies in research findings. In addition, the anti-inflammatory cytokine IL-10 is in charge of B-cell growth and differentiation, immune response suppression, and B-cell activity prevention. By inhibiting the synthesis and actions of significant pro-inflammatory cytokines, IL-4 has anti-inflammatory effects. IL-4 reduces IL-6 secretion and activity in addition to nearly totally blocking TNF- α 12 and IL-1 β synthesis and secretion (Shiau et al., 2019)

Methods

preparation of animals

Fifty mature male albino rats weighing 180–200 grams provided for this study by the University of Zakho / College of Veterinary Medicine. The animals were divided into five groups at random ($n = 10$) and housed in separate polypropylene cages lined with sawdust one week before the trial started to allow the animals to get used to their surroundings. The four or five rats in each cage were given tap water and the regular rat pellet diet. The animals were kept in a room with carefully managed conditions, including a 12-hour light/dark cycle, a temperature of $21 \pm 2^\circ\text{C}$, and a humidity of $30 \pm 11\%$. They received unrestricted access to water and their usual meals during the trial (N. F. Neamah, Naaem Khudair, & Al-Jadaan, 2019). Under chloroform anesthesia, blood was extracted from the rats' heart at the conclusion of the treatment period, giving about 6 milliliters. For twenty to thirty minutes, the gathered samples were stored at 21°C in gel tubes. Following that, serum was obtained by centrifugation at 4000 rpm for 15 minutes. The serum was then stored at -20°C for additional laboratory examination.

After being removed, the livers were cleaned using a 0.9% saline solution. The samples were embedded in paraffin wax, fixed in 10% formalin, and then cut into $5 \mu\text{m}$ slices. The slices (H and E) were then stained with hematoxylin and eosin.

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Oxidative Stress Model and Experimental Procedures

The University of Basrah's College of Pharmacy provided the infrastructure for the study, including the animal housing and labs. Ten rats each were randomly assigned to one of five groups, and each group was kept apart in its own cage. Rabeprazole was the reference anti-ulcer drug of choice for this investigation; the oral dosage was 20 mg/kg of body weight. The rats were given free rein to drink water prior to the experiment. The Indomethacin was given to the rats once a day for a maximum of seven days. The article was authorized by the Basrah University Animal Ethics Committee ((No.EC 30 updated to 1/9/2023)) for all animal handling protocols.

Design experimentation

Five groups of ten rats apiece were randomly assigned to the animals. The rats had twenty-four hours without food but freed access to water before the experiment. The exact same treatment, administered at a specific dose, was given to each group. For a maximum of seven days, rats received one dose per day:

1. Control Group (G1): for seven days, participants in this group were given 2 mL of distilled water (DW) orally via mouth gavage as a negative control.
2. Indomethacin treated group (G2): utilizing a magnetic hot plate, 50 mg/kg BW of pure indomethacin dissolved in DW as suspension was administered orally to the ulcers-induced group. The rats were given one dosage on the first day and two milliliters of DW six days later.
3. Rabeprazole treated group (G3): as positive group, indomethacin was administered orally on the first day, followed by Rabeprazole administration orally at a dose of 20 mg/kg BW two hours later. For the next six days, rabeprazole was given every day at 9:00 AM.
4. Sodium-Copper chlorophyll (SC-C) treated group (G4): first group received SC-C orally at a dose of 35 mg/kg on the first day, along with indomethacin. During the six days, just SC-C was administered.
5. Sodium-Copper chlorophyll (SC-C) treatment group (G5): Two hours after the first day's administration of Indomethacin, SC-C was administered at a dose of 70 mg/kg BW. In the six days that followed, only SC-C was given.

Training for Tissue Homogenates

Sections of liver were cut into pieces (each piece =500mg), homogenized in 4.5 ml phosphate buffer saline (PH:7.4), and centrifuged for 15 minutes at 4°C at 10,000 rpm. The supernatant was gathered and kept at -20°C in serum collection tubes to make analysis easier (Ibtihal J. Naoor, 2023).

Measurements of GPX activity, MDA, IL-4, IL-10, and TNF

The Sandwich enzyme-linked immunosorbent assay was used to assess the amounts of each of those enzymes (ELISA kit for rat: Elabscience Biotechnology Inc. China). In less than three hours, the measurements for GPX, MDA, IL-4, IL10, and TNF were completed.

Analytical Statistics

The One-way analysis of variance (ANOVA) method. Statistical significance was indicated by a value of $p < 0.05$. For every group, the data were presented as mean \pm standard deviation of the mean (SD).

Results and Discussion

Result

Antioxidant activity

Table (1) show that the G2 group had considerably lower serum GPx levels ($p < 0.05$) at both Tissue homogenies(T-Homo) and serum samples compared to the G1 group, while the G3 showed significant increase than G2 but not with G1. G4 and G5 groups performed significantly higher in terms of blood GSH levels ($p < 0.05$) than the G1,G2 and G3 groups.

According to table (1), MDA level was increased ($p < 0.05$) in group G2 compared with G1 at both serum and tissue homogenized samples. While, MDA T- homo levels were significantly ($p < 0.05$) decreased in the G3, G4 and G5 in comparison with G2 level.

MDA serum of G2 and G3 significantly increased in MDA than G1. While G4 and G5 showed significant ($p < 0.05$) decline in the MDA concentration than G2. G5 serum MDA concentration significantly decreased than G3 .

In G2,T-Homo MDA levels appeared to increased ($p < 0.05$)than in G1, while G3, G4, G5 the concentrations of MDA reduced($p < 0.05$) than in G2.

Groups	GPX(pg/mL) T-Homo	GPX(pg/mL)serum
G1(control)	806.8 \pm 238.958	193.17133 \pm 59.43
G2(Indol)	438.4588 \pm 255.3538 a	105.32 \pm 13.629a
G3(Indol+Rabe)	857.844 \pm 162.108b	187.255 \pm 74.564 b
G4(Indol+SC-C)	1155.60 \pm 295.573abc	248.226 \pm 61.792 abc
G5(Indol+ SC-C.H)	1244.66 \pm 521.408 abc	267.362 \pm 154.053 abc
LSD	378.9465	81.76866906
MDA(ng/mL) T- Homo	MDA(ng/mL) serum	
G1(control)	295.8875 \pm 162.3509	176.0714 \pm 106.4484
G2(Indol)	916.5156 \pm 491.7907a	538.275 \pm 102.3292 a
G3(Indol+Rabe)	361.5375 \pm 238.4519b	378.15 \pm 183.58911 a
G4(Indol+ SC-C)	211.0563 \pm 116.983b	230.96429 \pm 111.2651 b
G5(Indol+ SC-C H)	253.235 \pm 160.6692b	163.94 \pm 65.03902 bc
LSD	350.609	178.6923

Table 1. illustrated the antioxidant activity of SC-C in each experimental group in comparison with control groups

Ant inflammatory activity

According to the table (2) below, IL-4 serum levels significantly increased ($p < 0.05$) when the G2 and G3 in comparison with G1. The IL4 levels in G3, G4 and G5 significantly ($p < 0.05$) reduced in comparison with G2, with no significant differences with G1. Also there was significant reduction in G5- IL4 value than in G3. IL-4 ,T-Homo levels illustrate increased in IL4 value in G2 than G1. While G3,G4 and G5 the concentration of IL4 decreased than G2. Only G4 showed significant reduction than IL4 level in G3

IL 10 levels exhibit significant increase in G2 than in G1 at both serum and T.Homo samples. G3, G4, and G5 serum IL10 concentrations decreased significantly than G2 with no significant changes than in G1. While in T-Homo samples the results illustrate that G3 significantly differ than G1 levels, with no significant alteration with G2. While, G4 and G5 concentration levels decreased than G1.

TNF levels measurements

G2 in both serum and T.Homo samples showed statistically elevation than in G1. At serum samples, TNF concentrations in G3, G4, and G5 reduced significantly than that in G2. T-Homo samples at G4 and G5 TNF values significantly decline than G2, only G5 differ than G3.

Groups	IL4 (pg/mL) T-Homo	IL4 (pg/mL) Serum
G1(control)	163.1444±50.65829	166.1375±52.9208
G2(Indol)	632.6222±146.3119a	594.5625±86.3317 a
G3(Indol+Rabe)	269.1222±120.8833b	273.3375±200.3758 ab
G4(Indol+ SC-C)	156.61±80.02878bc	219.662±81.49277 b
G5(Indol+ SC-C.H)	207.8678±148.7483b	112.4625±43.8166 bc
LSD	110.3843	110.2306
Groups	IL10 (pg /mL) T -Homo	IL10 (pg /mL) Serum
G1(control)	792.3261±236.9118	57.123±4.762296
G2(Indol)	1013.064±28.55345a	70.74933±14.35233 a
G3(Indol+Rabe)	920.1285±33.78337a	56.59089±3.780668 b
G4(Indol+ SC-C)	892.8114±95.91915b	55.45189±8.405203 b
G5(Indol+ SC-C H)	898.7716±53.42018b	50.87733±7.578393 b
LSD	120.6991	8.207345
Groups	TNF (pg /mL) T-Homo	TNF (pg /mL) serum
G1(control)	256.75±69.7370	19.09192±5.18577
G2(Indol)	380.175±173.839 a	38.81944±23.15203 a
G3(Indol+Rabe)	293.3875±76.3141	18.12816±6.233827 b
G4(Indol+ SC-C)	191.1213±54.9944 b	26.28982±7.04467 b
G5(Indol+ SC-C.H)	183.0625±59.9631 b c	23.61029±8.609085 b
LSD	98.96339	12.22719

Table 2. showed proinflammatory and anti-inflammatory biomarkers level on all test groups

Histopathological liver examination

The liver of control rats is composed of up of hexagonal or pentagonal lobules with central veins, outer hepatic triplets or tetrads embedded in the connective tissue, and bands of organized hepatocytes radiating from the central vein, with Kupffer cells-containing sinusoidal structures acting as partitions between the bands. Its morphology is regular, they have a large spheroidal nucleus, a distinct nucleolus, and chromatin is distributed across the periphery. Certain hepatic cells have two nuclei in each(refers to cell division).

After consuming indomethacin concurrently, liver slices photographed under a microscope show dilated central veins, sinusoid congestion, and most hepatic lobules' trabecular structure obscured. Hepatocytes and pyknotic nuclei referred that the nuclei undergoing to necrosis or apoptosis, also some cells have empty areas that resemble the vacuoles of cytoplasmic fatty alterations. A significant number of Kupffer cells, sinusoid congestion, and dilated central veins are observed in the sinusoid walls. Additionally, the liver of a rat in the positive control group had a focal region with a microabscess composed of necrotic material and inflammatory cells.

The livers of rats given varying doses of SC-C for seven days in G4 and G5 were studied. The majority of hepatic lobules typically have substantially intact epithelium and no signs of blood vessel dilatation. The trabecular structures are somewhat deformed and split by sinusoids that contain Kupffer cells. There are occasionally cells in the cytoplasm that have empty spaces that resemble vacuoles. There are somewhat more Kupffer cells in the sinusoid walls than in other areas. Hepatocytes contain a large, spheroidal nucleus and are regular in shape.

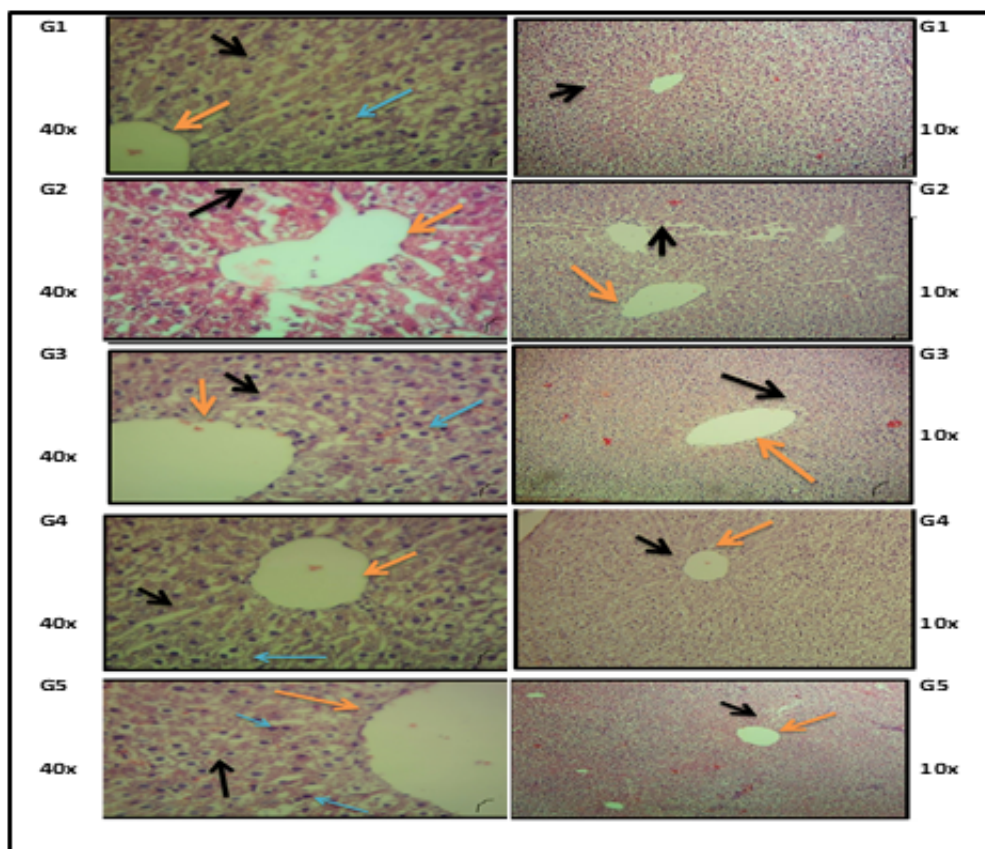


Figure 1. Representative microscopic tissue images of rat liver

Rats in the control group (G1) had liver slices that demonstrated a normal hepatic architecture. There are no symptoms of congestion, intact blood arteries (orange arrows), normal hepatocytes (black arrows), and Kupffer cells. Rats in the G2 had aberrant trabecular structure, fatty degeneration of the hepatocytes, necrotic debris and inflammatory cells, and a high concentration of Kupffer cells in their liver sections. Rats in the G3 group had liver sections that revealed mostly normal hepatic cells, with some fatty degenerated hepatic cells. An undamaged central vein with no longer any congestion, however inflammatory cells were present. Some of cell division are appear (blue arrow). Rats in the G4 and G5 had liver sections that displayed normal hepatic architecture, normal hepatic cells, and a normal central vein without any indications of congestion. Large number of hepatic cells were in cell division phase

Discussion

Antioxidant activity

One of the advantages of chlorophylls is their antioxidant activity, which works by chelating metal ions and decreasing reactive oxygen species (ROS) to prevent oxidative DNA damage and lipid peroxidation. Cu-chelated metal in the porphyrin ring gives Cu-chlorophyllin a claimed greater antioxidant activity than natural chlorophylls. It was discovered that the Cu-chelated compounds' antioxidant activity were significantly higher than those of Mg-free derivatives and naturally occurring chlorophylls (Suparmi, Fasitarsi, Martosupono, & Mangimbulude, 2016).

The potential of oxidative damage in cell suspensions from the liver, brain, and testis was significantly reduced when mice were given 1% of chlorophyll in their drinking water. The relationship between singlet oxygen and hydroxyl radical, two strong ROS, and chlorophyll was investigated in order for searching the potential causes for the stated antioxidant capabilities. The results demonstrated that chlorophyll is a very potent antioxidant that can shield mitochondria from oxidative damage brought on by different ROS (Nissa, Kartasurya, & Rahmawati, 2016).

According to another study results reported by (Lanfer-Marquez, Barros, & Sinnecker, 2005). Cu chlorophyllin had a greater antioxidant activity than natural chlorophylls, demonstrating the significance of the chelated metal's nature in the porphyrin ring, according to the results of the β -carotene bleaching procedure and the DPPH assay. The ability to donate hydrogen does not appear to be the mechanism behind the antioxidant activity exhibited by natural chlorophyll derivatives; instead, it may be related to linoleic acid's defense against oxidation and/or the prevention of hydroperoxide breakdown.

Lipid peroxides have a tendency to break down quickly into a range of sub products once they become unstable molecules. MDA is one of the most well-known lipid peroxidation secondary products that can be utilized as a marker for damage to cell membranes. An elevated MDA level is thought to be a sign of tissue damage and altered cell activity. Owing to the production of ROS or free radicals, this event manifests in the liver or kidneys. Natural anti-oxidants frequently reduce intracellular oxidative stress by preventing the production of free radicals (Neamah et al., 2020).

The study's findings showed that SC-C therapy helped to reoccurrence MDA levels to normal following indomethacin treatments. According to a previous study, rats given NaNO₂ had considerably higher serum MDA levels than control rats. This finding is consistent with earlier research that found higher MDA levels in rats given (Suparmi et al., 2016). The outcomes concur with the current study's findings. Additionally, oxidative stress caused by indomethacin was found in a trial where the drug was used to produce stomach ulcers. Rat stomach tissue treated with indomethacin showed a rise in MDA and a decrease in both enzymatic and nonenzymatic antioxidants, including catalase (CAT), glutathione peroxidase (GPX), SOD, GSH, and glutathione S-transferase (GST) (Suleyman, Albayrak, Bilici, Cadirci, & Halici, 2010).

Antinflammatory effects

Many pro-inflammatory mediators and nitric oxide are produced, which alters the microcirculation to bodily tissues by increasing vascular permeability and decreasing effective circulating volume. These responses to numerous organs primarily result in shock and the development of multiple organ dysfunction syndrome, which is associated with a high death rate. Immunosuppressive mediators with anti-inflammatory properties, such as prostaglandin E₂, IL-4, IL-10, IL-13, and others, prevent inflammatory cells from producing pro-inflammatory mediators. Thus, in order to control inflammation and promote its recovery, pro- and anti-inflammatory mediators must work together (Lee et al., 2002).

The primary source of necrotic cell death is the inflammatory reaction, which may be a biological role in healing. The two primary inflammatory cells responsible for induced liver damage are neutrophils and macrophages, which occur prior to cellular mitosis begins. Macrophage activation has been demonstrated to promote hepatocyte proliferation by eliminating damaged tissues that will be replaced by new cells (Sahar Muayad Noori; Nadheerah Falih Neamah, 2024).

The development of allergic inflammation is significantly aided by the cytokine interleukin (IL)-4. IL-4 augments IgE-mediated immune responses by upregulating IgE receptors on the cell surface, including the high-affinity IgE receptor on mast cells and basophils and the low-affinity IgE receptor on B lymphocytes and mononuclear phagocytic cells. Furthermore, by eliciting eosinophil chemotaxis and activation through elevated eotaxin expression (Steinke & Borish, 2001), IL-4 promotes eosinophilic inflammation and inhibits eosinophil apoptosis. IL-4 also has anti-inflammatory properties by lowering the production and activity of significant pro-inflammatory cytokines.

One of the key variables in the development of inflammation, degradation, and carcinogenesis in several types of tissues, including stomach ulcers, is proinflammatory cytokines, particularly TNF- α . Primarily all, TNF- α has complementary and cooperative actions that trigger the secretion of other cytokines. The inflammatory response was assessed by measuring TNF- α and IL-6 in serum using ELISA. In contrast to the normal groups, the mice treated with SC-C (20, 40, and 80 mg/kg) exhibited a significant reduction in TNF- α level ($P < 0.05$, $P < 0.01$, and $P < 0.01$) in a dose-dependent manner. Intra gastric administration of ethanol caused obviously increasing levels of pro-inflammatory cytokines TNF- α ($P < 0.01$) and IL-6 ($P < 0.01$). Concurrently, while administering 40 and 80 mg/kg of SC-C, the level of serum IL-6 was significantly reduced ($P < 0.05$ and $P < 0.01$, respectively) in comparison to the ulcer model group (Lv et al., 2019).

The source of the immune-suppressive cytokine IL-10 is human monocytes, macrophages, and T and B lymphocytes. IL-10 has been linked to the regulation of lymphoid and myeloid cell activities due to its capacity to inhibit T-cell and monocyte/macrophage production of pro-inflammatory cytokines. determined that IL-10 is a strong inhibitor of pro-inflammatory cytokines generated by PMNs, it was shown that IL-10 reduced the levels of TNF- and IL-8 mRNA after the start of PMN stimulation with LPS. These findings imply distinct anti-inflammatory mechanisms for IL-4 and IL-10. (Ciciliato et al., 2022).

When levels of pro-inflammatory cytokines in the blood rise, IL-10 can function as an internal "danger signal," meant to shield the body from the damaging effects of an unhealthy hyper inflammatory condition. As a master regulator of immunity during infection by bacteria, fungi, viruses, protozoa, and other pathogens, IL-10 is crucial for controlling or eliminating inflammation and the ensuing host defense. Innate immune cells often produce IL-10 later on in the inflammatory process than pro-inflammatory cytokines, which are released early on. In order to restore normal tissue homeostasis, IL-10, which is released at the site of continuing inflammation, must strike the correct balance between preventing harmful immune-mediated responses against infections and effectively eliminating pathogens. (Carlini et al., 2023)

T helper (Th1) pro-inflammatory cytokines, such as TNF- α , play a crucial role in the inflammatory response, both locally and systemically. TNF- α stimulates immune cell infiltration by increasing leukocyte adhesion molecules and

triggering the expression of vascular endothelial cells. By promoting lymphocyte infiltration to the infection site, it plays a critical role in the early response against viral infection.

IFN- γ or TNF- α are released when immune cells lyse or activate, primarily T cells, according to the pathophysiology of cytokine storm theory. As a result, endothelial cells, dendritic cells, macrophages, and other immune cells become activated. These cells release more pro-inflammatory cytokines after activation. T cells are activated by macrophages and endothelial cells that release large levels of IL-6. Macrophages and endothelial cells create large amounts of IL-6, which activates T cells and other immune cells. This leads to a positive feedback loop that causes a cytokine storm, which not only upregulates acute phase proteins but also triggers the release of many more cytokines and chemokines.

Histopathological study

In the present study, liver sections of SC-C treated groups showed normal hepatocytes. Rats in the liver damage-induced group exhibited abnormal trabecular architecture, fatty hepatocyte degeneration, inflammatory cells and necrotic debris in their liver sections, as well as a high number of Kupffer cells. Rats in the groups that received SC-C treatment, on the other hand, had liver sections with normal hepatic architecture, normal hepatic cells, and a normal central vein free of congestion. A significant portion of the liver cells were dividing.

Compared to a previous publication, the data indicate that there was an increasing score of hepatocyte injury in the group that had liver damage caused by NaNO₂. The rat liver cells in the NaNO₂-induced group showed hydropic degeneration, whereas the cells in the control group showed normal or non-changing histology. According to Suparmi, Fasitasari et al. (2016), the hepatocytes treated with Cu-chlorophyllin had the highest hepatocyte score, with minimal variation from the control group. The outcomes comparable to the current study's

Conclusion

The SC-C have antioxidant and anti-inflammatory activity against indomethacin induced oxidative stress.

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