Study the antioxidant effect of coconut oil in rabbits treated with sodium nitrate

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دراسة تأثير زيت جوز الهند كمضاد للأكسدة في الأرانب

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المستخلص

أجريت الدراسة الحالية لمعرفة تأثير تراكيز مختلفة من زيت جوز الهند كمضاد للأكسدة وبواقع (1.5, 2, 2.5 ml/kg) والمعطى عن طريق الفم على معايير الدم (كريات الدم البيضاء , الهيموكلوبين ,حجم الخلايا المرصوص والصفيحات الدموية) وعلى المعايير الكيموحيوية (الكوليسترول بأنواعه HDL,LDL, VLDL , الدهون الثلاثية) وعلى مستوى انزيمات الكبد (سوبر اوكساید دزميوتيز, الكلوتاثيون)في الأرانب التي تم إستحداث الأكسدة فيها بأستخدام نترات الصوديوم بتركيز (75 ملغم/ كغم) , وقورنت جميع النتائج مع حيوانات السيطرة . أظهرت النتائج نقصان في مستوى الدهون الثلاثية والكوليسترول الضار LDL والانزيمات وارتفاع مستوى الكوليسترول المفيد HDLوفي عدد خلايا الدم البيضاء والصفيحات الدموية والهيموكلوبين وانخفاض مستوى الانزيمات يعود لزيادة عدد الجذور الحرة نتيجة لعمليات الاكسدة جراء استخدام نترات الصوديوم .

الكلمات الافتتاحية:نترات الصوديوم , زيت جوز الهند , مستوى الدهون بالدم , المعايير الدموية .

Abstract

The present study was conducted to explore the effect of different concentrations of coconut oil as an antioxidant with rate of (1.5, 2 ,2.5ml/kg) of body weight given orally and measure Hematological parameters (white blood cells,PCV , Hb and platelets) and biochemical parameters (cholesterol and its different kinds (Total cholesterol, HDL, LDL, VLDL), triglycerides) and liver enzymes (Superoxide dismutase , Glutathione) in rabbits that demonstrated sodium nitrate as oxidative material use concentration (75 mg / kg), and compared to all results with control group . The results showed a decrease in triglycerides, LDL , SOD and GSH , increase HDL cholesterol levels and a raise in the number of white blood cells, platelets and Hb level this results belong to increase the number of free radicals as a result of oxidative stress caused by the use of sodium nitrate.

Key Words: sodium nitrate, coconut oil, lipid profile, hematological parameters.

Introduction

Coconut has been consumed for a long time, especially within Asian cuisine. The supply andinterest in coconut consumption has increased during the last few years. It is promoted as a dietary supplement said to optimize health (1).The edible part of the coconut consists of the white flesh (copra) and coconut milk (also called coconut water). It is from the flesh coconut fat is extracted (2). Nowadays, virgin coconut oil (VCO) has become popular due to its beneficial effects. VCO has been shown to have anti-inflammatory, analgesic, and antipyretic properties (3) . VCO has been shown to decrease lipid levels in serum and tissue as well as LDL lipid

peroxidation (4). Consumption of VCO enhances antithrombotic effects related to inhibition of platelet coagulation and low cholesterol level (5).VCO has been known to have higher antioxidant activity compared to refined coconut oil (6). Antioxidants' are substances that neutralize free radicals or their actions (7). Nature endowed each cell adequate protective mechanisms against any harmful effects of free radicals: superoxide dismutase (SOD), glutathione peroxidase, glutathione reductase , thioredoxin , thiols and disulfide bonding are buffering systems in every cell. α-Tocopherol (vitamin E) is an essential nutrient which functions as a chain-breaking antioxidant which prevents the propagation of free radical reactions in all cell membranes in the human body. Ascorbic acid (vitamin C) is also part of the normal protecting mechanism. Other non-enzymatic antioxidants include carotenoids, flavonoids and related polyphenols, α-lipoic acid, glutathione etc. Antioxidants, capable of neutralizing free radicals or their actions, act at different stages. They act at the levels of prevention. Preventive antioxidants attempt to stop the formation of ROS. These include superoxide dismutase (SOD) that catalysis the dismutation of superoxide to H2O2 and catalase that breaks it down to water (8).There are two main types of antioxidants:

1. Exogenous antioxidants like: vitamins A,C and E.
2. Endogenous antioxidants like: Glutathione (GSH), SOD.

Oxidative stress is an imbalance between the systemic manifestation of reactive oxygen species and a biological system's ability to readily detoxify the reactive intermediates or to repair the resulting damage. Disturbances in the normal redox state of cells can cause toxic effects through the production of peroxides and free radicals that damage all components of the cell, including proteins, lipids, and deoxyribonucleic acid (DNA). Oxidative stress can cause disruptions in normal mechanisms of cellular signaling. Chemically, oxidative stress is associated with increased production of oxidizing species or a significant decrease in the effectiveness of antioxidant defenses, such as glutathione (9,10). This imbalance can be an effect of depletion of endogenous antioxidants, low dietary intake of antioxidants and/or increased formation of free radicals and other reactive species. Free radicals are also known as Reactive Oxygen Species (ROS) and these compounds are formed when oxygen molecules combine with other molecules. An oxygen molecule with paired electrons is stable; however oxygen with an unpaired electron is reactive (11). The exposure to stress situations can stimulate numerous pathways, leading to increased production of oxygen free radicals .Free radicals generate a cascade producing lipid peroxidation. Lipid peroxidation is one of the main events induced by oxidative stress. Lipid peroxidation can produce a range of enzymatically damaging consequences .Extensive lipid peroxidation is shown to cause membrane disorganization, by peroxidizing mainly the polyunsaturated fatty acids and phospholipids leading to alterations in the ratio of polyunsaturated fatty acids to other fatty acids. Thus, lipid peroxidation is considered as a serious consequence of free radical toxicity leading to profound changes in the membrane structure and function that may even cause cellular death (12). Previous studies have indicated that stress stimulated numerous pathways leading to increased levels of free radicals (13). Toxic free radicals have been implicated as important pathologic factors in cardiovascular diseases. Oxidative stress arises when the balance between pro-oxidants and antioxidants is shifted toward the pro-oxidants (14). Sodium nitrite is an inorganic salt with both harmful and healthful effects (15, 16). It is known as E250 in the food industry and used as a common preservative and color fixative in fish and meats. Sodium nitrite is also used as pharmacological agent in cyanide

poisoning. Recent studies suggest that the vasodilator effect of NaNO2 may be of therapeutic benefit in the treatment of pulmonary hypertension (17), post hemorrhagic cerebral vasospasm (18), and myocardial infarction. Other common uses are in fertilizers, dyes, pyrotechnics, etc. Although NaNO2 can be found in drinking water, the diet is generally the most sufficient source of human exposure. Sodium nitrite in blood is highly reactive with hemoglobin, thus affecting hematopoiesis. A major concern in considering the toxicology of NaNO2 is the induction of methemoglobinemia – a condition in which there is a reduction in hemoglobin's ability to transport oxygen. The primary purpose of the present study was to determine the oxidative stress of sodium nitrate and virgin coconut oil as antioxidant in lipid parameters (total cholesterol, triglycerides, phospholipids, and lipoproteins) and superoxide dismutase and glutathione in the serum and in liver and kidney of the rabbits.

Materials and methods

Preparation of Coconut oil

Fermentation method

VCO was produced from coconut milk, using a 1:1 ratio of coconut meat : water. Coconut milk from the extraction was placed into a fermentation container controlled the temperature at 70-80°C and allowed to sit for 16 to 24 h for natural fermentation which generally 3 groups of cultures; lactic acid bacteria, yeast and mold were play a role of hydrolysis and breaking coconut milk emulsion. After fermenting, the oil was separated, filtered through an eight-layer filter cloth bag and then the water in oil was dried out under low heat at 65°C. The clear VCO was kept in a dry container at −20°C (19)

Cold pressing method

Dehusked coconut nuts were grated using a motorized grater; the coconut meat was then dried at 60°C in a hot-air drier until the moisture content was reduced to 10–12%. VCO was extracted by a pressurized stainless steel expeller; the oil was then filtered through a three-layer filter cloth bag. After drying under low heat (65°C), the clear VCO was kept in a dry container at −20°C.

Preparation of Sodium nitrate Dose

Sodium nitrate 75 mg / Kg of body weight (dissolved in 3 ml distilled water per 25mg of sodium nitrate) given orally daily for 1 week .

Animals and experimental design

Animals

32 of mature((six to nine months old) male rabbits were obtained from Biotechnology Researches Center of AL-Nahrain University (1500-1900)gm of local breed were acclimated for holding facilities for one week prior to commencement of dosing. Animals in all stages of experiment were housed in clear plastic cages in a conditional room (22-25) $^{\circ}$C 24 hours with controlled lightening using automatic electrical timer providing days length of twelve hours

(7:00 to 19:00) and twelve night cycle. Animals were fed standard laboratory pellets diets and along the period of experiment.

Study protocol

Six to nine months old male rabbits (1500- 1900) gm. were randomly divided in to eight groups (each group consist of four rabbits) and handled as follows for 8 weeks:

1-Group C (control): Rabbits of this group were received tap water daily and served as control group.
2-Group T1: Rabbits of this group were treated orally daily with 75mg/kg sodium nitrate (20).for 1 week
3-Group T2, T3 , and T4 : Rabbits of this groups were treated orally daily 1.5 , 2, 2.5 ml/kg of body weight/day respectively extract of virgin coconut oil (21), for 21 day
4-Group T5, T6 ,and T8: Rabbits of this group treated orally daily with sodium nitrate 75mg/kg of body weight/day with virgin coconut oil extract 1.5, 2, 2.5 ml/kg of body weight/day respectively.

Blood sampling

Blood samples were collected at 0,1 week of experiment . Blood was drawn by cardiac puncture technique by disposable syringes needles 3cm. divided in to 2 parts.

(a) Whole blood: for hematological by using anticoagulant tubes.
(b) Serum : Samples were collected by jell tube then centrifuged at 3000 round per minute (rpm) for 15 minutes , and then serum samples were stored in freezer at -18˚C until use.

Parameters used in study

Hematological parameters

1-White blood cells count (cell/mm^3).
2-Haemoglobin level measured (g/dl).

Biochemical parameters

1-Serum total cholesterol concentration (mg/dl).
2-Serum high density lipoproteins (HDL).(mg/dl).

Estimation of liver enzymes activity

1-Soperoxide Dismutase Enzyme (SOD).
2-Glutathione enzyme (GSH).

Hematological tests

Hemoglobin assay

Hb level was determined by colorimetric method using Drabkin's solution as described by Watson etal.(22)

White blood cells (WBCs) count

A sample of whole blood is mixed with a weak acid solution that lyses non nucleated red blood cells **(**23).

Biochemical parameters

Determination of total cholesterol (TC)

Total cholesterol in the serum was measured by enzymatic method (24, 25), with the (biolabo) kit, France).Total triglycerides in the serum were measured by enzymatic method (26 , 27) with the (biolabo kit, France).

***Measurement of serum high density lipoprotein cholesterol (HDL-C) concentration:**

In the serum was measured by enzymatic method (28, 29) using (biolabo kit, France).Very low density lipoprotein (VLDL) is estimated as triglyceride ÷5 .After the measurement of total cholesterol, HDL–cholesterol and

VLDL, LDL–cholesterol is calculated as total cholesterol minus Very Low Density Lipoprotein + High Density Lipoprotein (30). **LDL-C(mg/dl) = Total Cholesterol –(HDL + VLDL).** Atherogenic Index Level is calculated as LDL dividedHDL (31, 32).**Atherogenic index = LDL / HDL.**

Estimation of liver enzymes activity

Soperoxidedismutase enzyme

The method is based on the SOD ability to inhibit the epinephrine oxidation to adrenochrome. Assay reactions were performed at 37 centigrade in air.

Glutathione enzyme

The statistical analysis

The statistical analysis for using Gen stat 2012.

Results and discussions

Results the hematological parameters

The results in **(Table 1)** appeared the mean level of Hb in group(2) which were treatment by sodium nitrate (75mg) significantly decreased (9.47 ± 0.765) g/l compared with the control group (1) (11.55 ±0.433) g/l. While the mean level of HB significantly increased in groups (3,4,5) (13.22 ± 0.478, 13.32 ± 0.439 , 14.40 ± 0.471) g/l respectively which are treatment by

VCO (1.5,2.5 , 3ml/kg) respectively .But the results in groups (6,7,8) demonstrated no significant changes compared with control group . Which treatment by sodium nitrate and VCO.

Table (1): Effect of virgin coconut oil (VCO) and sodium nitrate on hematological parameters (Hb)

Treatment	**HB**g/dl
Control	11.55 ±0.433
Sodium nitrate	9.47 ± 0.765
Virgin coconut oil (1.5 ml/kg)	13.22 ± 0.478
Virgin coconut oil (2 ml/kg)	13.32 ± 0.439
Virgin coconut oil (2.5ml/kg)	14.40 ± 0.471
Sodium nitrate(75mg)+virgin coconut oil(1.5ml/kg)	11.05 ± 0.544
Sodium nitrate(75mg)+virgin coconut oil(2 ml/kg)	11.52 ± 0.539
Sodium nitrate(75mg)+virgin coconut oil(2.5ml/kg)	11.92 ± 0.607
LSD.	1.526

*small latters are significant (P<0.05) to difference between groups .

The results in **(Table 2)** showed the levels of WBC in group (2) significant decrease

(3200 ± 561.2)c/mm^{3}in compared with control group(1) (5675 ± 1242))but the results in groups (3,4,5) which are treatment by VCO and in groups (6,7,8) which are treatment by sodium nitrate (75mg) and VCO (1.5,2.5 , 3ml/kg) exhibited no significant difference with control group.

Table (2): Effect of virgin coconut oil (VCO) and sodium nitrate on hematological parameters (WBC)

Treatment	**WBC**
Control	5675. ± 1242
Sodium nitrate	3200. ± 561.2
Virgin coconut oil (1.5 ml/kg)	5950. ± 542.4
Virgin coconut oil (2 ml/kg)	6125. ± 381.6
Virgin coconut oil (2.5 ml/kg)	6900. ± 334.2
Sodium nitrate(75mg)+virgin coconut oil(1.5ml/kg)	4450. ± 202.1
Sodium nitrate(75mg)+virgin coconut oil(2 ml/kg)	4650 ± 607.6
Sodium nitrate(75mg)+virgin coconut oil(2.5ml/kg)	4625. ± 614.2
LSD.	1792.0

** small latters are significant (P<0.05) to difference between groups

Results the biochemical parameters

The statistical analysis for biochemical parameters in **(Table 3)** showed that there are a significant increase (p< 0.05) in the mean value of serum Cholesterol level in sodium nitrate (75mg) treated group (2)(172.2 ±21.33)mg/dl comparison with control group(1) (111.0 ±10.32) .Where as significant decrease in three groups (3,4,5) which treated virgin coconut oil (1.5 ,2.5 ,3ml /kg), (67.5±11.20 , 59.5±7.467, 59.2±5.963) mg/dl respectively. While the results of groups(6,7,8) which treated sodium nitrate and virgin coconut oil(VCO) appear

significant decrease in cholesterol level (81.5±4.031 , 78.2±5.618 , 73.8±8.290)mg/dl ,when compared with control group but the dose of virgin coconut oil (3ml/kg) observe more decrease in the cholesterol level (8)group than in group (6,7).

Table (3): Effect of virgin coconut oil (VCO) and sodium nitrate on serum lipid profile (Cholesterol)

Treatment	**Cholesterol**mg/dl
Control	111.0 ±10.32
Sodium nitrate (75mg/kg)	172.2 ±21.33
Virgin coconut oil (1.5 ml/kg)	67.5±11.20
Virgin coconut oil (2 ml/kg)	59.5±7.467
Virgin coconut oil (2.5ml/kg)	59.2±5.963
Sodium nitrate(75mg)+virgin coconut oil(1.5ml/kg)	81.5±4.031
Sodium nitrate(75mg)+virgin coconut oil(2 ml/kg)	78.2±5.618
Sodium nitrate(75mg)+virgin coconut oil(2.5ml/kg)	73.8±8.290
LSD.	30.81

* small latters are significant (P<0.05) to difference between groups .

The results in (Table 4) showed significant decrease in group (2) which treated by sodium nitrate (75mg) in the level of HDL (17.00 ± 2.121) mg/dl than in control group (22.50 ± 1.443)mg/dl ,while significant increase in the level of HDL in group (3,4,5) which were treated by (VCO) (1.5, 2.5 , 3ml/kg) (28.02 ± 1.890 ,31.75 ± 1.315 , 35.50 ± 3.428)mg/dl respectively than in control group . Also the results of group (6,7,8) exhibited significant increase in the level of serum HDL in group treated by sodium nitrate (75mg) and VCO) (1.5, 2.5, 3ml/kg) which were (28.00 ± 3.873 , 31.50 ± 3.5, 35.50 ± 1.887)mg/dl respectively.

Table (4): Effect of virgin coconut oil (VCO) and sodium nitrate on serum lipid profile (HDL)

Treatment	**HDL**
Control	21.50 ± 1.443
Sodium nitrate (75mg)	17.00 ± 2.121
Virgin coconut oil (1.5 ml/kg)	28.02 ± 1.890
Virgin coconut oil (2 ml/kg)	31.75 ± 1.315
Virgin coconut oil (2.5 ml/kg)	35.50 ± 3.428
Sodium nitrate(75mg)+virgin coconut oil(1.5ml/kg)	28.00 ± 3.873
Sodium nitrate(75mg)+virgin coconut oil(2ml/kg)	31.50 ± 3.5
Sodium nitrate(75mg)+virgin coconut oil(2.5ml/kg)	35.50 ± 1.887
LSD.	7.544

** small latters are significant (P<0.05) to difference between groups .

Results the antioxidants enzymes

The results in (Table 5) appears the mean levels of SOD is significant decrease in group (2) which treatment by sodium nitrate (75mg) (0.0852 ± 0.00239) compared with control group (0.1250 ± 0.00255) while significant increase in groups (3,4,5) which are treatment by VCO(1.5 ,2.5 , 3ml/kg) (0.342 ± 0.00278 , 0.638 ± 0.0534, 0.716 ± 0.0239) respectively .

Also a significant increase in groups (6,7,8) (0.300 ± 0.0631, 0.562 ± 0.0526 ,0.700 ± 0.0341) respectively.

Table (5): Effect of virgin coconut oil (VCO) and Sodium nitrate on enzymes concentration (SOD)

Treatment	SOD
Control	0.1250 ± 0.00255
Sodium nitrate	0.0852 ± 0.00239
Virgin coconut oil (1.5 ml/kg)	0.342 ± 0.00278
Virgin coconut oil (2 ml/kg)	0.638 ± 0.0534
Virgin coconut oil (2.5ml/kg)	0.716 ± 0.0239
Sodium nitrate(75mg)+virgin coconut oil(1.5ml/kg)	0.300 ± 0.0631
Sodium nitrate(75mg)+virgin coconut oil(2ml/kg)	0.562 ± 0.0526
Sodium nitrate(75mg)+virgin coconut oil(2.5ml/kg)	0.700 ± 0.0341
LSD.	0.01408

** small latters are significant (P<0.05) to difference between groups .

The mean levels of GSH shows in **(Table 6)** is significant decrease in group (2) which is treated by sodium nitrate (75mg) (3.53 ± 0.111) compared with control group (8.18 ± 1.739) but significant increase in groups (3,4,5) which are treatment by VCO ((1.5 ,2.5 , 3ml/kg) (13.03 ± 0.605 , 14.60 ± 1.061 , 17.18 ± 1.739) respectively ,and also significant increase in groups (6,7,8,) (11.80 ± 0.414 , 12.60 ± 0.061 , 15.38 ± 1.321) respectively ,in compared with control group.

Table (6): **Effect of virgin coconut oil (VCO) and Sodium nitrate on enzymes concentration(GSH)**

Treatment	GSH
Control	8.18 ± 1.739
Sodium nitrate	3.53 ± 0.111
Virgin coconut oil (1.5 ml/kg)	13.03 ± 0.605
Virgin coconut oil (2 ml/kg)	14.60 ± 1.061
Virgin coconut oil (2.5ml/kg)	17.18 ± 1.739
Sodium nitrate(75mg)+virgin coconut oil(1.5ml/kg)	11.80 ± 0.414
Sodium nitrate(75mg)+virgin coconut oil(2ml/kg)	12.60 ± 0.061
Sodium nitrate(75mg)+virgin coconut oil(2.5ml/kg)	15.38 ± 1.321
LSD.	2.651

** small latters are significant (P<0.05) to difference between groups .

Discussion the hematological results

Administration of sodium Nitrite induced a decrease of W.B.Cs, R. B.Cs, Hb% and Hct %. It is known that nitrites convert the ferrous ion of hemoglobin to ferric ion both in vivo and

vitro (33). This can explain the reduction of hemoglobin level. In other words, administration of both nitrite leads to hematopoietic tissue hypoxia resulting on the long term (one month in the present study) in a decrease of red blood cell production and hence to reduction of blood hemoglobin level. Differential leukocyte count showed significant increase in the number of lymphocytes and monocytes and decrease in the number of neutrophils and eosinophils (34). The present study was in accordance with results of (35) .Who found a reduction in total white blood cell counts as a result of caramel treatment. Nitrates in the body are converted into nitrite ions which can convert ferrous ions of the hemoglobin to ferric form, which is a stable oxidation product called met-hemoglobin and is no more able to carry oxygen for respiratory functions resulting in tissue anoxia. Oxidative stress of erythrocytes can cause destruction of iron complex and hemoglobin products formation (36),When the erythrocyte antioxidant defenses are overloaded, hemolysis can occur due to the inability of the erythrocytes to regenerate the affected components . Hereditary or acquired met-hemoglobinemia, is a clinical condition in which the hemoglobin is oxidized to met-hemoglobin that contains oxidized ferric iron Fe+3 rather than the reduced ferrous form Fe+2 found in hemoglobin. Ferric iron has slightly greater affinity for oxygen which shifts the oxygen dissociation curve to the left resulting in decreased release of oxygen in tissues (37, 38). Moreover, white blood cell (WBC) count and lymphocyte number is shown to decrease and this is associated with the failure of the hematopoietic tissues to produce new WBC (39).

The biochemical results

Through the results that have been obtained and compared with other studies relevant to the subject near the study was reached some studies that correspond to the results of this study: with (40), Indian biochemists set out to investigate the effect of virgin coconut oil on various lipid parameters in oil-fed rats. Virgin coconut oil, at 8 g/100 g weight, had a beneficial effect in lowering lipid component compared to copra oil and ground nut oil. It reduced total cholesterol, and increased HDL cholesterol in serum and tissues.(41). Who found that total cholesterol much lower in rats that feeding VCO, and another studies indicate that saturated fatty acids increase the plasma total cholesterol , whereas polyunsaturated fatty acids lower these parameters. Coconut oil contains up to 93% of saturated fatty acids. However, the important feature of coconut oil is that it is responsible for increasing serum HDL cholesterol concentrations more profoundly than other sources of saturated fat (42)**.** One noticeable drawback of coconut oil is due to its low level of essential fatty acids, with the percentage weight of linoleic acid ranging from to 2.6 (43). The reason for this is that coconut oil is composed of medium chain fatty acids which are rapidly metabolized in the liver into energy

and does not participate in the biosynthesis and transport of cholesterol (44).Coconut oil even with the High Density Lipoprotein (HDL) or the so called "good cholesterol", reducing the risk for coronary heart disease. Although studies may take years to prove the pharmacological effects of these substances that found in the virgin coconut oil, there is growing interest worldwide on the role of these biologically active substances to human health,(*Tocopherols*, which are already known as antioxidants, have a role in the prevention of certain chronic diseases like coronary heart disease and cancer, *Tocotrienols*, said to be better anti-oxidant than tocopherols This biologically active substance synonymous with tocopherols is collectively called tocols, Tocopherols are normally found in seeds and green parts of the plant while tocotrienols are found in germ and bran fraction, *Phytosterols* have been known to reduce blood cholesterol, specifically the LDL "bad" cholesterol , Plant sterols are plant compounds with chemical structures similar to that of cholesterol. Studies show that concentrated phytosterol extracts have lessened the discomfort of prostatic hyperplasia. Phytosterols help lower cholesterol levels.

The antioxidants enzymes

The action of $O^{-}\cdot$ scavengers is performed by a group of antioxidant enzymes called superoxide dismutases (SODs), which catalyze the dismutation of $O^{-}\cdot$ into hydrogen peroxide (H_2O_2) and oxygen (O_2) efficiently and specifically. Mammal tissues have 3 SODs isoforms: Cu/Zn superoxide dismutase (SOD1), Mn SOD (SOD2), and extracellular SOD (EC-SOD, or SOD3). SOD1 is a 32 kDAhomodimer cell protein containing copper and zinc, and it is present in the cytosol, nucleus, peroxisomes, and mitochondrial membrane. Its primary function is to reduce the steady intracellular concentration of superoxide (45).acts as a first line antioxidant defense. In the case of SOD deficiency or increased superoxide production, it reacts with nitric oxide to produce peroxynitrite ($ONOO^{-}$), which is a potent oxidant and nitrosating agent that can cause direct damage to proteins, lipids, and DNA ,Decreases of SOD may be due to excessive production and / or inadequate removal of ROS, especially superoxide anion which have been implicated in the pathogenesis of many cardiovascular diseases, including hypercholesterolemia, atherosclerosis, hypertension and may be due to progressive enzyme inactivation by its product H2O2. Free radical-scavenging enzymes such as SOD and GSH are the first line of cellular defense against oxidative injury, decomposing O2• and H2O2 before interacting to form the more reactive hydroxyl radical (SOH). These enzymes protect the red cells against O2• and H2O2-mediated lipid peroxidation (46).

Conclusion

In conclusion: Virgin coconut oil is a rich source of medium chain fatty acids especially lauric acid (49%) cause reduction in the biochemical parameters (cholesterol, triglyceride, LDL and VLDL) but induced HDL, So to prevent the risk of atherosclerosis and coronary heart disease , met-hemoglobinemia, intake the VCO inhibiting and removing ROS by antioxidants properties such as tochopherol (Vitamin E).

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