**The Influence of Various Toxic Effects on the Cornea and Lens Induced by Carbon Tetrachloride in Mice and the Antioxidant Effects of *Spirulina platensis***

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**Abstract**

**Objective:** The present study was directed to examine the toxicity of carbon tetrachloride (CCl₄) in ocular tissues of Swiss Albino mice and the strong antioxidant beneficial effects of *Spirulina platensis*. **Methods/Analysis:** Na⁺-K⁺-ATPase measurement was done on the lens membrane. Soluble lens proteins were extracted and the following techniques were made: evaluation of total soluble protein and gel filtration chromatography. Likewise, the corneal protein secondary structure was evaluated by Fourier transform infrared spectroscopy (FTIR). For comparison between multiple groups, analysis of variance was used with significance level set at $P < 0.001$. **Finding:** The results demonstrated a decrease in total soluble lens protein and Na⁺-K⁺-ATPase activity, increase in molecular weight of all lens protein fractions and there are different structural and conformational changes in cornea after injection of CCl₄. These changes were reduced in pretreated mice with *Spirulina*. **Novelty/Improvement:** Our study clearly concluded that *Spirulina platensis* shows a protective effect on the lens and cornea through its antioxidant activity on CCl₄-induced toxicity in mice.

**Keywords:** Antioxidant, Carbon Tetrachloride, Cornea, Lens, Spirulina

**1. Introduction**

Carbon tetrachloride (CCl₄) is utilized as a part of petrol additives, solvents, metal degradations and a cofactor in generation of polymers. Also CCl₄ is common in the production of pesticides. CCl₄ is liquid, has no color and nonflammable. It is known that CCl₄ is model compound induced tissue toxicity through producing free radicals such as in liver, blood, kidney, brain, lung and testis. The transformation of CCl₄ to trichloromethyl active free radicals (CCl₃OO) is by hepatic microsom cytochrome P450.

*Spirulina* is floating micro algae appeared with spiral owing the characteristics of filaments. It is known as Arthospira and referred to the class type of cyanobacteria that has the ability of photosynthesis. Initially Spirulina classification was in the plant kingdom since the presence of plant pigments in huge amount and its photosynthesis property. Later, based on its genetic characteristic and biochemical properties, it was grouping in bacteria kingdom. *Spirulina* develops in high-salt alkaline water reservoirs in subtropical and tropical areas.

A significant effect in decreasing plasma concentrations of total cholesterol, LDL-C, triglycerides and increasing of HDL-C were observed after supplementation with Spirulina. The utilization of Spirulina as eating regimen supplement has medical advantages in forestalling or dealing with certain incendiary maladies, hypersensitivities, tumor, and medication acted toxicities, viral contaminations, cardiovascular illnesses, diabetes and other metabolic sickness.

The present study was directed to look at the toxic effects of CCl₄ in ocular tissues of Swiss Albino mice and the strong antioxidant beneficial impacts of...
Spirulina platensis in an attempt to understand its mechanism of action, which may get the way for the possibility to utilize it for therapeutic medical application.

2. Materials and Methods

2.1 Animals
Fifty Swiss Albino mice from both sex and weighed 30±4 were chosen from Institute of Graduate Studies and Research (IGSR), Alexandria University. The animals were kept in very much ventilated house and under the control of departmental and ecological conditions. The animals were dispersed to five groups (10 mice each). Group I got a subcutaneous infusion of saline and served as control. Animals of group II got subcutaneous infusion of olive oil (0.5 ml/kg body weight/day). Groups III and IV got subcutaneously CCl₄ in a measurements of 1 ml/kg of body weight and CCl₄ diluted in olive oil by a proportion 1:1 as a dissolvable. Animals of group IV were pretreated with Spirulina (800 mg/kg body weight/0.5 ml in savoring water) oral route for 30 minutes after the infusion of CCl₄. Group V received Spirulina only in the same regime. All the animals were being fast 24 hours before the experiment that lasts for three weeks and the injection was day after day.

2.2 Na⁺⁻K⁺ ATPase Activity
After beheading of the animals, eyes were enucleated from the eye globe then, the lenses were taken from the eye and their capsules were removed. After weighing each lens capsule, adding an extraction medium that consists of 0.32 M sucrose, 1 Mm EDTA and deoxycholic acid. The activity of Na⁺⁻K⁺ ATPase were done on lens membrane according to previously described.

2.3 Estimation of Total Soluble Protein
Soluble lens protein was computed and evaluated by the previous method. Depending on the reaction between proteins with the alkaline copper reagent and foline reagent, a color was appeared that measured by thermo-fisher spectrophotometer at 750 nm.

2.4 Gel Filtration Chromatography
The column dimension was 1.6x100 cm and filled with Sephacryl G200 (Pharmacia, Uppsala, Sweden. Then eluted it with phosphate buffer (pH=7.4). Collection of fractions (7.5 ml/20 min) was made by fraction collector (type Haake Buchler, Inc.Saddle Brooke, USA). The absorbance was measured by spectrophotometer at wave length 280 nm. A curve was plotted between the fraction number and the absorbance of fractions for all the studied groups. The molecular weight of eluted protein fractions was calculated using the calibrations methods of the column.

2.5 Fourier Transform Infrared Spectroscopy (FTIR)
A cut was made through the ora serata to get cornea and weighed separately. Cornea was crushed to powder by liquid nitrogen and freeze dried for a day. A disk was prepared from the sample (5 mg cornea) and potassium bromide powder (95mg KBr). Infrared spectrophotometer type, Nicolet-iS5, thermo-fisher, USA at a resolution of 2cm⁻¹ was used to measure FTIR. By using origin (9.1), correction of baseline and smoothingthe spectra were done.

3. Statistical Analysis
Information were communicated as the mean ± SD. Correlation was performed utilizing investigation of change (ANOVA); economically accessible measurable programming bundle (SPSS-11 for windows) was utilized where the centrality level was set at p<0.05.

4. Results
Figure 1 illustrates the activity of Na⁺⁻K⁺ ATPase in μ Mpi/h/g wet wt for control lens membrane, olive oil, CCl₄, Spirulina+CCl₄, and Spirulina groups. For normal lens membrane, the enzyme activity was 30±2 μMpi/h/g wet wt. The activity of Na⁺⁻K⁺ ATPase for CCl₄ group was significant decrease (p< 0.05) compared to control group and reached to 20±0.5 μ Mpi/h/g. On the other hand, for the group IV that pretreated the mice with Spirulina, there was no significant difference in enzyme activity. Also olive oil group and Spirulina group were 29±2 and 31±1.5 μ Mpi/h/g respectively.
Figure 1. Activity of Na⁺-K⁺ ATPase (μ Mpi/h/g wet wt) of mice lens membrane for the different studied groups.

Figure 2 indicated the total soluble lens protein in mg/g lens for all the studied groups. For animals subcutaneously injected with CCl₄ (group III), the total soluble lens protein was significant decrease (p<0.05) from 200±4 which is the control value to 124±2 mg/g lens. The value of group III did not reach the control value and still statistically significant decrease (175±2). Groups II and V shows the same behavior as the Na⁺-K⁺ ATPase activity of the control.

Figure 2. Total soluble protein content of mice lens (mg/g lens) for all groups.

Figure 3 shows the chromatographic elution pattern for control animals, olive oil group, CCl₄ group, pretreated group with Spirulina before CCl₄ and Spirulina group only. The chromatographic elution pattern for control mice was eluted into four fractions: α, βH (high molecular weight), βL (low molecular weight), and γ-crystalline. The first peak (α-crystalline) eluted at molecular weight 484±6 kDa (kilodalton). This was followed by βH and βL crystalline with molecular weight 240±3 kDa for βH and 106±2 kDa for βL. The fourth peak (γ-crystalline) was eluted at molecular weight 41±1 kDa. For olive group, the four groups were eluted in the range of the control as indicated in Table 1 in which the molecular weights of α, βH, βL, and γ-crystalline for all studied groups were calculated. After subcutaneous injection of animals by CCl₄, the pattern changed and the four peaks were shifted towards high molecular weight reflecting a statistically significant increase (p<0.05) for all crystalline compared to control. A splitting of α-crystalline (833±7, 595±8 kDa) was observed. On the other hand, the chromatographic elution pattern of mice treated with Spirulina was shifted to lower molecular weight compared to CCl₄ group. These changes were given in Table 2 in which the molecular weights of α, βH, βL, and γ-crystalline for all the studied groups were calculated. From the table we observed that chromatographic elution pattern for crystalline of Spirulina group match the control.

Figure 3. Chromatographic elution pattern of soluble lens proteins for all groups.
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Table 1. Molecular weights of lens crystalline for control mice and different studied groups

| Groups     | Control | Olive oil | CCl₄ | Spirulina + CCl₄ | Spirulina |
|------------|---------|-----------|------|------------------|-----------|
| α-crytalline | 484±6   | 484±5     | 833±7, 595±8* | 536±6*, 460±8* | 484±5     |
| β₁-crytalline | 240±3   | 245±3     | 326±5* | 246±4            | 246±4     |
| β₂-crytalline | 106±2   | 106±4     | 178±4* | 94±2             | 106±3     |
| γ-crytalline | 41±1    | 41±1      | 60±3*  | 39±2             | 39±2      |

*Statistically significant (p<0.05).

Table 2. Band assignment, wavenumber (cm⁻¹) and absorbance for estimated components of corneal tissue for normal and all the studied groups

| Groups     | Control | Olive oil | CCl₄ | Spirulina + CCl₄ | Spirulina |
|------------|---------|-----------|------|------------------|-----------|
| 1-OH asym  | 3452±3  | 3458±4    | 3445±3* | 3451±3 | 3465±3    |
| 2- symCH₂  | 2961±2  | 2957±4    | 2930±3* | 2946±3 | 2954±4    |
| 3-asymCH₃  | 1405±4  | 1408±5    | 1396±3  | 1407±2 | 1403±4    |
| 4- Amide I | 1614±4  | 1658±3    | 1641±4* | 1651±5 | 1647±3    |
| 5- CH₂ bending | 1533±4 | 1550±4    | 1540±2*, 1456±3* | 1550±3 | 1547±4    |
| 6-COOsym   | 1405±4  | 1408±5    | 1396±3  | 1407±2 | 1403±4    |
| 7- asymPO₂ | 1237±3  | 1238±4    | 1234±3  | 1242±3 | 1243±2    |
| 8-symPO₂   | 1078±2  | 1077±1    | 1080±3  | 1087±3 | 1068±4    |

*Statistically significant (p<0.05). First line indicates the wavenumbers and second line is the absorbance.

Figure 4 shows the IR spectra of cornea for control, olive oil, CCl₄, *Spirulina*+CCl₄ and *Spirulina* groups in the range 4000–3000 cm⁻¹ (left panel) and in the range 3000-900 cm⁻¹ (right panel). The contour of control indicates the presence of seven bands (1) 3452 cm⁻¹(OHsym), (2) 2961 cm⁻¹(symCH₂), (4)1641 cm⁻¹ (Amide I ), (5) 1533 cm⁻¹(CH₂ bending), (6) 1405 cm⁻¹ (COOsym), (7) 1233 cm⁻¹(asymPO₂) and (8) 1078 cm⁻¹(symPO₂) respectively, as previously mentioned. Table 2 illustrated the assignment of the bands and their wavenumbers and absorbance. There were dramatic changes in the contour of CCl₄ group in contrast to all others groups compared to the control. These changes can be noticed in shifting of OHsym and symCH₂, to lower wave number, appear of asym CH₃ at 2854±3 cm⁻¹, splitting of amide I and CH₂ bending to two bands and statistically significant changes (p<0.05) of the absorbance of most of the bands.
4. Discussion

The eye is a critical organ that catches light rays and transforms the stimuli of light into the optic nerve then goes to be framed a picture direct to the brain. These days, there has been an expansion of eye disease in both human and veterinary. The fundamental driver of this expansion can be attributed to ecological contamination. Hepatotoxicity is the touchiest and the best concentrated on toxic end point for CCl₄ exposure. Notwithstanding its hepatocellular danger, CCl₄ has been considered to oxidative anxiety in tissue other than liver, for example, kidneys, heart, lung, testis, brain, blood and immune system by delivering free radicals.

The initial phase of CCl₄ metabolism is a one-electron reduction and hemolytic cleavage catalyzed by cytochrome P450 of the mixed function oxidase system (MFOS) to give trichloromethyl radical. P450 is kept in the reduced structure by reduced nicotinamide adenine dinucleotide (NADPH). The CCl₄ radical quickly reacts with molecular oxygen to form the trichloromethylperoxyl radical (OOCCl₂). This radical is more electrophilic than the CCl₃ radical and might be more in charge of assaults on unsaturated fatty acids, resulting lipid peroxidation. CCl₃ might be more included in covalent binding reactions of CCl₄.

In the present study the oxidative stress of CCl₄ to the lens is seen in the variations in the molecular weight fractions of various lens crystallins and aggregation of soluble lens protein which might be come about because of the blend between the native proteins and/or the breakdown results of the damaged proteins. Na⁺-K⁺ATPase activity was repressed after CCl₄ injection and this was because of the oxidative stress and this inhibition takes a part on the ionic transport in the cell membrane.

FTIR is a method gives a range of assimilation for solids, fluid and gas. It gives the structure and nature of any material. It gives data on the function groups of a cell or tissue and has been utilized as a part of numerous regions of therapeutic examination.

From FTIR data for cornea, the OHasym band was delicate to the CCl₄ and it is found in layer constituents as the lipid, protein and the hereditary material as well. Thusly CCl₄ treatment instigates changes in the membrane function groups that may be connected with the biohazard in tissue. SymCH₂ and asymCH₃ that showed up in the CH vibrational locale is utilized to describe for the lipid particles, after injection of CCl₄ the vibrational frequency was impacted exhibiting a natural change. The agreeable impact between the variety in the NH-OH locale and the CH vibrational area may in like manner be found in the finger print area which shows up at the extent 1600-900 cm⁻¹. FTIR was used to inspect the secondary structure of proteins in perspective of the amide I mode. The amide I absorption is associated with protein amide C=O vibrations, and it is reasonable as a test to decide the particular structures of proteins and polypeptides.

The results of Table 2 showed that the protein structure was affected after injection of CCl₄. Furthermore, the CH₃ bending is of essential enthusiasm, since it can be used to screen the disorder of the tissue.

It is especially documented that Spirulina feeding has no symptoms on the development and growth of embryo and fetus. Additionally, supplementation of Spirulina in the eating routine at the estimations much higher than any normal human usage did not bring any signs of embryotoxic effects. Moreover, Ingestion of Spirulina had no effects on conduct, water intake, development and survival. Hematologic and clinical science examinations recognized no variety from the typical. Additionally, no gross or microscopic changes were recognized with histological appraisal. As appeared in our outcomes, there is no adjustments in visual tissue (lens and cornea) watched for Spirulina assemble and came about about no signs of lethality quality. In addition, administration of Spirulina at 800 mg/kg body weight was found to apply a beneficial outcome spoke to by keeping the diminishing in the soluble lens proteins when contrasted and the CCl₄ group, significant (p<0.05) increase of Na⁺-K⁺ATPase activity and positive effects on the cornea constituents, as showed up by the movements toward control of the same Fourier change infrared spectroscopy groups. There are different pharmacological properties to Spirulina, as anti-carcogenic, immunostimulant, antigenotoxic, antihepatotoxic, and antioxidant activities. These properties ascribed to particles, for example, c-phycocyanin, β-carotene, tocopherol, Υ-linolenic, and phenolic compound. Spirulina had adequate power of free radical scavenging activity and can prevent or defer oxidative stress by decreasing the aggregation of ROS. Spirulina dramatically inhibited the production of thiobarbituric acid reactive substances (TBARS), such
as malondialdehyde (MDA), by almost 95% and this inhibition indicating the potent antioxidant activity of *Spirulina*

6. Conclusion

*Spirulina platensis* shows a protective effect on the lens and cornea through its antioxidant activity on CCl₄-induced toxicity in mice.

7. References

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