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Protective role of fucoxanthin from *Dilophys fasciola* on the kidneys against the detrimental effect of fast green pigment in albino rats

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Abstract
Synthetic fast green pigment (FG) is widely used worldwide as a food color for children, and it also promotes oxidative stress, attention deficit, and hyperactivity disorder in mammals. This experiment aims to evaluate oxidative stress and kidney function of rats exposed to Fast Green dye and the protective effects of algal fucoxanthin as an antioxidant and anti-inflammatory. Fucoxanthin was identified by HPLC. Antioxidant activity was determined by ferrous chelating and reducing power activities assays. Fucoxanthin showed potent antioxidant activity against Fe^{2+} chelating (IC_{50}= 820.48 ± 1.79 µg/ml) and reducing power (EC_{50} 37.3± 1.09 µg/ml). Fast green dye treatment resulted in significant renal insufficiency in rats after 28 days of treatment. This was demonstrated by elevated kidney levels of albumin, creatinine, urea, tumor necrosis factor-alpha (TNF-α), and lipid peroxidation. It's interesting to note that urea, creatinine, lipid peroxidation, and tumor necrosis factor-alpha (TNF-α) levels all dramatically dropped in rats given algal fucoxanthin treatment. This demonstrates that fucoxanthin is essential in preventing kidney damage while undergoing therapy. Our results indicate that fucoxanthin shields rats' kidneys from oxidative damage brought on by the fast green pigment.

***Keywords*:** Marine algae, Fucoxanthin, Synthetic pigment, Kidney function, Oxidative stress

1. Introduction

The usage of several chemicals as flavorings, preservatives, and colorants is extremely dependent on the rising demand for "convenience" food. Understanding the ramifications of these medications' adverse effects is very important. A triphenylmethane dye called fast green (FG) has been utilized as a food, medicine, and cosmetic coloring. Fast green azo dye was demonstrated to be hazardous, altering the immediate allergic reaction when consumed orally in food, and compromising liver and kidney functions [1].

Fucoxanthin is the most prevalent carotenoid in brown macroalgae and has been taken into consideration among seaweed components because of its distinctive structure. The metabolite of fucoxanthin is amarouciaxanthin A and fucoxanthinol in other tissues and adipose tissue, respectively [2]. Additionally, fucoxanthin is safe when used in experiments. Fucoxanthin was given orally to mice, but there was no mutagenicity or toxicity [3].

Numerous researches have proven that fucoxanthin has medical benefits, yet its structure is naturally somewhat brittle. Due to its unique chemical structure, which includes an allenic link, hydroxyl (OH), and epoxide group, fucoxanthin may be a potential antioxidant [4]. Numerous researches have demonstrated the anti-obesity [5], anti-inflammatory [6], anti-diabetes, anti-cancer [7], and hepatoprotective properties of fucoxanthin [8]. The antioxidant fucoxanthin may be able to stop kidney cell apoptosis [9]. To prevent and treat chronic kidney disorders, fucoxanthin functions as a dietary supplement [10].

The current investigation intended to examine the antioxidant and anti-inflammatory effects of fucoxanthin and the role of fucoxanthin in the

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prevention of the harmful effect of fast green dye on the kidney.

2. Experimental

Collection of marine algal material

In 2020, seaweed was harvested in the Marsa Matrouh governorate, specifically in the city of Matrouh. After preparing the herbal samples from algae, they were identified by Dr. Rawhia Abdel Latif, Professor of Botany Department, Faculty of Science, Al-Azhar University.

Extraction of fucoxanthin

Following ten to fifteen minutes of homogenizing; one kilogram of brown algae with cold acetone methanol (7:3 v/v), the solutions were filtered using filter paper [11]. On a rotary evaporator, the extract was evaporated to dryness at 30 to 35°C, and the residue was dissolved in methanol. Three times; the reconstituted residue was divided between 90% (v/v) aqueous methanol and n-hexane in a separating funnel. The phase of hexane was disposed of. Fucoxanthin was transferred to diethyl ether from the aqueous phase. On a rotating evaporator, the diethyl ether phase was evaporated until it was dry.

Quantitative assessment of fucoxanthin using HPLC analysis

The present study was performed to estimate qualitatively and quantitatively the fucoxanthin from *Dilophys fasciola* using HPLC analysis. Ten milligrams of dried extract and standard were thoroughly dissolved in one milliliter of methanol to create the sample and standard. For every sample solution, there was one injection volume of five microliters. An Agilent 1260 series was used for the HPLC analysis. The Shiseido SG 120 C_{18} column (4.6 mm x 250 mm i.d., 5 µm) was used to do the separation. MeOH: H_2O: DCM: CAN (70:4:13:13) was the composition of the mobile phase, with a flow rate of 1 ml/min. At 450 nm, the DAD detector was monitored. The temperature of the column was kept constant at 30°C.The obtained signals were compared to authentic fucoxanthin (Sigma) using the previous conditions. Quantitation in each gram of sample was carried out using an external standard method. The amount of fucoxanthin was expressed as micrograms per gram of dry weight (µg/g).

Antioxidant activity of crud fucoxanthin from *Dilophys fasciola*

Metal chelating action *in vitro*

The study investigated the effects of metal chelating agents on ferrous ions. One milliliter of fucoxanthin extracts or EDTA solution was used as a positive control at different concentrations (50, 100, and 200 µg/ml), which were spiked with 0.1 milliliter of 2 mM $FeCl_2$-$4H_2O$, 0.2 milliliter of 5 mM ferrozine solution, and 3.7 milliliters of methanol. The mixture was then mixed in a test tube and allowed to react for 10 minutes at room temperature. The absorbance was then measured at 562 nm.

The control combination was one that included no extract. Higher ferrous ion chelating activity is indicated by a decreased absorption [12]. The following formula was used to get the percentage of ferrous ion chelating activity: (Inhibition %) = [(Ac–AS/ Ac) ×100]; is the iron chelating activity (inhibition percentage), where AS was the absorbance in the presence of the algal extracts and Ac was the absorbance of the control reaction.

Reducing power

A mixture of 2.5 ml of phosphate buffer (50 mM, pH 7.0) and 2.5 ml of 1% potassium ferricyanide was combined with varying concentrations (50, 100, and 200 µg/ml) of fucoxanthin extracts (1.0 ml). After that, the mixture was incubated for 20 minutes at 50°C. Following the addition of 2.5 ml of 10% trichloroacetic acid to the mixture, the mixture was centrifuged for 10 minutes at 3000 rpm. Lastly, 0.25 ml of solution (0.1%, w/v) and 1.25 ml of distilled water were combined with 1.25 ml of the supernatant.

At 700 nm, the absorbance was measured. The tests were performed in triplicate, and the mean values ± standard deviations were reported as the results. A higher reducing power is shown by higher absorbance values. The graph of absorbance at 700 nm vs. extract concentrations was used to determine the extract concentrations that provided 0.5 of absorbance (EC_{50}). The industry standard was BHT.

Anti-inflammatory action *in vitro*

Bovine albumin serum (0.45) millilitres was mixed with different amounts of fucoxanthin extract or the prescription drug diclofenac sodium (50, 100, or 150 µg/ml). Samples were heated to 57°C for three minutes after being incubated for twenty minutes at 37°C. After cooling, 2.5 milliliters of 6.3 PH phosphate buffer was added to the samples. A UV-visible spectrophotometer set to 255 nm was used to measure the absorbance [13].

In-vivo Study

Animals

Twenty-four male rats, aged eight weeks, weighing 151.6 ± 7.2 g on average ± standard deviation were obtained from the National Research Center's animal house in Cairo, Egypt. The animals were kept in separate stainless steel cages with

unrestricted access to food and water, and typical laboratory settings (23-25°C, 12-hour light/dark cycle). The Ethics Committee of the National Research Center, No. 4421023, authorized the study's animal procedures.

Animals' diet

Reeves et al. [14] developed a balanced diet consisting of 12% protein from casein, 10% corn oil, 10% sucrose, 58.5% maize starch, 5% fiber, 3.5% AIN-93 salt mixture, and 1% AIN-93 vitamin mixture.

Animals' study design

Rats were placed into four groups of six rats each after a week of acclimatization, as follows:

The first group of rats was given no treatment; the second group of rats received fast green pigment orally (125 mg/kg body weight); the third group received fast green pigment orally (125 mg/kg body weight) plus fucoxanthin (100 mg/kg body weight according to Mao et al. [15], dissolved in olive oil; and the fourth group received fucoxanthin orally (100 mg/kg body weight, dissolved in olive oil).

All rats were fed on a balanced diet and the experiment lasted for 4 weeks. After the experiment period, blood samples were collected and analyzed for the following parameters:

Larsen; Fawcett and Scott; and Doumas et al. [16-18], were consulted for the determination of creatinine, urea, and albumin, respectively; Per Ohkawa et al. [19], the kidney was homogenized right away and examined for malondialdehyde (MDA) activity, and using an Eliza kit (Elabscience Biotechnology Co., Ltd., Wuhan, China) and following the manufacturer's instructions, the amount of kidney tumor necrosis factor-alpha (TNF-α) was measured.

Statistical analysis

The results were presented as mean and standard error (SE) and statistically assessed using one-way analysis of variance and the Duncan test, using SPSS version 20 for statistical analysis. To determine if the difference was statistically significant, the probability at $p < 0.05$ was employed.

3. Results

Quantitative assessment of fucoxanthin using HPLC analysis

HPLC profiles of the standard fucoxanthin and the isolated fucoxanthin are presented in Fig. 1(A, B). HPLC analysis showed the presence of the standard fucoxanthin and the isolated fucoxanthin peaks were observed at the same retention time of 3.281 min.

Total fucoxanthin content of *Dilophys fasciola*

Fucoxanthin of *Dilophys fasciola* was identified by HPLC as shown in Table (1), Figure (1). Fucoxanthin isolated was 0.07µg/g**.**

Table 1. HPLC of fucoxanthin content of *Dilophys fasciola.*

Sample	Area	Conc. (µg/ml)	Conc. (µg/g)
Fucoxanthin	0.43	0.0045	0.0714

Antioxidant activity

Fe^{2+} chelating activity of algal fucoxanthin

Depending on each antioxidant compound's capacity for reduction, the Fe^{3+} ferricyanide complex was reduced to the ferric form, changing the test solution's yellow hue to a blue-green hue. The results as shown in Table (2) indicate that fucoxanthin extract showed antioxidant activity against Fe^{2+} radical compared to the control.

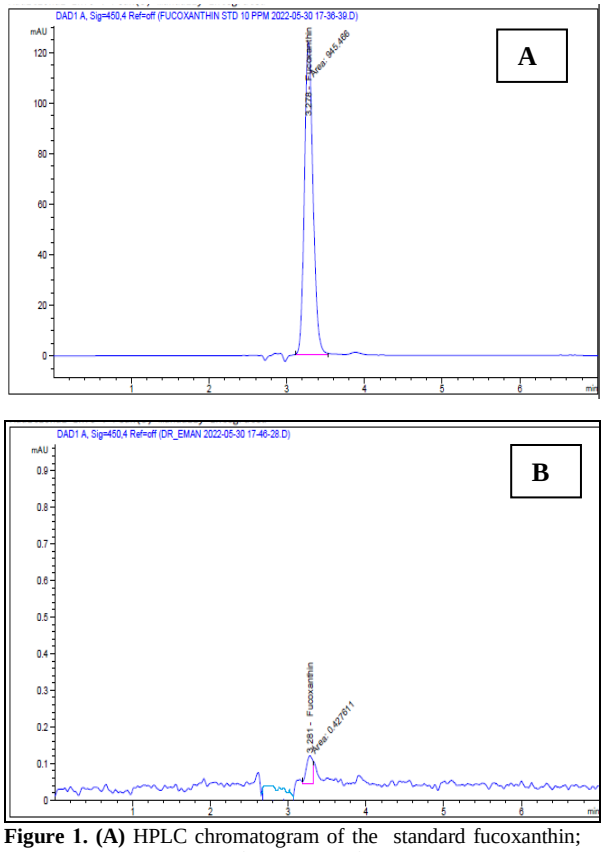

Figure 1. (A) HPLC chromatogram of the standard fucoxanthin; **(B)** HPLC chromatogram of the isolated fucoxanthin.

Table 2. Fe^{2+} chelating activity of algal fucoxanthin**.**

Samples	IC_{50} (µg/ml)
Algal fucoxanthin	820.48 ± 1.79
BHT standard	50.39 ± 0.44

Reducing power activity of algal fucoxanthin

Table 3 shows the reducing power of algal fucoxanthin. The algal fucoxanthin showed a high significant against reducing power with IC_{50} of 3.73±1.09.

Table 3. Reducing power activity of algal fucoxanthin.

Samples	EC_{50} (µg/ml)
Algal fucoxanthin	3.73± 1.09
BHT standard	4.74 ± 0.17

Anti-inflammatory activity of Algal fucoxanthin

In particular, Algal fucoxanthin showed anti-inflammatory activity and extract showed dose-dependent activity relationships as shown in Table (4).

Table 4. Anti-inflammatory activity of Algal fucoxanthin.

Samples	Inhibition		
	Samples concentration (µg/ml)		
	100	120	200
Algal fucoxanthin	48.30± 0.30	50.19±0.30	63.26±0.25
Diclofenac sodium	81.74±0.63	85.5±0.2	88.45±0.30

Influence of algal fucoxanthin and toxic fast green pigment on creatinine marker in kidney male rats

Fig. 2(A) summarizes the effects of the synthetic fast-green dye on creatinine in rat kidneys following a 28-day treatment. The rapid green group's blood serum creatinine value was 1.22 ± 0.05^{a}, higher than the 0.40 ± 0.02^{b} values of the control group. Rats given oral fucoxanthin in addition to fast green dye had lower creatinine readings (0.62 ± 0.02^{b}) than the fast green group. Rats administered oral fucoxanthin had creatinine readings (0.41 ± 0.01^{a}) similar to the control group.

Influence of algal fucoxanthin and toxic fast green pigment on kidney urea marker in male rats

In comparison to the control group, the fast green dye treatment group had a significantly higher blood serum urea level ($38.37^{c} \pm 0.61$). Furthermore, urea was found to be (30.87 ± 0.71^{b}) in a group of fucoxanthin mixed with fast green dye (Fig. 2(B)). Rats given fucoxanthin orally had urea levels that were nearly identical to those of the control group.

Influence of algal fucoxanthin and toxic fast green pigment on kidney albumin marker in male rats

Algal fucoxanthin mitigated the toxicity of the synthetic fast green dye treatment resulted in statistically significant drops in albumin relative to the control group ($P < 0.05$) (Fig. 2(C)).

Influence of algal fucoxanthin and toxic fast green pigment on kidney urinary protein marker in male rats

As seen in Fig. 2(D), the fast green group had higher urinary protein levels in their blood serum than the control group. Rats given oral fucoxanthin in addition to fast green pigment had lower urinary protein values than the fast green group. When rats were given fucoxanthin orally, their urine protein levels were similar to those of the control group.

Influence of algal fucoxanthin and toxic fast green pigment on Kidney lipid peroxidation in male rats

Polyunsaturated fatty acids, which may be quantified in terms of increases in malondialdehyde (MDA) and utilized as a biomarker of oxidative damage, inactivate to cause LPO. We observed that fast green pigment treatment causes significant increases in MDA content compared to control after 28 days of exposure. Treatment with fucoxanthin in addition to fast green pigment reduces the effect of oxidation (Fig. 2(E)).

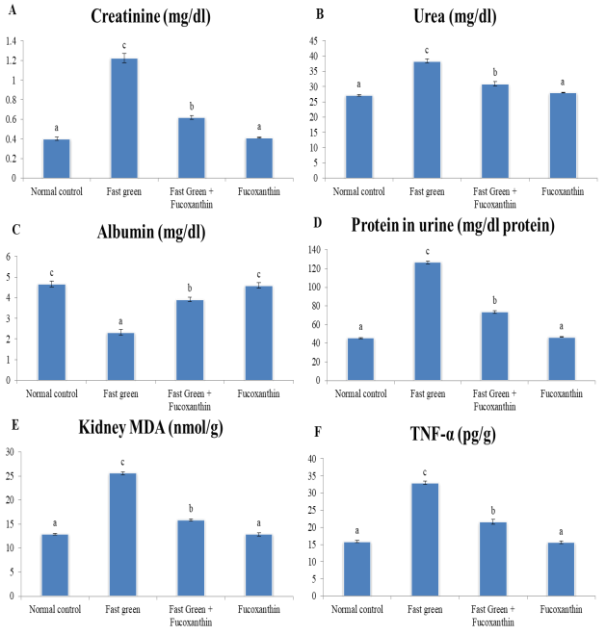

Figure 2. Creatinine, urea, albumin, protein in urine, kidney MDA and TNF-alpha values of the different experimental groups.

Influence of algal fucoxanthin and toxic fast green pigment on Kidney TNF-α in male rats

When compared to the control group, the kidney tissue under examination showed a statistically significant ($P < 0.05$) increase in TNF-α levels following treatment with the synthetic fast green dye. This result implies that mixing synthetic pigments with algae fucoxanthin can reduce the toxicity of artificial fast green dyes (Fig. 2(F)). Rats given oral fucoxanthin had renal TNF-α values that were similar to the control group's.

4. Discussion

Fucoxanthin is a kind of xanthophyll that accounts for over 10% of the total amount of carotenoids produced in nature. Fucoxanthin from *Undaria pinnatifida* has strong radical scavenging action [20]. Fucoxanthin is known to have higher antioxidant effects even more than that of beta-carotene in rat plasma and liver tests [21]. Fucoxanthin contains an active part for removing free radicals represented by the two hydroxyl groups that are arranged in a cyclic structure [22].

This work has shown the function of fucoxanthin, which is derived from *Dilophys fasciola*, in averting renal oxidative stress and inflammation caused by fast green pigment.

The synthetic organic food color fast green caused detrimental alterations in the blood parameters under investigation. These alterations may be related to the potential for the stomach or the nitrite-or nitrate-containing meals to produce carcinogenic nitrosamines. It was found previously that the activities of serum AST and ALT increased significantly following fast green treatment in rats [1].

It was found previously that the activities of serum AST and ALT increased significantly following fast green treatment in rats [1]. Raya et al. [23], found that kidney and liver functions as well as oxidative stress increased significantly following fast green treatment in rats. However, most of these changes showed signs of improvement with the treatments with fucoxanthin in combination with fast green compared to fast green treated rats alone as the antioxidants could protect cells and sub-cellular structures from oxidative damage.

According to Mao et al. [15], fucoxanthin is a marine carotenoid, that possesses a higher antioxidant capacity. It functions as a reactive oxygen species (ROS) inhibitor in many illnesses and mitigates oxidative damage by triggering the Sirt1/Nrf2/HO-1 signalling pathway, which shields the kidney from ischemia-reperfusion injury. One indication of lipid peroxidation that has been identified is MDA [24]. According to a prior study, GPx antioxidants can lower lipid peroxidase levels [25]. Numerous researches have indicated that fucoxanthin lowers MDA levels [26-28]. Edible *Dilophys fasciola's* fucoxanthin exhibited potent antioxidant properties against DPPH [29].

By blocking inducible nitric oxide synthase, fucoxanthin also has anti-inflammatory properties by lowering NO production [30]. Numerous academic publications have discussed the functional qualities of fucoxanthin, including its anti-inflammatory and antioxidant capabilities [31]. In a recent research, fucoxanthin-rich brown seaweed extract reduced the expression of interleukin (IL)-6 and tumor necrosis factor-alpha (TNF-α) on the testis and plasma of streptozotocin-induced diabetic rats [32].

Furthermore, an *in vitro* investigation revealed that fucoxanthin inhibited extracellular signal-related kinase (ERK) signalling to prevent human neuroblastoma (SH-SY5Y) cells from going into apoptosis and decreased several proinflammatory proteins (such as IL-1*β*, TNF-α, and IL-6) in LPS-induced inflammation in RAW 264.7 macrophage cells. Additionally, it prevented the production of the transcription factor nuclear-factor kappa B (NF-κB) and the phosphorylation of mitogen-activated protein kinases (MAPKs) [31]. Furthermore, via altering the AMPK-NF-κB signalling pathway, fucoxanthin also decreased the levels of cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS) [32].

5. Conclusion

According to our findings, the synthetic pigment fast green pigment (FG), damages the kidneys by raising blood levels of TNF-α, MAD, albumin, urea, and creatinine. However, after the rats were given the natural dye fucoxanthin, we found that they resembled the control group more. It is feasible to conclude that fucoxanthin, which is derived from *Dilophys fasciola*, acts as an antioxidant and an anti-inflammatory to shield the kidneys from the harmful effects of fast green pigment after giving fast green dye and fucoxanthin to rats.

Abbreviations

ALT: Alanine aminotransferase; AST: Aspartate aminotransferase, COX-2: Cyclooxygenase-2, ERK: extracellular signal-related kinase, iNOS: inducible nitric oxide synthase, IL-1*β*, TNF-α, and IL-6: Proinflammatory proteins, MAPKs: Mitogen-activated protein kinases, MDA: Malondialdehyde, NF-Kb: transcription factor nuclear-factor kappa B, ROS: Reactive oxygen species, SH-SY5Y: Human neuroblastoma cells, TNF-α: Tumor necrosis factor-alpha.

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Conflict of interest

There is no conflict of interest.

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