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Potential protective role of spirulina algae and/or aminoguanidine on carbon tetrachloride induced liver fibrosis in rats.

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Abstract

The present study focuses on fibrotic effects on rat liver tissue induced by carbon tetrachloride (CCl_4) injection and the possible protective effects of spirulina algae (SP) and/or aminoguanidine (AG) on induced histological and biochemical changes.

To achieve this goal, a comparison was conducted between control and fibrotic groups (60 rats each). Fibrotic rats model was induced by the injection with an intraperitoneal dose of 2.0 ml/kg body weight CCl_4 twice/ week for four weeks to induce fibrosis. Control group was divided into four subgroups (n=15) as follows: control given saline through orogastric tube; SP administered with SP at a dose of 1g/kg/b.wt; AG administered with AG at a dose of 100 mg AG/kg/b.wt; double treatment group with SP+AG at the aforementioned doses (1g/kg/b.wt of SP+ 100mg/kg/b.wt of AG). Fibrotic groups were similarly subdivided into 4 subgroups (n=15): fibrotic non-treated; SP; AG and SP + AG as a mixture treated subgroups. The study extended for 3 time intervals, 2, 4 and 6 weeks (n=5/ intervals).

Histological alterations in liver tissue of fibrotic rats included hepatocytes degeneration with pyknotic nuclei and connective tissue fibers proliferation using haemtoxylin & eosin and silver impregnation technique for investigations. Biochemical results showed significant ($P<0.05$) increase in serum levels of α-fetoprotein (AFP), carcino embryonic antigen (CEA), tumor necrosis factor-alpha (TNF-α). On the other hand, significant ($P<0.05$) decrease in activity of cytochrome P_{450} content (CYP-450) was recorded in fibrotic rats compared with the normal controls.

When fibrotic subgroups were treated with SP and/or AG, considerable protective effects in previous biochemical and histological parameters were recorded especially in mixture treated group.

Keywords: Aminoguanidine, Carbon tetrachloride, Fibrosis, Spirulina, Rats.

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1. Introduction

The liver is a vital organ admitted in more than five hundred metabolic responses within the biological system and one of its major capacities is the detoxification of injurious substances or poison. However, it can be damaged by toxicants, which within the process may mutilate the metabolic actions of the liver, thus giving rise to liver injury [1, 2]. Liver fibrosis is attached to inflammation, tissue remodeling under wound healing and excessive accumulation of extracellular matrix [3].

Carbon tetrachloride (CCl_4) is a known ideal for causing liver damage involving two phases; in the 1st phase, CCl_4 is enzymatically converted by cytochrome P_{450} activity (CYP2E1) in hepatocytes to create trichloromethyl free radicals that are fatal metabolite [4]. It causes lipid peroxidation of the endoplasmic reticulum of the membranes of hepatocytes and initiates oxidative damage. The 2nd phase of hepatotoxicity involves the inflammatory reactions that perform a critical function. Certain hepatic cells including sinusoidal endothelial cells, Küpffer cells and hepatic stellate cells (HSCs) generate exude cytokines that referee liver fibrogenesis [5]. Both radicals (trichloromethyl and trichloromethylperoxy free radicals) are highly reactive and initiate complex cellular modifications that result in hepatotoxic damage, inflammation, cirrhosis, fibrosis, and hepatocellular carcinoma (HCC) [6].

Spirulina protects liver tissues from damage by reducing the level of lipid peroxidation and increasing antioxidant activities. In this regard, [7] reported the hepatoprotective potential of spirulina in fibrosis and accredited this to the antioxidant effects of spirulina and to its ability to potentiate the antioxidant system. In addition, Spirulina improved activity of the antioxidant system and decreased lipid peroxidation in the liver of cadmium-intoxicated rats [8].

The defense impact of aminoguanidine (AG) may result from its coordinate antioxidant properties [9]. AG has been depicted to be a successful hydroxyl radical scavenger. AG shows a critical dosage subordinate impact against free radical damage. Concomitantly, AG acting as a strong free radical scavenger anticipates the exhaustion of cellular antioxidants that help the cell from damaging and lose their internal enzymes such as hepatic enzymes [10]. Moreover, according to [11] it was noticed that plasma concentration of nitrates/nitrites, considered of nitric oxide (NO) generation that altogether expanded within the fibrotic formation and diminished with AG treatment.

Since; the liver is one of the major target organs of long term fibrosis, the aim of the present study was undertaken to show the possible effectiveness of the above mentioned

nutrients (SP and/or AG) to adjust the disturbance in certain blood biochemical components and changes in histological liver studies.

2. Material and methods

2.1 Animals housing:

A total number of 120 adult male albino rats (Rattus rattus) approximately of the same age (12±1 week old) and of weights (170±10gm) were obtained from the Breeding unit, Serum and Antigen Laboratories at Helwan farm. They were caged in metal boxes with wire top galvanized metal covers under controlled environmental conditions, and were supplied with diet and tap water ad libitum for one week prior to the experiment for acclimatization. Experimental protocol were taken after the Rules for the Care and Use of Laboratory Animals affirmed by the Institutional Ethics Committee of Ain Shams University RE (174)20.

2.2. Chemicals and antioxidant agents:

CCl_4 was purchased from el Goumhoiria company, Cairo, Egypt, with case number (1001/120/559/32179) and product number (UN: 1846 IATA: 6.). Spirulina was purchased from National Research Centre, Guiza, Egypt. Aminoguanidine was purchased from Sigma Aldrich Chemicals Co., St. Louis, Missouri, United States, with case number (2582-30-1) and product number (1043B). Diethyl ether was purchased from el Goumhoiria company, Cairo, Egypt, with case number (1134627) and product number (67-66-3).

2.3. Experimental design:

After acclimatization to the laboratory conditions, a comparison was executed between normal control rats group (60 rats) and fibrotic rats group (60 rats). The control rats group (60 rats) were divided into four subgroups of 15 rats each as follows: normal control given saline through oro-gastric tube; SP algae subgroup administered with SP at a dose of 1g /kg/b.wt /day [13]; AG subgroup administered with AG at a dose of 100mg AG/kg/b.wt /day [14]; and double treatment group with both SP 1g /kg/b.wt /day and AG 100 mg/kg/b.wt /day. The fibrotic rats group (60 rats) were divided into four subgroups of 15 rats each as follows: fibrosis subgroup were injected with a dose of 2.0ml/kg body weight of carbon tetrachloride (CCl_4) intraperitonealy (i.p.) twice a week [6, 12]; fibrotic treated with SP algae subgroup administered with SP at a dose of 1g /kg/b.wt /day [13]; fibrotic treated with AG subgroup administered with AG at a dose of 100mg AG/kg/b.wt /day [14]; and fibrotic treated with double treatment group with both SP 1g /kg/b.wt /day and AG 100 mg/kg/b.wt /day. Five rats were used in each interval (2, 4 and 6 weeks).

2.4. Samples collection:

At the end of each experimental period, rats were slightly anaesthetized by diethyl ether in transparent glass jars. As a nose cone, 50 ml centrifuge tubes inserted with cotton was used for every 20 ml volume of jar. Blood samples were collected from the heart in clean dry test tubes. Blood samples were centrifuged at 10,000 rpm for 20 minutes to obtain the serum. Sera were separated and kept at -20°C for the biochemical parameters. First, rats were anesthetized and then sacrificed in accordance with ethical rules and immediately livers were carefully removed aseptically, and washed with cooling saline solution (0.9% NaCl).

2.5. Histological studies:

The right lobe of liver was fixed in 10% buffered formalin and processed in paraffin wax. Paraffin sections were cut (6μm thick) for histopathological investigations using the routine staining (haematoxylin and eosin) [15] and silver impregnation technique [16]. Histological profiles of hepatic tissues were microscopically observed and photographed by digital camera (Model CX22RFS1, Nikon, Tokyo, Japan)

2.6. Biochemical studies:

The left lobe of the livers was kept at -80°C for biochemical parameters.

Liver tumor markers: included serum α-fetoprotein (AFP) and carcino-embryonic antigen (CEA) level estimated kinetically using kits from BQ kits, Inc. San Diego, Califorina, United States [17, 18].

Cytokines profile: included serum tumor necrosis factor-alpha (TNF-α) level using quantitative method kits from Kamiya biomedical company, Gateway Drive, Seattle [19].

Cytochrome profile: included tissue cytochrome P_{450} (CYP-450) content using quantitative determination using kits from CUSABIO, Houston, USA [20].

2.7. Data analysis:

Data were statistically analyzed using Two Way Analysis of Variance (ANOVA) by the aid of SPSS (version 20.0) program to measure the differences in the variables dependent on both time and treatment. Values were considered statistically significant when $p \leq 0.05$.

Results

3.1. Histological studies:

The results of the histopathological examination by H&E and silver impregnation technique are represented in figures 1 and 2. These figures show the histopathological effects of CCl_4 on the rat liver, as well as rats treated by SP and/or AG post liver fibrosis.

The pathological sections stained by H&E and silver impregnation technique showed no fat or fibers accumulation in the liver sections of the control group (Fig. 1A). Connective tissue (Fig. 1B) and reticulin fibers were normally distributed around the areas of the central veins (Fig. 1C). On the contrary, the CCl_4 model group showed serious histopathological alterations, including fatty degeneration, massive necrosis, amplified infiltration of mononuclear cells (Fig. 1D), and expanded space of sinusoids with fibrous bridges between cells (Fig. 1E) most likely due to the unusual arrangement of liver pseudolobules, and deviated area of central veins. The buildup of liver reticular fibers stained by silver appeared as brown fibers that increased by the exposure to CCl_4. Fiber accumulation and, slender septa that links between the central veins were also detected (Fig. 1F).

Livers of rats treated with SP showed attenuated appearance of degenerative necrotic changes (Fig. 2A) and collagen fibers (Fig. 2B). Liver sections obtained from animals administered with AG and treated with CCl_4 showed consistent reduction of liver necrosis, inflammation (Fig. 2C) and partially regained fibers around blood vessels (Fig. 2D). The inflammation of liver cells, fatty degeneration (Fig. 2E) and bridging of fibers were significantly reduced in the CCl_4 treated with SP and Ag as a mixture (Fig. 2F).

3.2. Biochemical parameters:

3.2.1. Liver tumor markers profile:

There was a significant ($p<0.05$) elevation in the levels of liver tumor markers profile levels [Alpha-fetoprotein (AFP) and carcino-embryonic antigen (CEA)] in fibrotic rats group compared with normal control rats group (Table 1). A remarkable correction occurred in the previous parameters after the fibrotic rats groups were treated with SP or AG dependent on the duration of treatment (2, 4 and 6 weeks). The best corrections were reported in the serum levels of AFP and CEA of fibrotic rats group which were treated with both antioxidants (SP and AG) compared to their corresponding normal control rats group (Table 1).

3.2.2. Cytokines profile:

Induction of fibrosis in rats, injected with a dose of 2.0ml/kg of body weight of CCl_4 intraperitonealy (i.p.) twice a week, led to a significant ($p<0.05$) elevation in the serum cytokines level TNF-α (Table 2). Fibrotic rats treated with 1g SP/kg/b.wt/day or 100mg AG/kg b.wt/day showed considerable protection effects on all studied TNF-α levels depending on duration of treatment (Table 2). The best amelioration effects were reported in the serum levels of TNF-α of fibrotic rats group that were treated with SP + AG compared to their corresponding normal control rats group at 2, 4 and 6 weeks interval (Table 2).

3.2.3. Cytochrome profile:

Significant ($P<0.05$) decrease of the liver cytochrome P_{450} activity in fibrotic rats group with 2.0ml/kg CCl_4 twice a week led to pronounced liver damage and fibrosis. Treating fibrotic rats with 1g SP/kg/b.wt/day or 100mg AG/kg b.wt/day led to considerable modulation effects on CYP-450 activity dependent on duration of treatment (Table 2). The maximum corrections were recorded in the CYP-450 activity in tissue of fibrotic rats group which were treated with a mixture of SP and AG compared to their corresponding normal control rats group at 2, 4 and 6 weeks (Table 2).

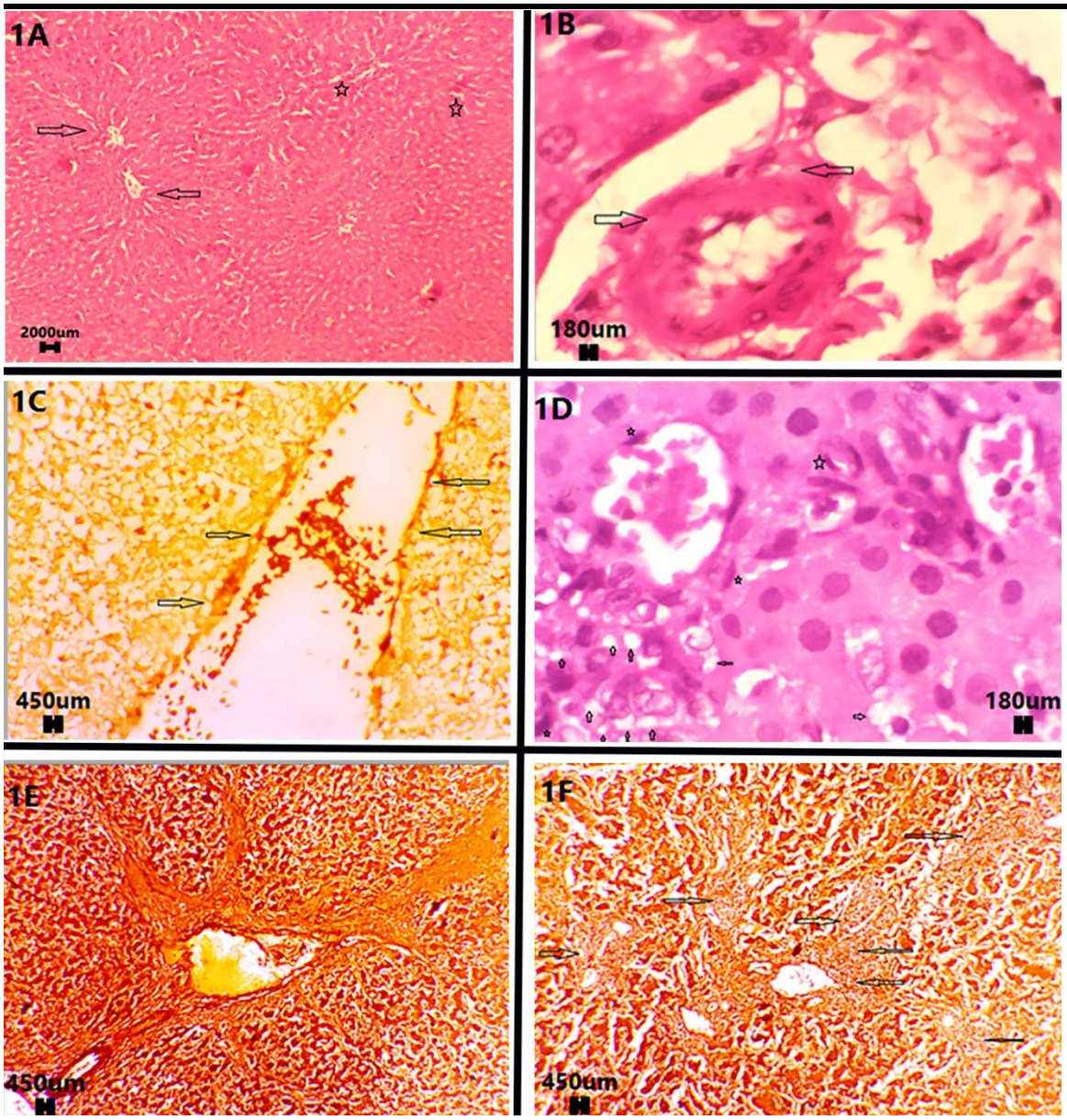

Figure 1: photomicrographs of sections from control and fibrotic rats (haematoxylin & eosin (H&E) and silver impregnation technique) and (X= magnification).

1A- Liver section of normal control rat showing normal architecture of hepatic cord cells (⇧) and sinusoids (☆) (H&E; X100).

1B- Liver section of normal rat showing connective tissue fibers and blood vessels encircled by fibres (⇧) (H&E; X1000).

1C- Liver section of control rat group showing reticulin fibers around the areas of central veins (⇧) (Silver impregnation technique; X400).

1D- Liver section of fibrotic rat treated with CCl_4 showing hepatocellular necrosis (☆) and massive fatty infiltration changes (⇧) (H&E; X 1000).

1E- Liver section of fibrotic rat treated with CCl_4 showing fibrous bridges between cells (Silver impregnation technique; X400).

1F- Liver section of fibrotic rat treated with CCl_4 showing slender septa between central veins (⇧) (Silver impregnation technique; X400).

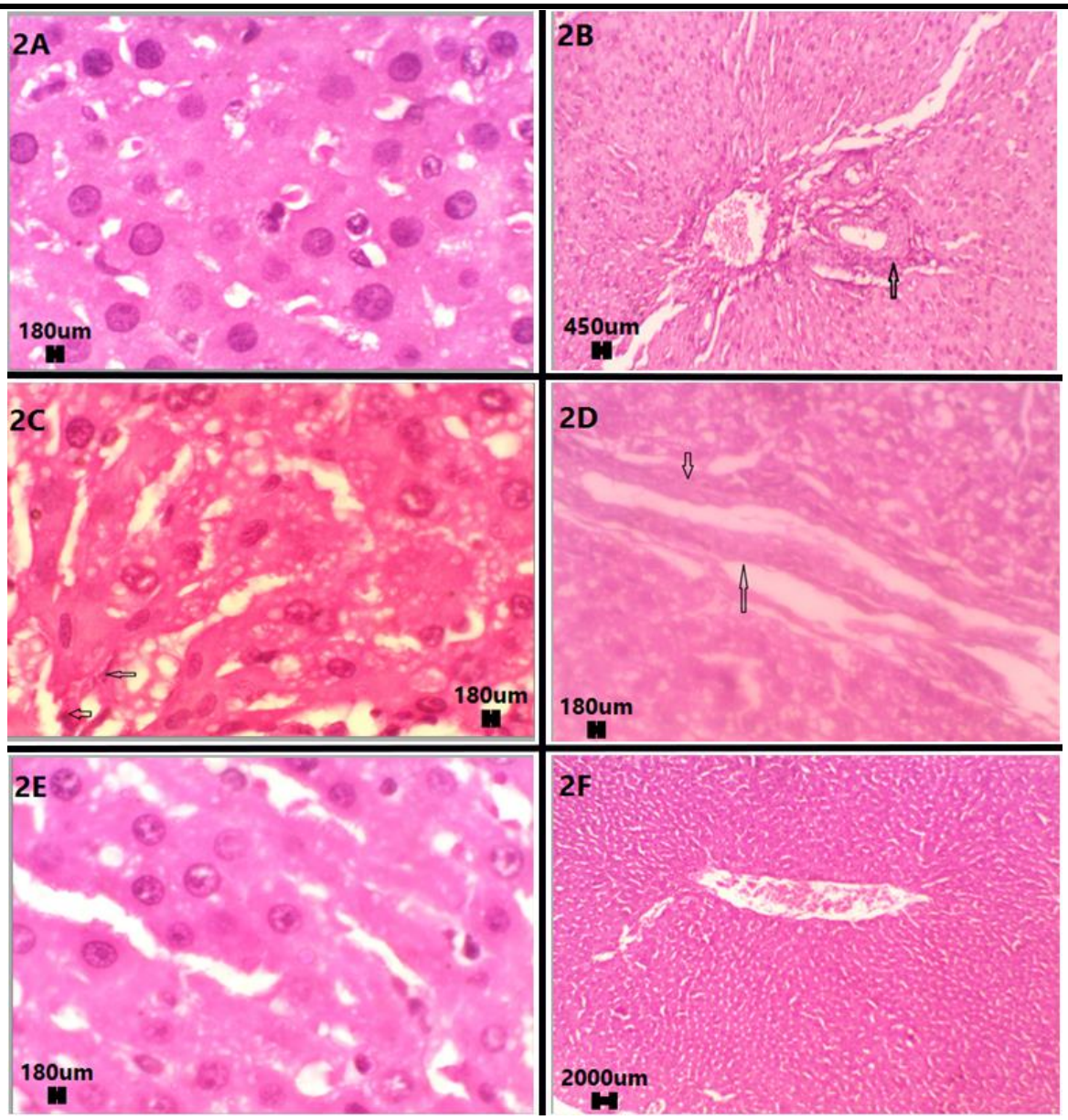

Figure 2: photomicrographs of sections from fibrotic rats treated with spirulina algae (SP) and/or aminoguamidine (AG).
(H&E).
2A- Liver section of treated rat with SP showing attenuated appearance of degenerative necrotic changes (X1000).
2B- Liver section of treated rat with SP showing attenuated appearance of collagen fibers (⇧) (X400).
2C- Liver section of treated rat with AG showing reduction of liver necrosis and inflammation (X1000).
2D- Liver section of treated rat with AG showing partly regained fibers (⇧) (X1000).
2E- Liver section of treated rat with SP and AG showing regain to normal structure(X1000).
2F- Liver section of treated rat with SP and AG showing recovery of tissue (X100).

Table (1): Changes in the studied parameters of α-fetoprotein and carcino-embryonic antigen in normal and fibrotic rats treated with spirulina algae and/or aminoguanidine at 2, 4 and 6 weeks.

Groups / Time	Normal				CCl_4(Fibrotic)			
	Control	**SP**	**AG**	**Mix**	**CCl_4**	**SP**	**AG**	**Mix**
α-fetoprotein (AFP) (ng/ml)								
2 weeks	$1.09\pm 0.124^{E}{}_{c}$	$1.06\pm 0.009^{E}{}_{a}$	$1.07\pm 0.011^{E}{}_{a}$	$1.1\pm 0.019^{E}{}_{a}$	$4.92\pm 0.022^{A}{}_{c}$	$3.73\pm 0.027^{B}{}_{a}$	$3.18\pm 0.045^{C}{}_{a}$	$2.63\pm 0.052^{D}{}_{a}$
4 weeks	$1.04\pm 0.021^{E}{}_{a}$	$1.07\pm 0.005^{E}{}_{a}$	$1.09\pm 0.005^{E}{}_{b}$	$1.07\pm 0.005^{E}{}_{b}$	$5.45\pm 0.023^{A}{}_{b}$	$3.29\pm 0.030^{B}{}_{b}$	$2.56\pm 0.049^{C}{}_{b}$	$2.11\pm 0.030^{D}{}_{b}$
6 weeks	$1.06\pm 0.008^{E}{}_{b}$	$1.06\pm 0.007^{E}{}_{a}$	$1.07\pm 0.004^{E}{}_{a}$	$1.07\pm 0.006^{E}{}_{b}$	$5.99\pm 0.052^{A}{}_{a}$	$2.93\pm 0.036^{B}{}_{c}$	$2.10\pm 0.033^{C}{}_{c}$	$1.68\pm 0.364^{D}{}_{c}$
Carcinoembryonic antigen (CEA) (ng/ml)								
2 weeks	$0.11\pm 0.005^{E}{}_{a}$	$0.10\pm 0.005^{E}{}_{a}$	$0.10\pm 0.005^{E}{}_{a}$	$0.09\pm 0.000^{E}{}_{b}$	$2.10\pm 0.052^{A}{}_{c}$	$1.06\pm 0.163^{B}{}_{a}$	$0.92\pm 0.012^{C}{}_{a}$	$0.71\pm 0.022^{D}{}_{a}$
4 weeks	$0.10\pm 0.005^{E}{}_{a}$	$0.10\pm 0.005^{E}{}_{a}$	$0.09\pm 0.004^{E}{}_{b}$	$0.09\pm 0.003^{E}{}_{b}$	$2.73\pm 0.023^{A}{}_{b}$	$0.81\pm 0.199^{B}{}_{b}$	$0.64\pm 0.013^{C}{}_{b}$	$0.53\pm 0.013^{D}{}_{b}$
6 weeks	$0.10\pm 0.005^{E}{}_{a}$	$0.08\pm 0.002^{E}{}_{b}$	$0.09\pm 0.002^{E}{}_{b}$	$0.10\pm 0.007^{E}{}_{a}$	$3.24\pm 0.030^{A}{}_{a}$	$0.68\pm 0.018^{B}{}_{c}$	$0.47\pm 0.018^{C}{}_{c}$	$0.30\pm 0.02^{D}{}_{c}$

- Data are expressed as Mean±standard error (SE).

- $^{A, B, C, D, E}$ Means with a common superscript within a row are significantly ($P<0.05$). n=120.

- $_{a, b, c}$ Means with a common subscript within a column are significantly ($P<0.05$).

SP = Spirulina algae. AG = Aminoguanidine.

Mix = Spirulina algae + Aminoguanidine as a mixture. CCl_4 = Carbon tetrachloride.

Table (2): Changes in the studied parameters of tumor necrosis factor-alpha and cytochrome P_{450} in normal and fibrotic rats treated with spirulina algae and/or aminoguanidine at 2, 4 and 6 weeks.

Groups / Time	Normal				CCl_4(Fibrotic)			
	Control	**SP**	**AG**	**Mix**	**CCl_4**	**SP**	**AG**	**Mix**
Tumor necrosis factor-alpha (TNF-α) (pg/ml)								
2 weeks	5.21± 0.019$^{E}_{a}$	5.21± 0.008$^{E}_{b}$	5.22± 0.004$^{E}_{a}$	5.21± 0.009$^{E}_{b}$	19.59 ± 0.052$^{A}_{c}$	10.55± 0.119$^{B}_{a}$	9.52± 0.048$^{C}_{a}$	8.68± 0.019$^{D}_{b}$
4 weeks	5.20± 0.022$^{E}_{a}$	5.19± 0.014$^{E}_{c}$	5.21± 0.010$^{E}_{a}$	5.24± 0.009$^{E}_{a}$	26.62 ± 0.023$^{A}_{b}$	9.14± 0.027$^{B}_{b}$	8.48± 0.042$^{C}_{b}$	7.53± 0.022$^{D}_{b}$
6 weeks	5.19± 0.012$^{E}_{a}$	5.23± 0.016$^{E}_{a}$	5.21± 0.006$^{E}_{a}$	5.15± 0.119$^{E}_{c}$	31.86 ± 0.030$^{A}_{a}$	8.16± 0.050$^{B}_{c}$	7.69± 0.058$^{C}_{c}$	6.71± 0.062$^{D}_{c}$
Cytochrome P_{450} (CYP-450) (pg/mg)								
2 weeks	483± 1.86$^{A}_{b}$	481± 0.837$^{A}_{b}$	483± 1.95$^{A}_{b}$	483± 0.800$^{A}_{b}$	337± 4.30$^{E}_{b}$	410± 0.510$^{D}_{c}$	422± 0.748$^{C}_{c}$	433± 0.860$^{B}_{c}$
4 weeks	486± 2.22$^{A}_{a}$	479± 1.07$^{B}_{c}$	485± 1.07$^{A}_{a}$	485± 1.28$^{A}_{a}$	281± 2.42$^{E}_{c}$	420± 0.837$^{D}_{b}$	430± 0.860$^{C}_{b}$	449± 1.33 $^{B}_{b}$
6 weeks	484± 1.50$^{A}_{b}$	486± 1.36$^{A}_{a}$	483± 1.36$^{A}_{b}$	481± 1.69$^{A}_{c}$	245± 3.04$^{E}_{d}$	429± 1.14$^{D}_{a}$	451± 1.21$^{C}_{a}$	471± 3.01$^{B}_{a}$

- Data are expressed as Mean±standard error (SE).

- $^{A, B, C, D, E}$ Means with a common superscript within a row are significantly ($P<0.05$). n=120.

- $_{a, b, c}$ Means with a common subscript within a column are significantly ($P<0.05$).

SP = Spirulina algae. AG = Aminoguanidine.

Mix = Spirulina algae + Aminoguanidine as a mixture. CCl_4 = Carbon tetrachloride.

Discussion

Hepatic fibrosis to a great extent comes from the clutter within the homeostasis of synthesis, degeneration, deposition and absorption of collagens [21]. HSCs are one of the foremost origin of extra cellular matrix (ECM) proteins within the liver; hepatic damage, HSCs create a myofibroblast-like phenotype, distinguished by a diminished substance of vitamin A, enhanced expression of network protein, increased multiplication and migration; as well as generation of lattice metalloproteinases (MMPs) [22]. Thus they characteristically accept the capacity to redesign extracellular matrix through their generation of ECM proteins in liver. The

neurotic progression of liver fibrosis is exceedingly correlated with the fibrotic reaction and the proliferation of connective tissue. Amid the fibrotic process, HSCs are enacted and lose their lipid drops [23].

This study reports the protective effect of SP and/or AG against hepatic damage and fibrosis created by CCl_4 in rats. CCl_4 is hepatotoxic agent, and protection of liver from its damage effect has been broadly utilized as a marker of liver defensive action of drugs in common. In agreement with the present study, the value of biochemical parameters for diagnosing liver fibrosis are very limited, and final histopathological examination is still the golden criterion for the diagnosis of liver fibrosis. In recent decades, in spite of diverse etiology, liver injuries are as often as possible related with overabundance oxidative stress. Oxidative stress initiated by CCl_4 metabolism might advance stimulation of invasiveness and proliferation of HSCs [24].

The overlapped genes/proteins involved in process of oxidation reduction and response to oxidative stress, may finally lead to liver injury and fibrosis. When the liver is subjected to chronic injury, hepatic stellate cells (HSCs) are activated and proliferate, causing extra cellular matrix (ECM) deposition, and hepatocytes are replaced with abundant ECM which leads to scar formation and fibrosis [25].

Küpffer cells enacted by oxidative stress secrete cytokines, such as TNF-α to invigorate the expression of soluble intercellular adhesion molecule-1 (sICAM-1) which actuates neutrophils activation [26]. H&E stained sections of CCl_4 group showed aggregation of inflammatory cells. Moreover, silver impregnated sections of this group displayed reticular fibers within necrotic areas and fibrotic bridges. In the liver tissue of SP algae and aminoguanidine groups, the areas occupied by the reticular fibers and necrotic tissues were reduced in addition to the reduction of TNFα were reduced.

Hepatic damage initiated by CCl_4, has been broadly utilized for evaluation of hepatoprotective action. The mechanism is included in free radicals which are created amid CCl_4 metabolism by cytochrome P_{450}, counting trichloromethyl and oxygen-centered lipid radicals, lipid peroxidation, DNA alteration mitochondrial damage, and indeed cell death in organisms which play critical role in liver injury and elevated AFP and CEA [27].

The current results revealed significant–increase in the liver tumor cytokines profile including tumor necrosis factor-alpha (TNF-α) of hepatotoxicated rats injected with CCl_4. Such result runs in full agreement with and have been thoroughly confirmed by a relative previously

reported study [28]. The cellular signaling organize are activated by reactive oxygen species (ROS) that elevate level of TNF-α to cause apoptosis and includes protein-protein interactions. TNF-α apoptotic signaling could be a complex handle that includes protein-protein intuitive and the cooperation of a few intermediates. Hepatocytes mitochondria play a fundamental part in apoptosis, and TNF-α– mediated apoptosis leads to numerous forms of liver damage [29].

The current study showed a significant reduction of liver cytochrome profile including liver cytochrome P_{450} (CYP-450) content activity. These results are attributed to the destructive effect of CCl_4 on the hepatocytes [30].

Recent data propose that lipid peroxidative forms moreover influence collagen synthesis [31]. In reality, Collagen synthesis by fibroblasts stimulated by lipid peroxidation and antioxidants can inhibit this incitement, apparently by hindering lipid peroxidation. Additionally, responsive oxygen species created by inflammation and free radicals reactions are included within the inflammatory response and can contribute to liver fibrosis and injury [32, 33].

Spirulina algae causes significant reduction in the rate of lipid peroxidation which is most likely attributed to the presence of antioxidants which have important beneficial effects on the liver regeneration [34]. Spirulina has a significant effect on scavenging free radicals, thereby decreasing fibrosis bridging of liver. SP has been recognized as a potent antioxidant that decreases the level of liver function markers profile [35]. These parameters were elevated in the current study due to the toxic effect of CCl_4. These results are in consistent with those reported by **Chaklader et al. (2021) and Lin et al. (2021)** [36, 37]

In consistent with **Khafaga et al., (2018)** [38], treating hepatotoxic rat with spirulina produced a significant decrease in the serum cytokines TNF-α and increased cytochrome P_{450}.

In fact, carotenoids of SP act as protective agent against oxidative damage [39]. Beta-carotene of SP may decrease cell damage, particularly the damage to DNA particles, hence it plays part within the repair of recovery process of harmed liver cells [40].

SP has inhibitory effect on metabolic activation of carcinogenic chemicals and different toxins metabolized by cytochrome P450 1A2 (CYP1A2), which may in turn diminish the individual risk of organ harmfulness, carcinogenesis and mutagenesis initiated by chemicals and increased CYP-450 activity in liver tissue [41].

Thus it may be noticed from this study the proof of the antioxidant action of SP. Similarly it was also concluded that the nourishing of SP at a dosage of 1 g/kg essentially

diminishes the toxic impacts, by improving the body defense system through scavenging the free radicals and progressing the viability of endogenous antioxidants [42].

The present recovery manifestations were seen as hepatotoxicated animals were treated with AG (100 mg/kg b.wt per day) showed lesser degenerative fibrotic cells; partial regain of elastic fibers of blood vessels and loss of invagination reticular fibers. These were duration dependent than SP treatment. Moreover, according to **Apinar et al. (2007)** [43], animals were essentially expanded within the fibrotic animals and diminished with AG treatment.

Another study has shown that AG significantly reduced the liver necrogensis. This may be maintained through cytokine generation, decreasing liver oxidative damage and antioxidant defenses, and portal and central inflammation in rat thus preserving liver function [44]. Also, these results are supported by the ability of AG to prevent the hepatotoxic necrotic damage in rat livers [45].

Inducible nitric oxide synthase (iNOS) protein is communicated in inflammatory cells and hepatocytes amid fibrosis development. Subsequently, reserve of iNOS expression may have a vital part in both endotoxaemia and cirrhosis. AG has been observed with antioxidant enzymes activation and detoxification components through diminishing the metabolic activation of CCl_4 by specifically inhibiting P4502E1, the isoenzyme (paraoxonase-1, superoxide dismutase, and catalase) most effective within the activation of CCl_4 [46].

AG inhibits oxidative stress that occur when the production of ROS and reactive nitrogen species (RNS) appear beyond antioxidant protection mechanisms and thus preventing mitochondrial failure [47]. Additionally, **Panchal et al. (2017)** [48] mentioned that AG was shown to decrease AFP and CEA in hepatotoxicated animals and inhibit tumorigenic effect.

The antioxidant effect of AG lies upon its ability to prevent hepatotoxicity and prevent the elevation of serum cytokine (TNF-α) that induced liver failure [49]. Meanwhile, treatment with aminoguanidine to hepatotoxic animals produced a significant decrease in CYP-450 activity when compared with hepatotoxicity group accordance with **Ingawale et al. (2014)** [50].

Conclusions

On the basis of previous investigations and current data, the maximum corrections in all studied histological investigations and biochemical parameters were recorded in fibrotic rats group treated with a mixture of antioxidants (SP and AG) dependent on the duration of treatment (2, 4, and 6 weeks).

The present investigation could help to encourage the clinical use of these compounds as a treatment for fibrosis. Yet it is recommended that the mixture of SP and AG should be further evaluated for their protective potentials in fibrotic clinical setting. So, future researches are needed to address the potential adverse effects of SP and AG on the treatment of fibrosis subjects.

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Conflicts of interest
Declared none.

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الملخص العربى

الدور العلاجى الممكن لطحلب الأسبيرولينا و الأمينوجوانيدين و خليطيهما على تليف الكبد الناجم عن رابع كلوريد الكربون في الجرذان.

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تركز الدراسة الحالية على التأثيرات الليفية على أنسجة الكبد والتأثيرات المحسنة المحتملة لطحالب الأسبيرولينا (SP) أو الأمينوجوانيدين (AG) وخليطهما على التغيرات النسيجية والفسيولوجية المستحثة في كبد الجرذان.

ولتحقيق هذا الغرض أجريت مقارنة بين مجموعة الجرذان الضابطة (٦٠ جرذآ) ومجموعة الجرذان المتليف كبدها (٦٠ جرذآ). تم حقن الجرذان فى المجموعة المتليف كبدها بجرعة تبلغ ٢,٠ مل / كجم من وزن الجسم من رابع كلوريد الكربون (CCl_4) مرتين في الأسبوع لمدة أربعة أسابيع للحث على التليف. تم تقسيم مجموعة الجرذان الضابطة (٦٠ جرذآ) الخالية من علاج رابع كلوريد الكربون إلى أربع مجموعات فرعية مكونة من ١٥ جرذآ لكل منها على النحو التالي: المجموعة العادية التى تم حقنها بمحلول ملحي من خلال أنبوب المعدة. المجموعة الفرعية التى تم حقنها بطحلب الأسبيرولينا بجرعة ١ جم / كجم / وزن / يومياً ؛ المجموعة الفرعية التى تم حقنها بالأمينوجوانيدين بجرعة ١٠٠ ملجم لكل كيلو جرام من وزن الجسم يومياً؛ المجموعة المختلطة التى تم علاجها بكلا من الأسبيرولينا و الأمينوجوانيدين معآ. وبالمثل ، تم تقسيم مجموعات الجرذان المتليف كبدها (٦٠ جرذآ) إلى ٤ مجموعات فرعية: المتليفة بدون معالجة ؛ المجموعة التى تم علاجها بالأسبيرولينا؛ و التى تم علاجها بالأمينوجوانيدين؛ والتى تم علاجها بخليط من الأسبيرولينا و الأمينوجوانيدين. تم أستخدام خمسة جرذان في كل فترة (٢ و ٤ و ٦ أسابيع).

وشملت التغيرات النسيجية في أنسجة الكبد من الجرذان المتليفة وتشمل (تكرار) الأنوية المحللة فى الخلايا الكبدية؛ ضيق الأوعية الدموية. تسلل الخلايا الأحادية النواة فى الخلايا الموسعة فى الفراغ الخلوى وأنتشار النسيج الضام والألياف. بالمقارنة مع الجرذان الطبيعية الضابطة، أظهرت النتائج زيادة كبيرة ($P<0.05$) في مستويات مصل البروتين الممرض (AFP) ، المستضد السرطاني المضغي (CEA) ، عامل نخر الورم ألفا (TNF-α). من ناحية أخرى ، تم تسجيل أنخفاض كبير ($P<0.05$) في نشاط مستوى السيتوكروم P_{450} (CYP-450) في الجرذان المتليف كبدها مقارنة مع المجموعة الضابطة.

عند معالجة المجموعات المرضية فى الجرذان المتليف كبدها بطحلب الأسبيرولينا او/و الأمينوجوانيدين، وجد تأثير تحسينى كبير في جميع الدراسات السابقة كانت واضحة معتمداً على بعض الآليات التى تمت مناقشتها وفقا للأبحاث الحديثة المتاحة. علاوة على ذلك ، كشفت الدراسات النسيجية للكبد تأثيراً تحسينياً واضحاً من هذه المضادات المؤكسدة فيما يتعلق بتلف الأنسجة والسلامة الهيكلية.