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Spirulina platensis, reduced liver and kidney injuries induced by Sodium arsenite

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Abstract : *Spirulina platensis***,** a blue-green alga, has vastly therapeutic properties and it is used as food supplement. This study aims to evaluate the protective effect of *Spirulina platensis* against arsenic hepatorenal toxicity based on its antioxidantproperties,free-radical scavenger and immunological properties. Oral administration of sodium arsenite (6.3mg/Kg, p.o BW) to rats for eight weeks led to a significant increase in serum monocyte chemoattractant protein-1 (MCP-1), tumor necrosis factor alpha (TNF-α), interleukin 6 (IL-6), cholesterol, triglycerides, liver (AST,ALT, and ALP), and kidney function parameters (urea, uric acid, and creatinine) except serum protein level which was significantly reduced. In addition, arsenic-induced oxidative stress and lipid peroxidation process as evidenced by elevation of malondialdehyde (MDA) and declinein the activities of hepatic and renal catalase (CAT),superoxide dismutase(SOD) enzymes, and glutathione (GSH) levels in which reflected on the final body and liver weights.Hepatorenal pathological changes were observed in the arsenic group.Treatment of rats with *spirulina platensis*(300mg/kg, p.o BW) mitigates indices of atherosclerosis, hepato-renal oxidative stress and pathological changes of liver and kidney induced by arsenic. These results support the *spirulina* protective effect of by which it lessens several factors involved in the progression of atherosclerosis and liver and kidney toxicity.
Keywords : *Spirulina platensis*, Sodium arsenite, Atherosclerosis, Kidney toxicity, Livertoxicity.

Introduction:

The cyanobacterium *Spirulina* is a filamentous blue-green alga belonging to the Oscillatoriaceae family that is commonly found in tropical and subtropical areas in warm chalky waterThe spirulina is characterized by plenty of nutritional components including high protein content [60–70% by dry weight], and a plenty of [vitamins, amino acids, gamma-linoleic acid, and minerals][1]. *Spirulina platensis* as a diet supplement has a major benefits in improving hyperlipidemia[2], type 2 diabetes[3], inflammation[4], cancer[5], and vascular diseases[4]. Furthermore, *Spirulina platensis* affords a defense against many toxicants related to oxidative

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stress and changes in hepatic antioxidant defense mechanism[6]. These effects were highly linked to its active constituent phycocyanin[4]. Phycocyanin, is a biliprotein, contains a tetrapyrrole phycocyanobilin, which is related to the antioxidant and free radical scavenging properties of phycocyanin[4].

Longtime exposure to arsenic has numerous harmful side effects, inclusive cardiovascular disease, peripheral vascular disease, kidney and liver disorders[7-9]. Nevertheless, many studies confirm its role in the progression of oxidative stress and inflammation with consequent release of proinflammatory cytokines induced organs damage[9,10].

Spirulina offers a protection against aluminum-induced nephrotoxicity and DNA damage through the inhibition of oxidative stress[11]. Spirulina has a potential hepato-protective effect against diclofenac-induced hepatic injury in rats[4] through maintaining antioxidant enzymes and reduction of lipid peroxidation. These effects were highly linked to its active constituentphycocyanin (4). Phycocyanin, is a biliprotein, contains a tetrapyrrole phycocyanobilin, which is related to the antioxidant and free radical scavenging properties of phycocyanin[4].

Arsenic-induced oxidative hepatic and renal dysfunctions[12]. Arsenic-induced oxidative hepatic and renal dysfunctions[12]. Arsenic has several toxicity mechanisms associated with damaging of tissues, including oxidative stress,inflammation, impaired protein degradation, autophagy,endoplasmic reticulum stress, and mitochondrial dysfunction[13].

Since *Spirulina platensis* is a source of bioactive compounds,particularly antioxidants , sothe present study was designed to investigateits ability to alleviate hepatorenal intoxication,oxidative stress and hyperlipidemia induced by sodium arsenite. which may flatten the way for the possibility to use it for therapeutic application.

Material and Methods:

1. Drugs and Chemicals:

Sodium arsenite was obtained from [Merck, Germany], while *Spirulina platensis* from [Alibaba Comp., China] in a powder form. The other chemicals used were of high analytical grades. Sodium arsenite and *Spirulina platensis* were administered orally, which they were suspended in distilled water.

2. Animals:

Mature male Wistar albino rats weighing 120-140 g were obtained from the National Research Centre Laboratory (Dokki, Giza, Egypt) and were kept in standard polypropylene cages and saved under stable environmental conditions with equal light-dark cycles. Rats were fed normal pellet diet and water *ad libitum*.

3.Ethics Statement:

This experiment was carried out in accordingto the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health (NIH publication No. 85–23, revised 1996) andunder regulations ofAnimal Care and Use of National Research Centre in Egypt. All surgery was performed under deep sodium pentobarbital anesthesia and all efforts were made to minimize suffering.

4. Experimental design:

This model was carried out in according to a preceding study[14]. 40 rats were divided into 4 groups, the first group [10 rats]was received the vehicle (distilled water) to be considered as normal control group. While, the second group [10 rats]administered orally sodium arsenite in a dose of 6.3 mg/kg equivalent to 15% of LD50[41mg/kg] [15] and considered as arsenic control group. The third group was administered orally 300mg/Kg of *Spirulina platensis*(16)followed by oral administration of sodium arsenite 6.3mg/kg and served as *Spirulina platensis*/ arsenic-treated group, while the fourth group was received300mg/kg of *Spirulina platensis* orally only and considered as *Spirulina platensis* treated group to test if there are any alterations or side effects due its consumption. Treatments were continued daily for 8 weeks.

5. Determination of body, liver and kidney weights:

Body weight of rats was determined before and after treatment. Also, liver and kidney weights were determined after washing with a saline solution in all groups at the end of treatments after animal sacrifice.

6. Serum Collection for Analysis:

Blood was collected from the retro-orbital plexus of veins under sodium pentobarbital anesthesia and was centrifuged (700×g, 4°C, 25 min) to separate serum. Serum was used to estimate liver enzymes activities(AST, ALT, and ALP), kidney function (urea, uric acid, creatinine, and protein), and total cholesterol and triglycerides levels by using colorimetric reagent kits[Salucea Co., Netherlands]. ELISA technique was performed for the assessment of Tumor Necrosis Factor-α (TNF-α) and Interleukin-6 (IL-6) (R&D Systems, USA.) and Monocyte Chemoattractant Protein-1(MCP-1) (Cusabio Biotech, Wuhan, China).

7. Liver and kidney tissue extracts:

After serum collection, rats were sacrificed under a deep sodium pentobarbital anesthesia. Liver and kidney were separated out, washed, weighed and homogenized in phosphate buffer saline[PBS] [10%]. One portion of the aliquot was centrifuged at 1500×g at 4C° for 20 minutes and the supernatant was collected and kept at -80C° for the direct determination of some parameters and the rest parts of the aliquot were needed to be subjected to two repeated freeze-thaw cycle to break the cell membranes, then centrifuged at 5000×g for 5 minutes and kept at -80C° for the assessment of the rest of parameters.

8. Assessment of liver and kidney parameters:

Colorimetric kits were obtained from Biodiagnostic, Egypt and used in the estimation of Malondialdehyde (MDA), Glutathione (GSH), Catalase (CAT), and Super Oxide Dismutase (SOD). Meanwhile, arsenic level in both liver and kidney was estimated by atomic absorption (Perkin-Elmer, UK).

9. Liver and kidney histopathological examination:

The liver and kidney of different groups were removed and fixed in 10% formol saline, 5um thick paraffin sections were stained with hematoxylin and eosin[17] and investigated by light microscopy.

10. Statistical Analysis:

The values were stated as mean ± S.E. of 8-10 rats and the variances between groups were tested for significance using analysis of variance (ANOVA), followed by Tukey-Kramer posthoc test estimated by SPSS software, version 21 (IBM).The level of statistical significance was at $P<0.05$.

Results:

1. Body and organ weights:

The arsenic control group displayed a significantly apparent decrease in final body weight, while liver weight was increased compared with the normal control group as shown in Table 1.*Spirulina platensis* treated group showed a significant enhancement in both final body and liver weights compared with the arsenic control group due to the absence of arsenic toxicity and didn't affect the final body and liver weights. Meanwhile, *Spirulina platensis* / arsenic-treated group didn't present any significance in the final body and liver weights related to the arsenic control group. Kidney weight didn't affect by arsenic toxicity in this experiment.

Table 1.Effect of *Spirulina platensis* either alone or concomitant with sodium arsenite after eight weeks of administration on the body, liver and kidney weights in sodium arsenite-induced toxicity in rats.

		Normal control	Arsenic control	Arsenic + *Sp. platensis*	*Sp. platensis*
Body weight	Initial	220.87±7.26	228.75±5.41	222.66±8.89	220.63±4.97
	Final	321.29±10.64	245.56±18.85*	270.86±12.50*	294.13±13.94@
	Weight gain	31.25%	6.84%	17.79%	24.98%
Liver weight		8.85±0.42	12.19±0.49*	10.69±0.47*@	8.60±0.32
Kidney weight		1.00 ±0.03	0.98±0.04	0.95±0.03	0.95±0.04

Values are means ± S.E of 8-10 animals. As compared with normal control (*), arsenic control (@)and the variances between groups were tested for significance using analysis of variance (ANOVA), followed by Tukey-Kramer posthoc test, P<0.05.

Table 2.Effect of *Spirulina platensis* either alone or concomitant with sodium arsenite after eight weeks of administration on the kidney functions in sodium arsenite-induced toxicity in rats.

	Normal control	Arsenic control	Arsenic + *Sp. platensis*	*Sp. platensis*
Urea(mg/dl)	37.54±1.06	50.11±2.85*	40.13±1.96@	39.44±3.00
Uric acid(mg/dl)	1.21±0.09	2.06±0.07*	1.18±0.12@	1.29±0.80
Creatinine(mg/dl)	0.48±0.01	0.67±0.03*	0.44±0.02@	0.43±0.01
Protein(g/dl)	6.95±0.21	5.81±0.16*	6.07±0.18*@	6.80±0.53

Values are means ± S.E of 8-10 animals. As compared with normal control (*), arsenic control (@) and the variances between groups were tested for significance using analysis of variance (ANOVA), followed by Tukey-Kramer posthoc test, P<0.05.

2. Kidney function parameters:

The arsenic control group showed a significantly obvious increase in serum urea, uric acid, and creatinine levels, while serum protein level was decreased in comparison with the normal control group as shown in Table 2. *Spirulina platensis* / arsenic-treated group revealed a significant improvement in these parameters level compared with the arsenic control group, but the previous parameters did not change significantly in rats treated with *Spirulina platensis* only.

Table 3.Effect of *Spirulina platensis* either alone or concomitant with sodium arsenite after eight weeks of administration on the liver functions in sodium arsenite-induced toxicity in rats.

	Normal control	Arsenic control	Arsenic + *Sp. platensis*	*Sp. platensis*
AST(U/l)	51.29±1.47	68.32±2.44*	49.57±1.38@	46.57±3.92
ALT(U/l)	32.65±0.87	80.55±3.00*	67.24±2.46*@	30.89±3.75
ALP(U/l)	98.34±3.66	240.71±9.68*	161.38±7.42*@	88.34±2.45
Cholesterol (mg/dl)	71.36±3.29	112.18±5.42*	84.64±4.32*@	78.54±2.01
Triglycerides (mg/dl)	56.94±1.86	86.37±3.71*	70.58±3.07*@	53.42±2.25

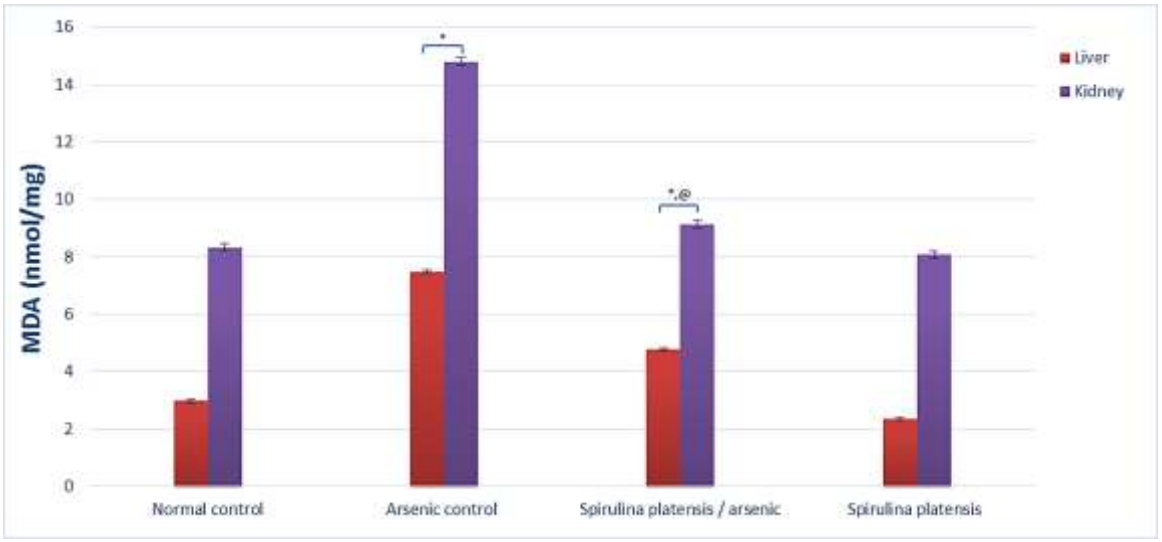
Values are means ± S.E of 8-10 animals. As compared with normal control (*), arsenic control (@) and the variances between groups were tested for significance using analysis of variance (ANOVA), followed by Tukey-Kramer posthoc test, P<0.05.

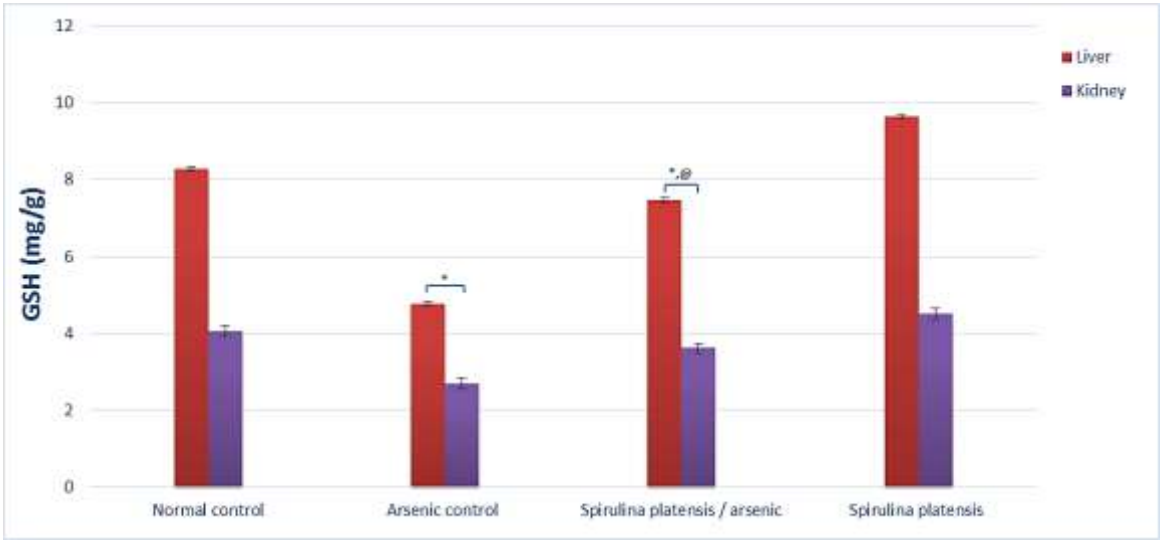
3. Liver function parameters:

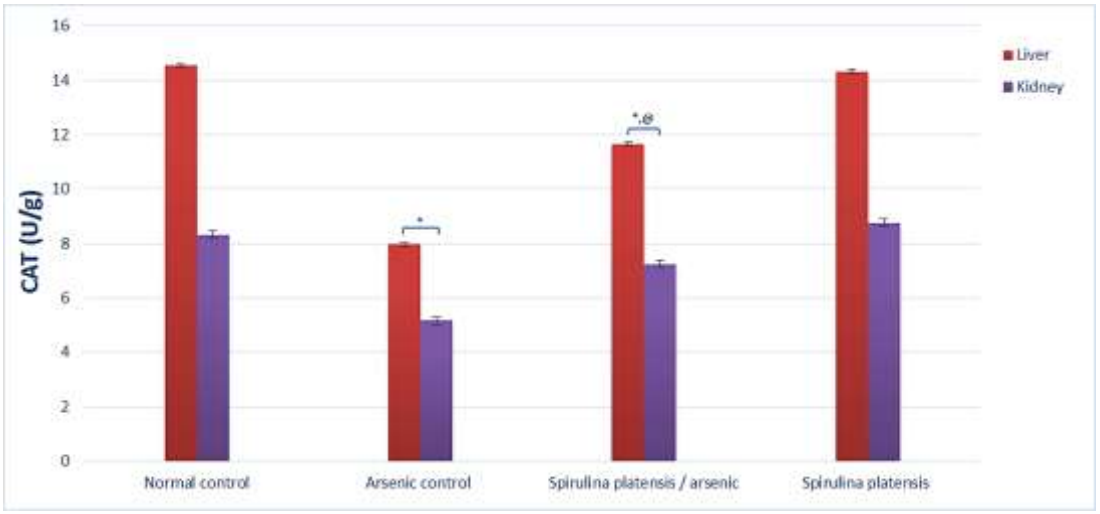
Serum AST, ALT, ALP, total cholesterol and triglycerides levels in the arsenic control group presented a significantly noticeable elevationin relation with the normal control group as shown in Table 3. *Spirulina platensis* / arsenic-treated group revealed a significant decline in these parameters level in comparison with the arsenic control group, but the aforementioned parameters levels did not alter significantly in rats treated with *Spirulina platensis* only.

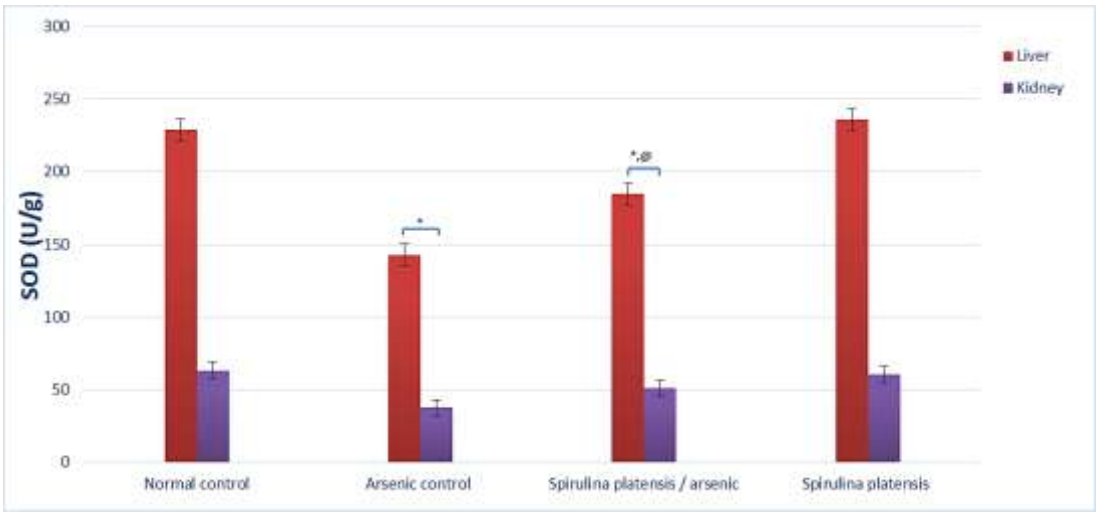
4. Liver and kidney oxidative stress parameters and arsenic concentration:

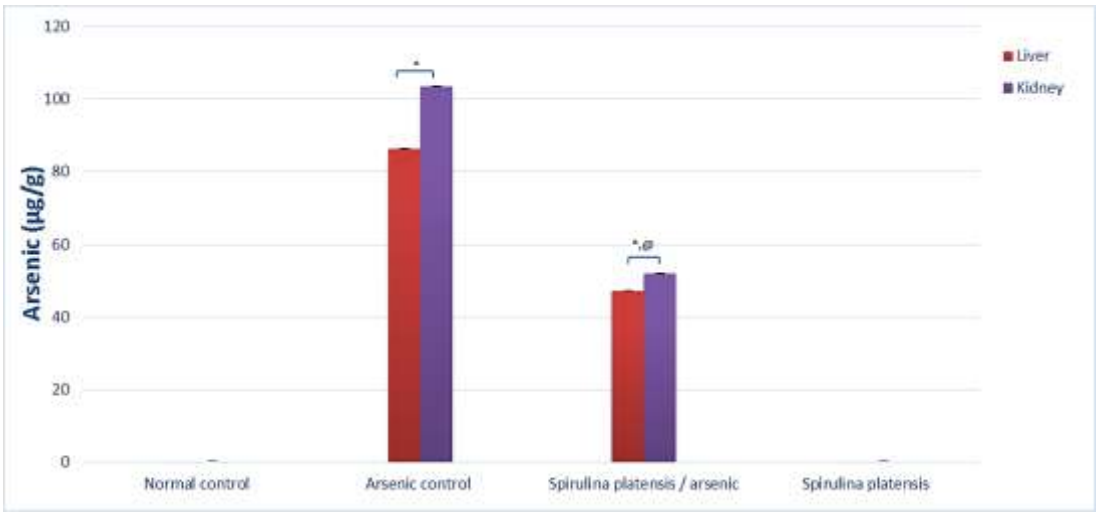
GSH level, CAT andSOD activities in the tissue of the arsenic control group exhibited a significantly clear decrease, while tissue MDA and arsenic levels were increased compared with the normal control group as presented in Figure 1. Either *Spirulina platensis* / arsenic-treated group or *Spirulina platensis* treated group revealed a significant advance in these parameters level in relation with the arsenic control group, but the effect of *Spirulina platensis* treated group was more noticeable and significant in all these parameters due to the absence of arsenic toxicity and didn't affect the oxidative balance.





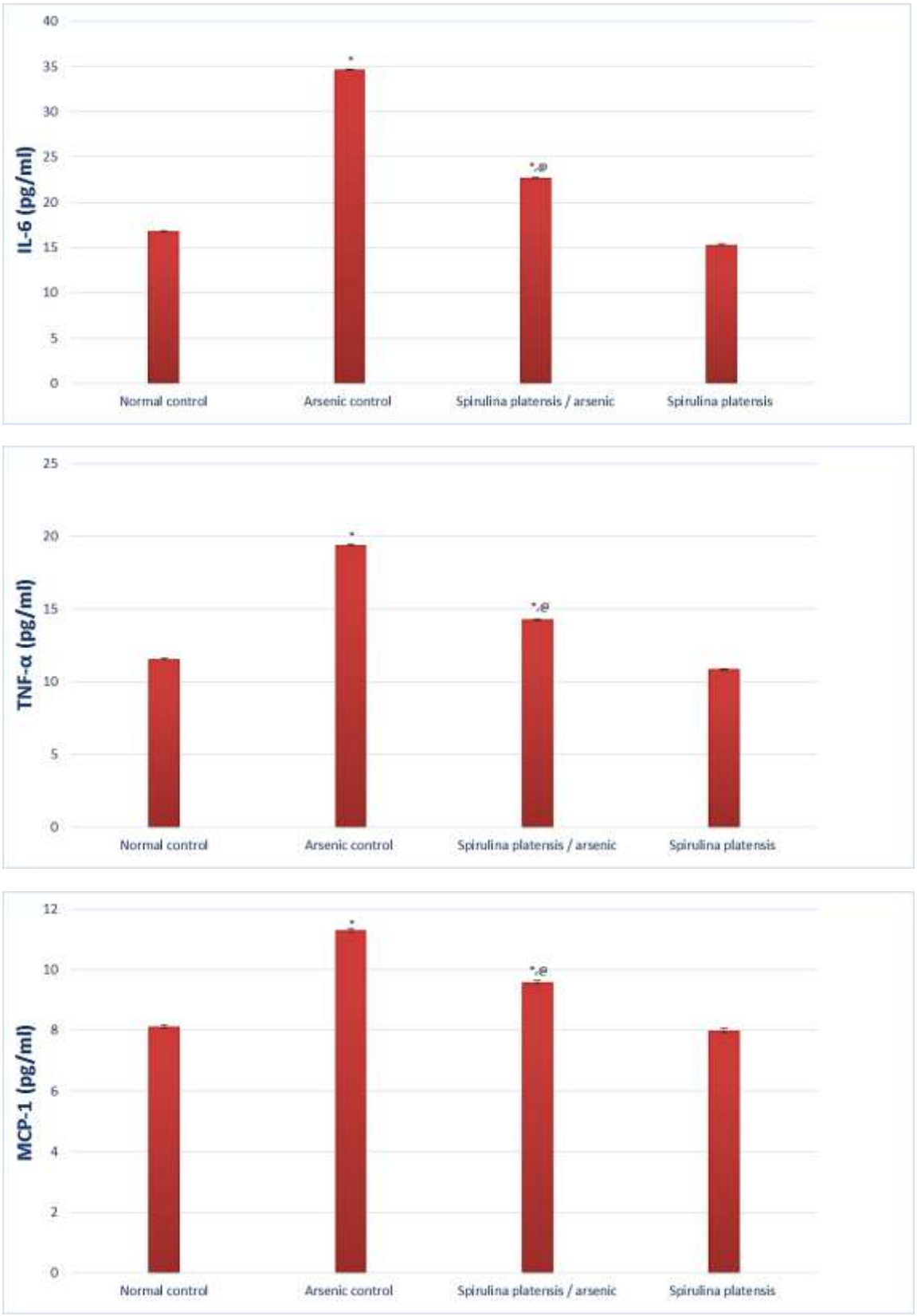






Values are means ± S.E of 8-10 animals. As compared with normal control (*), arsenic control (@) and the variances between groups were tested for significance using analysis of variance (ANOVA), followed by Tukey-Kramer posthoc test, $P<0.05$.

Figure 1.Effect of *Spirulina platensis* either alone or concomitant with sodium arsenite after eight weeks of administration on the Liver and kidney oxidative stress parameters (MDA, GSH, CAT, and SOD) and arsenic concentration in sodium arsenite-induced toxicity in rats.



Values are means ± S.E of 8-10 animals. As compared with normal control (*), arsenic control (@) and the variances between groups were tested for significance using analysis of variance (ANOVA), followed by Tukey-Kramer posthoc test, $P<0.05$.

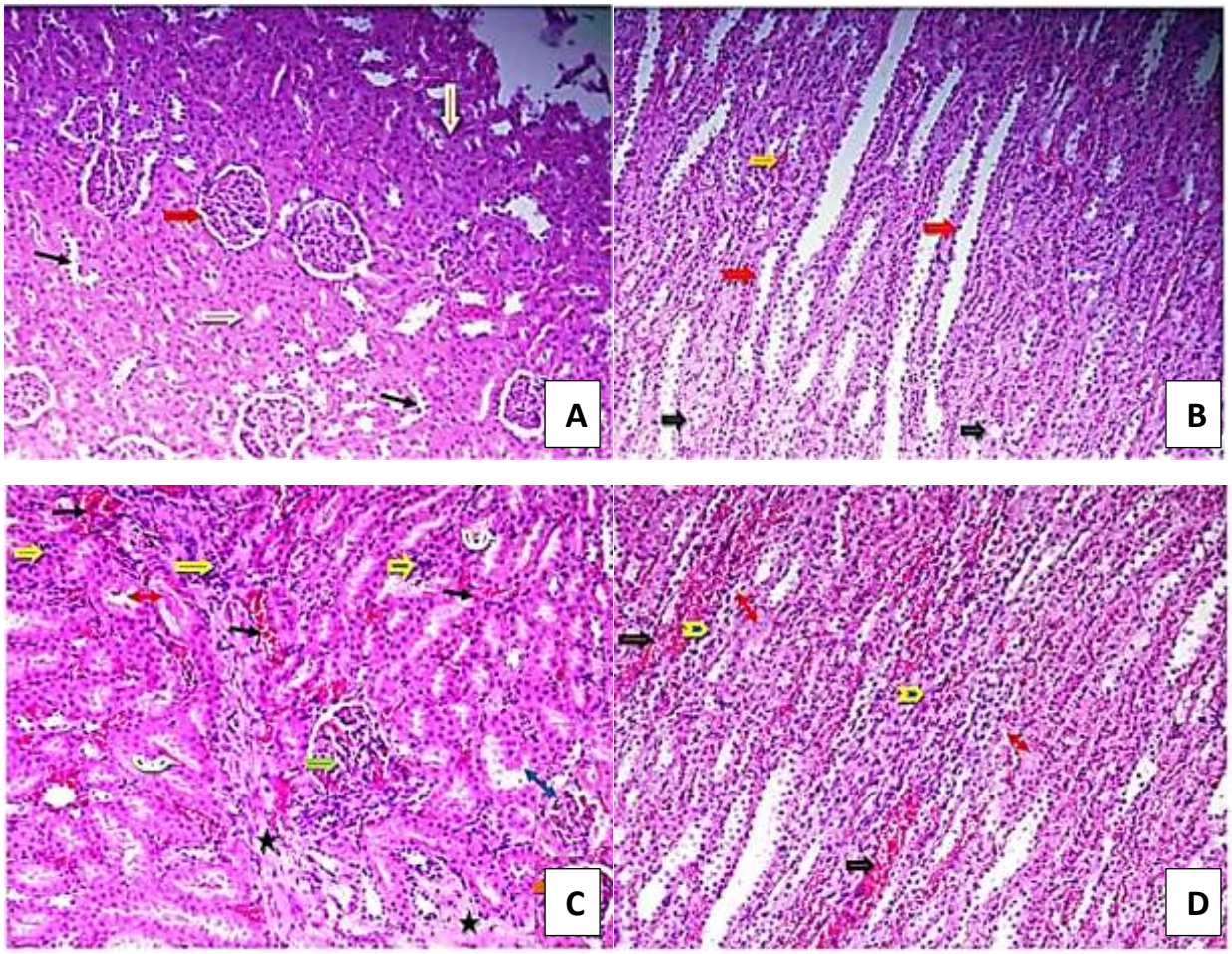
Figure 2.Effect of *Spirulina platensis* either alone or concomitant with sodium arsenite after eight weeks of administration on the serum inflammatory cytokines (IL-6 and TNF-α) and atherosclerotic chemokine's (MCP-1) in sodium arsenite-induced toxicity in rats.

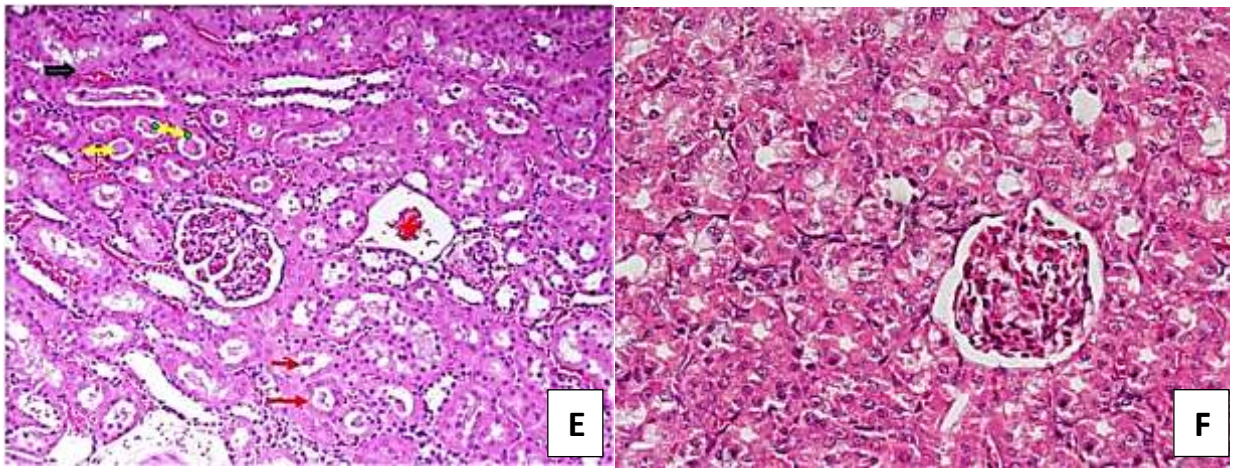
5. Inflammatory cytokines and atherosclerotic chemokines:

The arsenic control group showed a significantly obvious elevation in serum IL-6, TNF-α, and MCP-1 levels compared to the normal control group as shown in Figure 2. Both *Spirulina platensis* / arsenic-treated group and *Spirulina platensis* treated group exhibited a significant amelioration in these parameters level related to the arsenic control group, but the effect of *Spirulina platensis* treated group was more distinct and significant in the aforementioned parameters due to the relief of arsenic toxicity and neither affect the inflammatory nor atherosclerotic parameters.

6. Kidney histopathological examination:

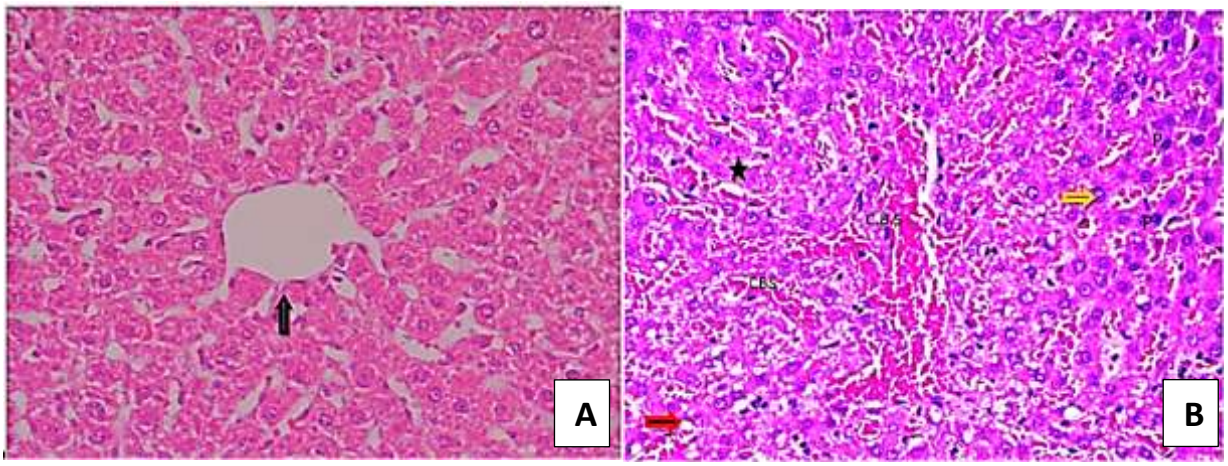
The normal control group showed a normal histological structure of the renal cortex and medulla of the kidney (Figs.3A, B).Histopathological changes of the kidney from rats exposed to arsenic only at dose level 6.3mg/Kg displayed a marked interstitial hemorrhage among renal tubules, edema, and thickening of lining epithelium of renal tubules with narrowing lumen. Hyaline casts and cells debris in some renal tubules with mononuclear cellular infiltration in interstitial tissue and perivascular area were observed in arsenic control group(Figs3C, D). Moreover, arsenic toxicity led to a degeneration in the proximal convoluted tubules, distal tubules, and hyalinization cytoplasm. In addition, some glomeruli appeared with interglomerular hemorrhage with mesangial hypercellularity and others appeared degenerated. Also, arsenic control group exhibited a renal medulla of thin tubules and collecting ducts displayed severe interstitial hemorrhages, inflammatory cells and narrowing the lumen of most tubules(Figs3C, D). Histological findings in the kidney of rats exposed to arsenic and subjected to *Spirulina platensis* revealed some improvement in pathological changes reflected on the absence of edema, inflammatory cells and degenerated glomeruli although the kidney still suffers from mild interstitial tissue hemorrhage and dilated of interstitial tissue (Figs3E). Meanwhile, Histological findings in the kidney of rats exposed to *Spirulina platensis*only showed the normal structure and renal cortex (Figs3F).

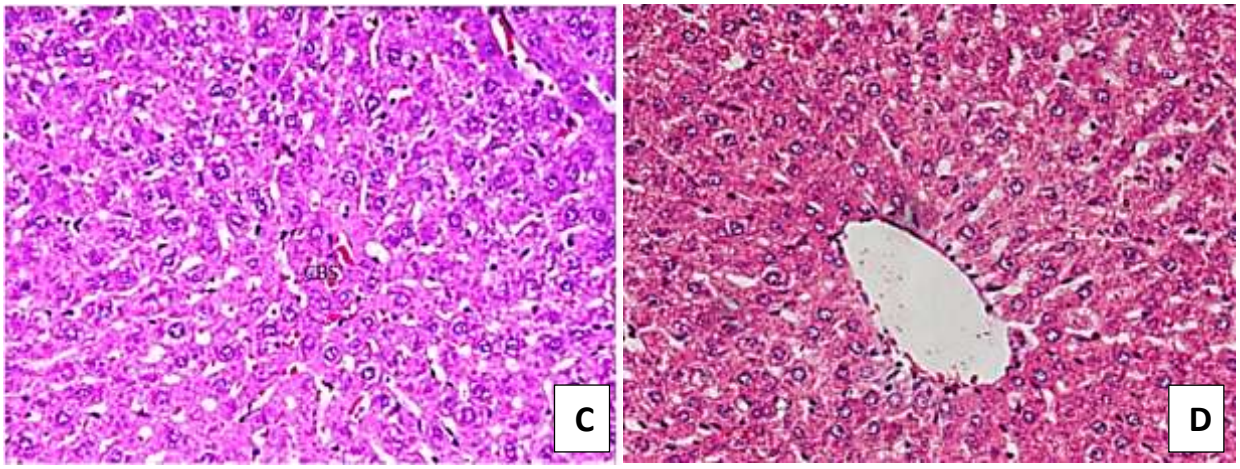




Photomicrographs of H&E stained sections of kidney from rats showing,(A): normal kidney section of renal of cortex of glomeruli(red arrow), proximal (white arrow) and distal (black arrow) tubules,(B): normal renal medulla showing thin tubules (black arrow), collecting ducts (red arrow) and inter-tubular blood capillaries(orange arrow),(C): kidney from rats exposed to arsenic revealed marked interstitial hemorrhage among renal tubules (black arrow)and edema(star).Hyaline casts in some renal tubules (red arrow). Some glomeruli appeared with interglomerular hemorrhage with mesangial hypercellularity (green arrow) and others appeared degenerated (orange arrow)or lobulation(blue arrow),(D): kidney from rats of renal medulla exposed to arsenic showed severe interstitial hemorrhages(black arrow),inflammatory cells (yellow arrow)and edema(red arrow),(E): of kidney from rats exposed to arsenic and subjected to *Spirulina platensis* revealed some improvement in pathological changes in renal cortex in the form of no edema no inflammatory cells no degenerated glomeruli. Kidney tissue showed mild interstitial tissue hemorrhage (black arrow) and dilated of interstitium(star). The hyaline casts (yellow arrow) and cell debris in the lumen of some tubules (red arrow),(F): kidney of rats exposed to *Spirulina platensis* only showed the normal structure and renal cortex.(Hx&Ex200)

Figure 3.Effect of *Spirulina platensis* either alone or concomitant with sodium arsenite after eight weeks of administration on kidney histopathological examination in sodium arsenite-induced toxicity in rats.





Photomicrographs of H&E stained sections of liver from rats showing,(A):normal histological structure of hepatic lobules and central vein(arrow),(B): liver from rats exposed to arsenic displaying the hepatic cords separated by dilated and congested blood sinusoids. Signs of degeneration in the form pyknosis (P),foci of necrosis(star),minute vacuolar degeneration(red arrow) and swelling of some hepatocytes(orange arrow), (C): liver from rats exposed to arsenic and subjected to *Spirulina platensis* revealed some improvement in pathological changes in the form of no fibrosis, no inflammatory infiltrate, no signs of degeneration. Most of the hepatocytes appeared normal but minimal congestion of blood sinusoids still present (CBS), (D): liver of rats exposed to *Spirulina platensis* only showed normal characteristic architecture.(Hx&Ex200)

Figure 4.Effect of *Spirulina platensis* either alone or concomitant with sodium arsenite after eight weeks of administration on liver histopathological examination in sodium arsenite-induced toxicity in rats.

7. Liver histopathological examination:

The liver of normal control group rats revealed a normal characteristic architecture (Figure 4A).Histopathological examination of H & E sections of theliver of rats intoxicated with arsenic only revealed hepatic cords separated by dilated and congested sinusoids. In addition to,signs of degeneration in the form pyknosis, foci of necrosis, minute vacuolar degeneration, and swelling of some hepatocytes were seen (Figs.4B).Microscopic examination of liver tissue of rats subjected to arsenic along with *Spirulina platensis*, showed some improvement through the absence of fibrosis, inflammatory infiltrate, and signs of degeneration and most of thehepatocytes appeared normal but minimal congestion of blood sinusoids still present (Figure 4C). Meanwhile, Histological findings in liver of rats exposed to *Spirulina platensis* only showed normal characteristic architecture(Figure 4D).

Discussion:

A high percentage of farmers in many countries are subjected to high arsenic levels in their drinking water or in the manufacturing of cigarettes, herbicides, and woods for a long time(7). Exposure to arsenic has many harmful impacts, by increasing the risk factor for the pathogenesis of cardiovascular disease, peripheral vascular complications, hypertension, and cancers[18].

Oxidative stress is a pathological state that arises when free radicals chemically interact with and damage biological molecules. Many studies show that oxidative stress plays an important role in several clinical conditions such as atherosclerosis[19], liver and kidney injuries[20].It was reported that oxidative stress was elevated in arsenic-exposed mice[21].

In this study, the serum activities of liver functions(AST, ALT and ALP), total cholesterol and triglycerides levels were high in arsenic control group as they are sensitive indices of hepatic injury which attributed to cellular leakage and loss of the functional integrity of liver membrane architecture[22] due to arsenic intoxication. Also, as a confirmatory liver histopathological examination, we found dilated and congested hepatic sinusoids and many signs of degeneration including pyknosis, foci of necrosis and vacuolar degeneration[23].

By the same token, the serum parameters of kidney functions (creatinine, urea, and uric acid) were elevated in thearsenic control group and at the same time, serum protein level was declined due to its loss in urine. All of these parameters considered as a highly indicators for kidney damage[24,25]. Earlier studies showed that arsenic is a heavy metal ion and increases the production of Reactive Oxygen Species(ROS) concomitant with increases the production ofpro-inflammatory cytokines and increased activities of the oxidant enzymes such as heme oxygenase-1 (HO-1), cyclooxygenase-2 (COX-2) and Nicotinamide Adenine Dinucleotide Phosphate (NADPH) [26,27] Paralleled with these findings, kidney histopathological examination revealed a marked interstitial hemorrhage among renal tubules with thickening of lining epithelium of renal tubules and degeneration in proximal convoluted tubules and distal tubules[28].

On the same scenario, arsenic control group exhibited a significant decrease in GSH, SOD, and CAT tissue levels with a subsequent increase in MDA tissue level. The probable toxicity of arsenic can therefore be related to its inhibitory action on the antioxidant defense system (GSH, SOD and CAT) [29,30] through ROS produced by arsenic leading to destabilization of cell membranes by lipid peroxidation and MDA utilization, which is a basic cellular deteriorating process. Thus, the reduction of GSH level, SOD, and CAT activities accelerates arsenic predisposition in liver and kidney and causing oxidative stress[31] besides the variations in final body and liver weights.

Co-administration of *spirulina* along with arsenic increased the activities of hepatic and renal SOD and CAT and GSH content when compared to rats treated with arsenic alone. Moreover, it inhibits hepatic and renal MDA production induced by arsenic. This could be attributed to the antioxidant properties of *spirulina* that have attracted the attention of many researchers due to its active ingredients, notably phycocyanin, β-carotene, tocopherol, selenium, and phenolic compounds that haveoperative antioxidant and anti-inflammatory activities. The antioxidant property of phycocyanin,a major water-soluble antioxidant constituent in *Spirulina*, is more efficient than vitamin C by 20 times[32].The active principals of *spirulina* can act synergistically leading to intensive antioxidant effect.

Pro-inflammatory cytokines (IL-6 and TNF-α) in this study plays an important role in the pathogenesis of arsenic toxicity, showing high serum levels in the arsenic control group. Arsenic is a well-known inflammatory agent leading to up-regulation of Nuclear Factor Kappa-light-chain-enhancer of activated B cells (NF-kB) transcription factor which regulates the expression of different inflammatory response genes[33]. Moreover, most target genes of the NF-kB transcription factor are basically pro-inflammatory including IL-6 and TNF-α[34]. So that, these findings are too related to the state of oxidative stress in liver and kidney besides ROS generation as a result of the reduction of the antioxidant defense system and increased the level of lipid peroxidation.

MCP-1 is a potent chemotactic factor for monocytes, and is believed to play a pivotal role in the trafficking of macrophages and were found in endothelial cells, foam cells and vascular smooth muscle cells (VSMCs) of atherosclerotic lesions. IL-6 can also induce MCP-1 expression[35] and induce lesion inflammation. In this experiment, administration of arsenic (arsenic control group)induces both vascular inflammation and circulating concentrations of serum MCP-1 and IL-6. Many studies have approved that MCP-1 is induced in VSMCs by many triggers which uric acid is one of them[36].

Hypercholesterolemia, characterized by altered plasma lipids and increased oxidative stress and inflammation, requires both cholesterol-lowering and antioxidant therapies, which were helpful to control atherosclerosis. C-phycocyanin (C-PC) seems to play master roles in the hypolipidemic effect of spirulina since it has been found to inhibit jejunal cholesterol absorption[37] and lipase activity[38].The hypolipidemic effect of *Spirulina* in our investigation could be referred to its essential polyunsaturated fatty acids as omega-6, omega-3 and linolenic acid and vitamin B3[39]. Moreover, dietary supplementation with C-PC or *Spirulina platensis* significantly inhibits pro-inflammatory cytokine formation, such as TNF-α, IL-1b and suppresses cyclooxygenase-2 (COX-2) expression induced by the salicylate[40]. Based on our results, it is suggested that the inhibition of TNF-alpha,IL-6, MCP-1 and lipids over-production (induced by arsenic) by *spirulina* may contribute, at least in part, to its anti-atherosclerotic activity.

The protective effect of *spirulina* against arsenic toxicity in the present study can be referred to the enhancement of antioxidant enzymes and glutathione or to inhibiting lipid peroxidation that is responsible for initiating and developing liver cirrhosis and nephrotoxicity(20). The aqueous extract of *Spirulina* could reduce

significantly apoptotic cell death induced by the free radicals. The antioxidant activity based on the DPPH assay, showed that a mixture of the extract was more active than a single pure compound, phycocyanin[41].The protective effect of *spirulina* against galactosamine motivated hepatotoxicity was reported[42]. In addition, *Spirulina* has been shown to have protective effects against oxidative stress induced by lead acetate in the liver and kidney of rats[42].

Spirulina platensis either administered alone (to determine any side effects) or in combination with sodium arsenite intoxicated rats, showed a hopeful significant improvement in liver and kidney functions through its modulation to the oxidative stress parameters and the inflammatory cytokines. This improvement in the aforementioned parameters specially IL-6 and uric acid levels reflected on the significant decrease in MCP-1 level which is the target of our experimental study in ameliorating atherosclerotic indices. In the future, Spirulina may be used as a natural adjuvant cheap and safe therapy for atherosclerosis rather than highly expensive standard drug therapy with many side effects[43].

Conclusion:

The present study provides decisive evidence for the protective effects of *spirulina platensis* against arsenic provoked oxidative stress and hepatorenal pathological changes. However, the exact and detailed mechanism of *Spirulina platensis* (enriched with phycocyanin)is not completely elucidated in earlier studies, so, we hope that we give a new insight on its effect through different biomarkers to help to know some of its mechanistic role in arsenic toxicity and as a natural competitor for the standard anti-atherosclerotic drugs.

Conflict of interest:

The authors have declared that no competing interests exist.

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Author Contributions:

All authors of the current manuscript contributed equally to accomplish different parts of this work.

References:

1. Hoseini, S. M., Khosravi-Darani, K., & Mozafari, M. R. (2013). Nutritional and medical applications of spirulina microalgae. *Mini-Reviews in Medicinal Chemistry*, 13(8), 1231–1237. doi:10.2174/1389557511313080009.
2. Abdel-Daim, M. M., Abuzead, S. M., & Halawa, S. M. (2013). Protective role of Spirulinaplatensis against acute deltamethrin-induced toxicity in rats. *PLoS One*, 8(9), e72991. doi:10.1371/journal.pone.0072991.
3. Ma, Q. Y., Fang, M., Zheng, J. H., Ren, D. F., & Lu, J. (2016). Optimised extraction of beta-carotene from Spirulinaplatensis and hypoglycaemic effect in streptozotocin-induced diabetic mice. *Journal of the Science of Food and Agriculture*, 96(5), 1783–1789. doi:10.1002/jsfa.7286.
4. Ali, E. A., Barakat, B. M., & Hassan, R. (2015). Antioxidant and angiostatic effect of Spirulinaplatensis suspension in complete Freund's adjuvant-induced arthritis in rats. *PLoS One*, 10(4), e0121523. doi:10.1371/journal.pone.0121523.
5. Kurd, F., & Samavati, V. (2015). Water soluble polysaccharides from Spirulinaplatensis: extraction and in vitro anti-cancer activity. *International Journal of Biological Macromolecules*, 74, 498–506. doi:10.1016/j.ijbiomac.2015.01.005.
6. El-Tantawy, W. H., & the El-TantawyWH. (2016). Antioxidant effects of Spirulina supplement against lead acetate-induced hepatic injury in rats. *Journal of Traditional and Complementary Medicine*, 6(4), 327–331. doi:10.1016/j.jtcme.2015.02.001.
7. Zheng, L., Kuo, C. C., Fadrowski, J., Agnew, J., Weaver, V. M., & Navas-Acien, A. (2014). Arsenic and Chronic Kidney Disease: A Systematic Review. *Current Environmental Health Reports*, 1(3), 192–207. doi:10.1007/s40572-014-0024-x.

8. Li S, Ding Y, Niu Q, Xu S, Pang L, Ma R, (2015). Lutein Has a Protective Effect on Hepatotoxicity Induced by Arsenic via Nrf2 Signaling, *BioMed Research International*, 2015, 10.
9. Krohn, R. M., Lemaire, M., Negro Silva, L. F., Lemarie, C., Bolt, A., Mann, K. K., & Smits, J. E. (2016). High-selenium lentil diet protects against arsenic-induced atherosclerosis in a mouse model. *The Journal of Nutritional Biochemistry*, 27, 9–15. doi:10.1016/j.jnutbio.2015.07.003.
10. Dutta K, Prasad P, Sinha D., (2015). Chronic low level arsenic exposure evokes inflammatory responses and DNA damage, *International Journal of Hygine and Environmental Health*, 218, 564-574.
11. Yigit F., Gurel-Gurevin E., Isbilen-Basok B., Esener O.B., Bilal T., Keser O., (2016). Protective effect of Spirulinaplatensis against cell damage and apoptosis in hepatic tissue caused by high fat diet, *Biotechic and Histochemistry*, 91, 182-194.
12. Mershiba, S. D., Dassprakash, M. V., & Saraswathy, S. D. (2013). Protective effect of naringenin on hepatic and renal dysfunction and oxidative stress in arsenic intoxicated rats. *Molecular Biology Reports*, 40(5), 3681–3691. doi:10.1007/s11033-012-2444-8.
13. Escudero-Lourdes, C. (2016). Toxicity mechanisms of arsenic that are shared with neurodegenerative diseases and cognitive impairment: Role of oxidative stress and inflammatory responses. *Neurotoxicology*, 53, 223–235. doi:10.1016/j.neuro.2016.02.002.
14. Bashandy, S. A., El Awdan, S. A., Ebaid, H., & Alhazza, I. M. Antioxidant Potential of Spirulinaplatensis Mitigates Oxidative Stress and Reprotoxicity Induced by Sodium Arsenite in Male Rats, Oxidative Medicine and Cell Longevify, 2016: 7174351.
15. Lewis, R., & Saxs, J. Dangerous Properties of Industrial Materials, V. N. Reinhold, New York, NY, USA, 9th edition (1996).
16. Simsek, N., Karadeniz, A., Kalkan, Y., Keles, O. N., & Unal, B. (2009). Spirulinaplatensis feeding inhibited the anemia- and leucopenia-induced lead and cadmium in rats. *Journal of Hazardous Materials*, 164(2-3), 1304–1309. doi:10.1016/j.jhazmat.2008.09.041.
17. Drury, R. A. B., & Wallington, E. A. Preparation and fixation of tissues. In: Drury RAB, Wallington EA, editors. Carleton's Histological Technique. 5. Oxford: Oxford University Press; 1980. pp. 41–54.
18. Moon, K., Guallar, E., & Navas-Acien, A. (2012). Arsenic exposure and cardiovascular disease: an updated systematic review. *Current Atherosclerosis Reports*, 14(6), 542–555. doi:10.1007/s11883-012-0280-x.
19. Perrotta I, Aquila S., (2015).The role of oxidative stress and autophagy in atherosclerosis, *Oxidative Medicine and Cell Longevify*, 2015, 130315.
20. Palipoch, S., & Punsawad, C. (2013). Biochemical and histological study of rat liver and kidney injury induced by Cisplatin. *Journal of Toxicologic Pathology*, 26(3), 293–299. doi:10.1293/tox.26.293.
21. Srivastava, S., Vladykovskaya, E. N., Haberzettl, P., Sithu, S. D., D'Souza, S. E., & States, J. C. (2009). Arsenic exacerbates atherosclerotic lesion formation and inflammation in ApoE-/- mice. *Toxicology and Applied Pharmacology*, 241(1), 90–100. doi:10.1016/j.taap.2009.08.004.
22. Kotyzova, D., Bludovska, M., & Eybl, V. (2013). Differential influences of various arsenic compounds on antioxidant defense system in liver and kidney of rats. *Environmental Toxicology and Pharmacology*, 36(3), 1015–1021. doi:10.1016/j.etap.2013.09.003.
23. Jalaludeen, A. M., Ha, W. T., Lee, R., Kim, J. H., Do, J. T., Park, C., & Song, H. (2016). Ameliorates Arsenic-Induced Hepato- and Hematotoxicity in Rats. *Molecules (Basel, Switzerland)*, 21(1), 69. doi:10.3390/molecules21010069.
24. Rizwan, S., Naqshbandi, A., Farooqui, Z., Khan, A. A., & Khan, F. (2014). Protective effect of dietary flaxseed oil on arsenic-induced nephrotoxicity and oxidative damage in rat kidney. *Food and Chemical Toxicology*, 68, 99–107. doi:10.1016/j.fct.2014.03.011.
25. Zhang, W., Liu, Y., Ge, M., Jing, J., Chen, Y., Jiang, H., . . ., & Zhang, Z. (2014). Protective effect of resveratrol on arsenic trioxide-induced nephrotoxicity in rats. *Nutrition Research and Practice*, 8(2), 220–226. doi:10.4162/nrp.2014.8.2.220.
26. Manimaran, A., Sarkar, S. N., & Sankar, P. (2011). Repeatedpreexposure or coexposure to arsenic differentially alters acetaminophen-induced oxidative stress in rat kidney. *Environmental Toxicology*, 26(3), 250–259. doi:10.1002/tox.20551.
27. Messarah, M., Saoudi, M., Boumendjel, A., Kadeche, L., Boulakoud, M. S., & Feki, A. E. (2013). Green tea extract alleviates arsenic-induced biochemical toxicity and lipid peroxidation in rats. *Toxicology and Industrial Health*, 29(4), 349–359. doi:10.1177/0748233711433934.

28. Sener, U., Uygur, R., Aktas, C., Uygur, E., Erboga, M., Balkas, G., . . ., & Erdogan, H. (2016). Protective effects of thymoquinone against apoptosis and oxidative stress by arsenic in rat kidney. *Renal Failure*, 38(1), 117–123. doi:10.3109/0886022X.2015.1103601.
29. Rana, T., Bera, A. K., Das, S., Bhattacharya, D., Pan, D., Bandyopadhyay, S., . . ., & Das, S. K. (2012). Pleurotusfloridalectin normalizes duration dependent hepatic oxidative stress responses caused by arsenic in rat. *Experimental and Toxicologic Pathology*, 64(7-8), 665–671. doi:10.1016/j.etp.2010.12.010.
30. Hall, M. N., Niedzwiecki, M., Liu, X., Harper, K. N., Alam, S., & Slavkovich, V. (2013). Chronic arsenic exposure and blood glutathione and glutathione disulfide concentrations in Bangladeshi adults. *Environmental Health Perspectives*, 121, 1068–1074.
31. Tayman C, Cekmez F, Kafa IM, Canpolat FE, Cetinkaya M, Tonbul A, (2013). Protective Effects of Nigella sativa Oil in Hyperoxia-Induced Lung Injury, *Archivos deBronconeumologia*, 49, 15-21.
32. Ismail, M., Hossain, M. F., Tanu, A. R., & Shekhar, H. U. (2015). Effect of spirulina intervention on oxidative stress, antioxidant status, and lipid profile in chronic obstructive pulmonary disease patients. *BioMed Research International*, 2015, 1. doi:10.1155/2015/486120.
33. Jin, Y., Wan, Y., Chen, G., Chen, L., Zhang, M. Q., Deng, L., & Xin, J.-B. (2014). Treg/IL-17 ratio and Treg differentiation in patients with COPD. *PLoS One*, 9(10), e111044. doi:10.1371/journal.pone.0111044.
34. Ive, E. C., Couchman, I. M., & Reddy, L. (2012). Therapeutic effect of Arsenicum album on leukocytes. *International Journal of Molecular Sciences*, 13(3), 3979–3987. doi:10.3390/ijms13033979.
35. Nishikawa, Y., Kajiura, Y., Lew, J. H., Kido, J. I., Nagata, T., & Naruishi, K. (2017). Calprotectin Induces IL-6 and MCP-1 Production via Toll-Like Receptor 4 Signaling in Human Gingival Fibroblasts. *Journal of Cellular Physiology*, 232(7), 1862–1871. doi:10.1002/jcp.25724.
36. Kanellis, J., Watanabe, S., Li, J. H., Kang, D. H., Li, P., Nakagawa, T., & Johnson, R. J. (2003). Uric acid stimulates monocyte chemoattractant protein-1 production in vascular smooth muscle cells via mitogen-activated protein kinase and cyclooxygenase-2. *Hypertension*, 41(6), 1287–1293. doi:10.1161/01.HYP.0000072820.07472.3B.
37. Abdelkhalek, N. K. M., Eissa, I. A. M., Ahmed, E., Kilany, O. E., El-Adl, M., Dawood, M. A. O., & Abdel-Daim, M. M. (2017). Protective role of dietary Spirulinaplatensis against diazinon-induced Oxidative damage in Nile tilapia; Oreochromisniloticus. *Environmental Toxicology and Pharmacology*, 54, 99–104. doi:10.1016/j.etap.2017.07.002.
38. Nawrocka, D., Kornicka, K., Smieszek, A., & Marycz, K. (2017). Spirulinaplatensis Improves Mitochondrial Function Impaired by Elevated Oxidative Stress in Adipose-Derived Mesenchymal Stromal Cells (ASCs) and Intestinal Epithelial Cells (IECs), and Enhances Insulin Sensitivity in Equine Metabolic Syndrome (EMS) Horses. *Marine Drugs*, •••, 15.
39. Mazokopakis, E. E., Starakis, I. K., Papadomanolaki, M. G., Mavroeidi, N. G., & Ganotakis, E. S. (2014). The hypolipidaemic effects of Spirulina (Arthrospiraplatensis) supplementation in a Cretan population: a prospective study. *Journal of the Science of Food and Agriculture*, 94(3), 432–437. doi:10.1002/jsfa.6261.
40. Hwang, J. H., Chen, J. C., & Chan, Y. C. (2013). Effects of C-phycocyanin and Spirulina on salicylate-induced tinnitus, expression of NMDA receptor and inflammatory genes. *PLoS One*, 8(3), e58215. doi:10.1371/journal.pone.0058215.
41. Lima, F. A. V., Joventino, I. P., Joventino, F. P., de Almeida, A. C., Neves, K. R. T., do Carmo, M. R., . . ., & de Barros Viana, G. S. (2017). Neuroprotective Activities of Spirulinaplatensis in the 6-OHDA Model of Parkinson's Disease Are Related to Its Anti-Inflammatory Effects. *Neurochemical Research*, 42(12), 3390–3400. doi:10.1007/s11064-017-2379-5.
42. Ponce-Canchihuaman, J. C., Perez-Mendez, O., Hernandez-Munoz, R., Torres-Duran, P. V., & Juarez-Oropeza, M. A. (2010). Protective effects of Spirulina maxima on hyperlipidemia and oxidative-stress induced by lead acetate in the liver and kidney. *Lipids in Health and Disease*, 9(1), 35. doi:10.1186/1476-511X-9-35.
43. Jensen, G. S., Drapeau, C., Lenninger, M., & Benson, K. F., Clinical Safety of a High Dose of Phycocyanin-Enriched Aqueous Extract from Arthrospira. (2016). (Spirulina) platensis: Results from a Randomized, Double-Blind, Placebo-Controlled Study with a Focus on Anticoagulant Activity and Platelet Activation. *Journal of Medicinal Food*, 19(7), 645–653. doi:10.1089/jmf.2015.0143.

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