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The Protective Effects of Blue-Green Algae (*Spirulina*) Against Arsenic-Induced Differences in Lipid Panel and Hematological Parameters in Female Rats (*Rattus norvegicus*)

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Arsenic (As), a notorious human poison, originates from natural sources. The rising presence of arsenic pollution in groundwater, used for irrigation, human consumption, and industry, has become a major public health concern. This study aimed to assess Spirulina's impact on hematological and lipid parameters in rats exposed to arsenate. Forty-eight female rats were categorized into six groups: a control group with a standard diet, a group receiving 5mg/kg sodium arsenate (As), two groups taking 300mg/kg and 600mg/kg Spirulina (Sp), and two groups combining As and Sp at the same doses. After 28 days, blood samples were collected after an overnight fast and anesthesia for hematological and lipid profile tests. As exposure led to a significant reduction in hemoglobin (Hb) and packed cell volume (PCV) and an increase in white blood cell count (WBCs). However, red blood cell (RBC) parameters, including mean corpuscular hemoglobin (MCH), mean corpuscular volume (MCV) and mean corpuscular hemoglobin concentration (MCHC), showed minor non-significant changes. Conversely, treatment with 300mg/kg or 600mg/kg Spirulina resulted in slight increases in RBC parameters, with a notable PCV increase in the 600mg/kg group. Furthermore, As exposure elevated cholesterol, triglyceride, and very low-density lipoprotein (VLDL) levels, while reducing high-density lipoprotein (HDL). However, Sp supplementation at 300mg/kg and 600mg/kg levels restored these lipid parameters to levels similar to the control group, suggesting Spirulina's potential to alleviate arsenic-induced adverse effects on hematological and lipid parameters in rats.

Keywords: Albino rats, Arsenate, Hematological parameters, lipid panel, Spirulina.

Introduction

Arsenic (As) is a natural metalloid found in various compounds and naturally occurring states across the Earth's crust. Numerous studies in epidemiology have demonstrated that As poses a significant threat to both the environment and the overall population, even when present at minimal levels within human bodies [1]. It is estimated that more than 100 million people in South and Southeast Asia are believed to encounter dangerous levels of As in their groundwater [2]. Arsenic primarily infiltrates the ecological food chain by predominantly originating from contaminated groundwater. There are also fewer inputs from sources like agricultural pesticides and fertilizers, supplements added to chicken feed, and releases from smelting and mining operations [3].

Blue-green algae are rich in crucial amino acids, minerals, fibers, carotenoids, and various other bioactive constituents [4,5]. *Arthrospira*, formerly identified as *Spirulina platensis*, is a type of filamentous cyanobacterium that has a significant historical tradition of being harnessed for its nutritional value. It contains a diverse array of vital constituents, encompassing proteins, fats, and carbohydrates, as well as essential nutrients like zinc, magnesium, manganese, selenium, β-carotene, riboflavin, α-tocopherol, and linoleic acid [6]. Spirulina (Sp) thrives in elevated temperatures and alkaline environments. In 1967, the International Association of Applied Microbiology (IAAM) warmly embraced Sp as a promising answer for ensuring high-quality food production in the future [7]. Spirulina can neutralize hydroxyl radicals due to its antioxidant properties [8]. Spirulina has

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demonstrated diverse biological effects, including antihypertensive and antihyperlipidemic [9,10], and chemoprevention of tumors [11], Additionally, it offers protection to the liver from the harmful effects of cadmium toxicity [12]. These actions were linked principally to phycocyanin, a physiologically active protein present in Sp [13]. This study examines how Sp may offer protection against hemato-biochemical changes induced by As- Exposure in a preclinical rat model.

Materials and Methods

Ethical approval

This research received ethical clearance from the Animal Care and Use Committee at Zakho University, with the approval of AEC-020

Chemicals

All materials used in the study were of the utmost purity. Sodium Arsenate (Na_2HAsO_4) in powdered form, was obtained from Sigma in the United States, while the spirulina platensis powder, sourced from the Netherlands-based company Natura Vitalis,

Sample Selection and Feeding

Forty-eight female Wister rats weighing between 205.2±8.22grams (gm) were housed in polypropylene cages under a 12-hour dark/12-hour light cycle and housed in the animal house at the Biology Department of the University of Zakho. the rats were provided with unrestricted access to both food and water [14]. These rats were then allocated randomly into six groups, which included one control group and five experimental groups. The control group was fed a standard basal diet, while the experimental groups received different diets in addition to the basal diet as follows: Group 2 received oral gavage of sodium arsenate at a concentration of 5mg/kg of body weight (BW). Group 3 received oral gavage of Sp at a concentration of 300 mg/kg BW. Group 4 received oral gavage of Sp at a concentration of 600 mg/kg BW. Group 5 received a gavage containing a combination of 5 mg/kg of sodium arsenate and 300 mg/kg Sp, whereas Group 6 received a gavage containing a combination of 5 mg/kg sodium arsenate and 600 mg/kg of Sp.

Blood Collection

After subjecting the animals to 28 days of arsenic exposure, they underwent an overnight fasting period followed by diethyl ether anesthesia. Subsequently, each animal had 5 ml of blood samples collected via cardiac puncture. Out of this total volume, 2 ml were transferred to sample containers with Ethylene diamine tetra acetic acid (EDTA) for the evaluation of hematological parameters. Meanwhile, the remaining 3 ml of blood were placed in plasma gel tubes and then underwent centrifugation at 4000 rpm for 5 minutes. This centrifugation step efficiently separated and collected plasma, which was later analyzed to determine the lipid profile of the samples.

Determination of Hematological Parameters

The hematological analysis in this study followed the methods described by Onuh and Igwemma. [15]. WBC was assessed using a 1:20 dilution with Turk's fluid and counted in an improved Neubauer counting chamber under an Olympus microscope, while RBC was determined by diluting blood with Hayem's fluid (1:200) and counted in the same chamber with a x40 objective. PCV was measured using the macrohematocrit method, and Hb content was estimated employing Sahli's haemoglobinometer. Furthermore, specific formulas outlined by various authors [16, 17, 18, 19, 20] were employed to calculate parameters such as RBC, WBCs, PCV, and Hb, as well as several RBC indices including MCH, MCHC, and MCV.

Determination of Lipid Panel

Lipid panel parameters including cholesterol, triglyceride, HDL, LDL, and VLDL were done using FUJIFILM (DRI_CHEM NX500- Czech Republic) along with slide reagent kits, following the guidelines provided by the manufacturer [21].

Statistical analysis

GraphPad Prism 9 was employed to perform statistical analysis, encompassing a one-way analysis of variance (ANOVA) and subsequently conducting a Dunnett test to compare all groups vs the control group. Significance between groups was determined at a threshold of p-values below 0.05. The results are presented in the form of means and their corresponding standard error.

Results

Influence of Spirulina on Complete Blood Count (CBC)

To study the influence of Sp supplementation on As toxicity, the primary objective was to assess how As exposure affected the hematological parameters in rats. Rats exposed to As exhibited a significant decrease in some hematological measures, including

Hb: 10.62±0.2, PCV: 34.2±0.45 in comparison to the healthy control group, Hb: 13.07±0.46, and PCV: 41.83±1.48, as illustrated in Table 1 and Figure 1 (panels C, and D,). Conversely, WBCs: exhibited a significant increase ($p \leq 0.001$), as shown in the same Figure (panels B). However, the levels of RBCs and their markers, such as MCH, MCV, and MCHC, showed a slight decrease but were statistically nonsignificant ($P \geq 0.05$) when compared to the control group.

On the contrary, when 300mg/kg of spirulina was administered to the animals, there was a negligible increase in the levels of PCV, Hb, RBCs, and their markers (MCH, MCV, and MCHC). A similar outcome was observed when rats were treated with 600mg/kg of spirulina, except for a significant increase ($P \leq 0.05$) in PCV compared to the control rats. Importantly, the deviations in blood parameters observed in the As-exposed group were nearly restored to normal levels through co-treatment with 300mg and 600mg of Sp.

Influence of Spirulina on Plasma-Lipid Profile Measurements

Table 2 and Figure 2 (panels A, B, E) illustrated that the administration of 5 mg of As led to a significant rise in plasma cholesterol ($p \leq 0.05$; 101.7±2.74), triglyceride ($p \leq 0.001$; 86.86±2.14), and VLDL ($p \leq 0.001$; 17.37±0.42) compared to the control values (cholesterol: 92±1.83, triglyceride: 76.72±2.02, VLDL: 15.34±0.4). Conversely, there was a noteworthy reduction ($P \leq 0.05$) in HDL (50.33±1.2) when compared to rats fed on regular pellets, as depicted in Figure 2 (panel C). The levels of LDL did not exhibit any significant change compared to rats fed on normal pellets.

Rats receiving 300mg/kg of Sp exhibited noteworthy reductions in their cholesterol, triglyceride, and VLDL levels. Furthermore, when rats were given 600mg/kg of Sp, there was a decrease in cholesterol levels ($p \leq 0.05$), while the levels of triglyceride and VLDL remained unchanged ($p \geq 0.05$) when compared to the control group.

Simultaneous administration of 300mg/kg and 600mg/kg of Sp along with 5mg of As restored the lipid profile parameters to their baseline values, similar to those observed in the control animals.

Discussions

Arsenic is a chemical element, that can be found in small amounts in various places including the atmosphere, soil, and water [22, 23, 24]. The presence of arsenic contamination in both groundwater and animal feed poses a health risk to both humans and animals. This contamination is a source of threat to wildlife as well [25]. The toxicity is commonly associated with serious health disruptions [26]. Skin, lung, kidney, liver, and urinary bladder cancers are significant malignancies linked to these harmful consequences [1, 27]. Numerous studies have consistently shown that exposure to arsenic, regardless of its specific inorganic salt form, leads to a significant elevation in the production of free radicals [28]. While the exact mechanism by which As exerts its toxic effects remains not completely understood, it is generally believed that the generation of reactive oxygen species (ROS) by arsenic plays a substantial role in this process [29].

In our current study, we observed anemia in rats when they were exposed to 5mg/kg body weight of arsenic. This exposure ultimately led to a reduction in levels of Hb, and PCV in the rats treated with As. The potential cause for the erythropoiesis might be attributed to the effect of As on the erythrocyte membrane [30]. Previous studies have also reported a decline in hematological parameters in both animal models and human populations following exposure to toxicants [31]. Earlier research has reported that the decrease in Hb levels could be attributed to the inactivation of the δ-aminolevulinic acid dehydrase enzyme, which plays a crucial role in Hb production [32, 33]. Furthermore, this reduction in Hb might be a result of the apoptosis of plasma cells induced by As [34]. Arsenic can potentially impact bone marrow, kidney function, and Hb metabolism. This is supported by the findings of Gnanaraj et al. [35], who demonstrated that any substance that significantly alters the values of RBCs and related parameters is likely to have effects on the bone marrow, kidney function, and the metabolism of Hb. Conversely, the increase in WBC counts in rats subjected to As treatment was also noted in the study, These may be indicative of As-induced leukocytosis, lymphocytosis, monocytosis, basophilia, eosinophilia, and neutrophilia. WBCs provide immunity against antigen invasion. The significant increase in WBC may be a result of the necrotic activities of As in the cells, as reported by Efosa et al. [36].

Spirulina platensis is often referred to as a superfood renowned for its diverse impacts on growth, antioxidant processes, well-being, and overall quality of life additionally, it plays a significant role in promoting growth and supporting cellular rejuvenation [37, 38]. Based on the findings of the current observations, the rats' feds on control feed supplemented with a dose of 300mg and 600mg/kg Sp produced a positive impact on WBCs, Hb, PVC, and RBCs and their indices. These results agree with the research on rats, in which authors discovered that the addition of Sp in rat foods enhanced markedly the hematological parameters [39]. The obtained results also agreed with the results of Arrari et al [40] and Ramesh, et al [41], who

observed a significant increase in Hb when using Sp powder for five weeks. Also, Bléyéré *et al.,* [42] showed that consumption of Sp by rabbits led to an increase in values of RBCs and Hb. Kambou et al [43] studied the anti anemic effect of Sp in rabbits and showed that Sp is a rich source of nutrients. Notably, Sp has the ability to hinder the onset of leucopenia and anemia induced by exposure to lead and cadmium in rats [44].

It was noted that Sp demonstrates interleukin and tumor necrosis factor, which are responsible for controlling cellular processes in carp. Additionally, it aids in the production of red and white blood cells as well as interferons in rats [45]. Spirulina is a good iron-rich meal, with a whopping 20 times the iron content of grain. So, Sp may help cases of anemia [46]. Abed et al. [47] showed that Sp improved Hb levels in children's blood when used for 12 weeks. These results have demonstrated that utilizing spirulina instead of iron supplements not only alleviates pregnancy-related anemia but also enhances fetal and mom health via altering the gut microbiome [48].

Exposing the rats to 5mg/kg of As can have a noteworthy impact on lipid metabolism, leading to a substantial decrease in serum HDL levels and a notable increase in serum cholesterol, triglycerides, and VLDL levels. These changes can be attributed to the disruptive effects of As on lipid metabolism, resulting in perturbations in hepatic lipid synthesis, impaired clearance of lipoproteins, and heightened oxidative stress. These findings highlight the potential cardiovascular risks associated with As exposure and underscore the importance of understanding its impact on lipid metabolism [49]. The current study findings indicated that rats exposed to both 300mg/kg and 600mg/kg of Sp exhibited functional changes when compared to the control group. However, a noteworthy shift was observed in the serum lipid profiles following the feeding regimen of Sp in these rats. This regimen resulted in a decrease in serum cholesterol, triglyceride, LDL, and VLDL levels, while there was an observable increase in the serum HDL level. Gentscheva et al. [50] reported that Sp reduced serum cholesterol, triglyceride, LDL, and VLDL when volunteers were given a 4gm/day oral dose. In line with these studies, Sp (4.2 gm/day) was added for eight weeks to the diet of 30 Japanese males with high cholesterol, mild hypertension, and hyperlipidemia. Spirulina resulted in significant changes in blood pressure, lowered cholesterol levels, increased HDL, lowered triglycerides, and lowered systolic and diastolic blood pressure [10]. Spirulina may also have therapeutic effects such as preventing and decreasing the damages caused by hyperlipidemia and oxidative stress [51].

Conclusion

The current study demonstrates the potent antioxidant effects of Sp on rats exposed to As-induced toxicity. Spirulina supplementation significantly improved hematological parameters, restoring WBCs, Hb, and PVC to healthier levels. Additionally, it effectively countered the adverse effect of As on lipid profile reducing cholesterol, triglycerides, and VLDL while increasing HDL levels. These results underscore Spirulina's potential as a protective agent against As-induced hematological and lipid disturbances, highlighting its promising role in mitigating the oxidative stress and health risks associated with As exposure. Further research should explore its clinical applicability and mechanisms underlying these beneficial effects.

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Conflicts of Interest

We declare that we have no conflict of interest.

Funding statement

None

Contribution of authors

The authors, BSA, made significant contributions to the study, which encompassed its development, design, statistical analysis, and writing process.

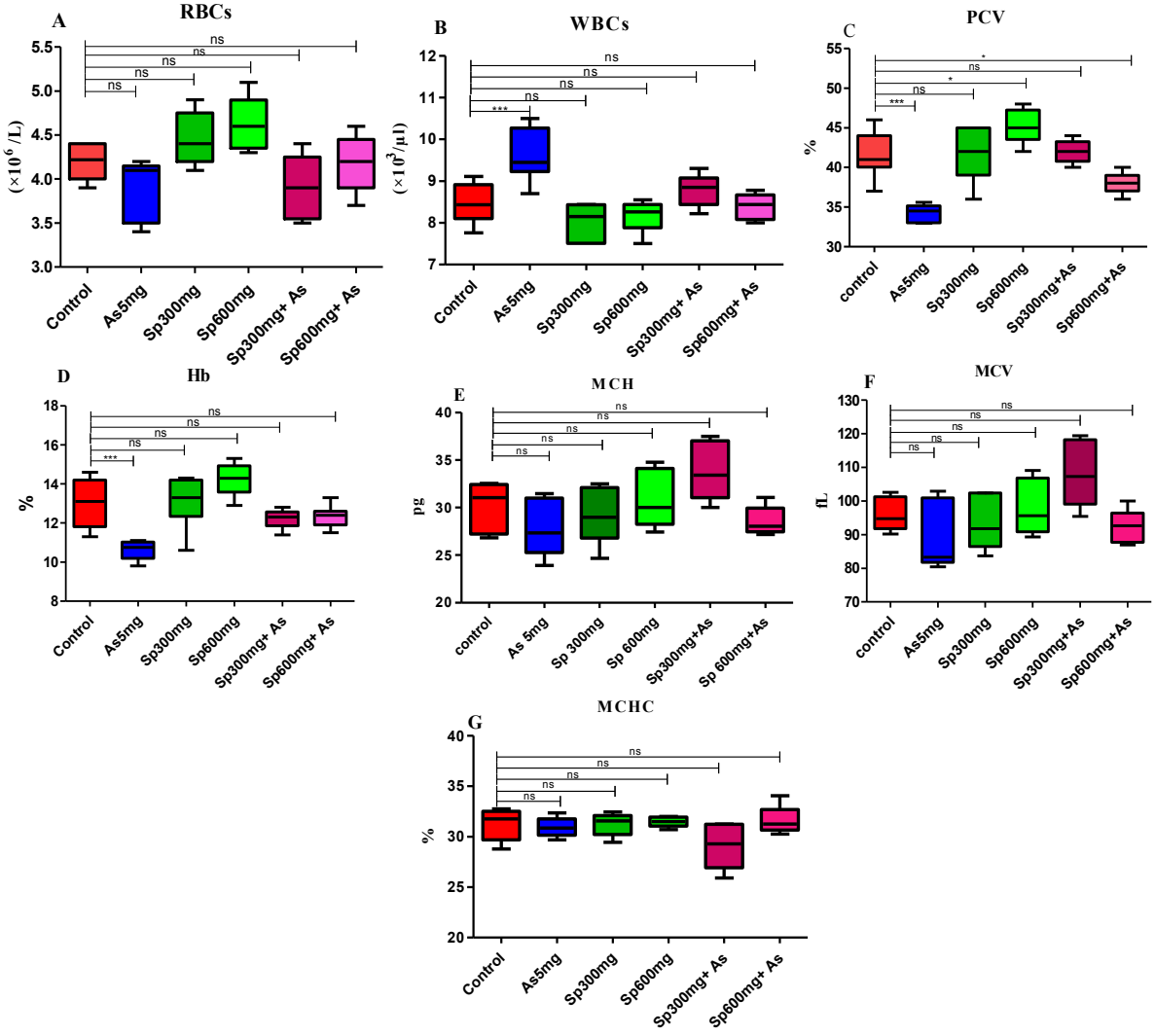

Fig. 1. Illustrates the Hematological counts in both the control and experimental groups.: (A) RBCs, (B) WBCs, (C) PCV, (D) Hb, (E) MCH, (F) MCV, and (G) MCHC.

TABLE 1. Demonstrates the hematological counts in both the control and experimental groups.

CBC	Control	Arsenate 5mg	Spirulina 300mg	Spirulina 600mg	Sp 300mg+ As 5mg	Sp600mg+As 5mg
RBCs (10^6/L)	4.20±0.09	3.88±0.16	4.46±0.13	4.62±0.13	3.9±0.16	4.18±0.14
WBCs (10^3/µl)	8.46±0.19	9.61±0.26***	8.02±0.18	8.16±0.15	8.78±0.15	8.39±0.12
PCV (%)	41.4±1.11	34.2±0.45***	41.4±1.21	45.2±0.87*	42±0.57	38.1±0.51*
Hb (%)	13.0±0.46	10.6±0.2***	13.1±0.48	14.2±0.33	12.2±0.19	12.3±0.22
MCH (Pg)	30.0±1.2	27.7±1.19	29.4±1.37	30.9±1.37	33.9±1.39	28.6±0.68
MCV (fL)	96.2±2.25	89.7±4.64	93.8±3.69	98.2±3.75	108±4.47	92.2±2.27
MCHC (%)	31.2±0.71	30.9±0.43	31.2±0.50	31.5±0.22	29.1±1.02	31.6±0.64

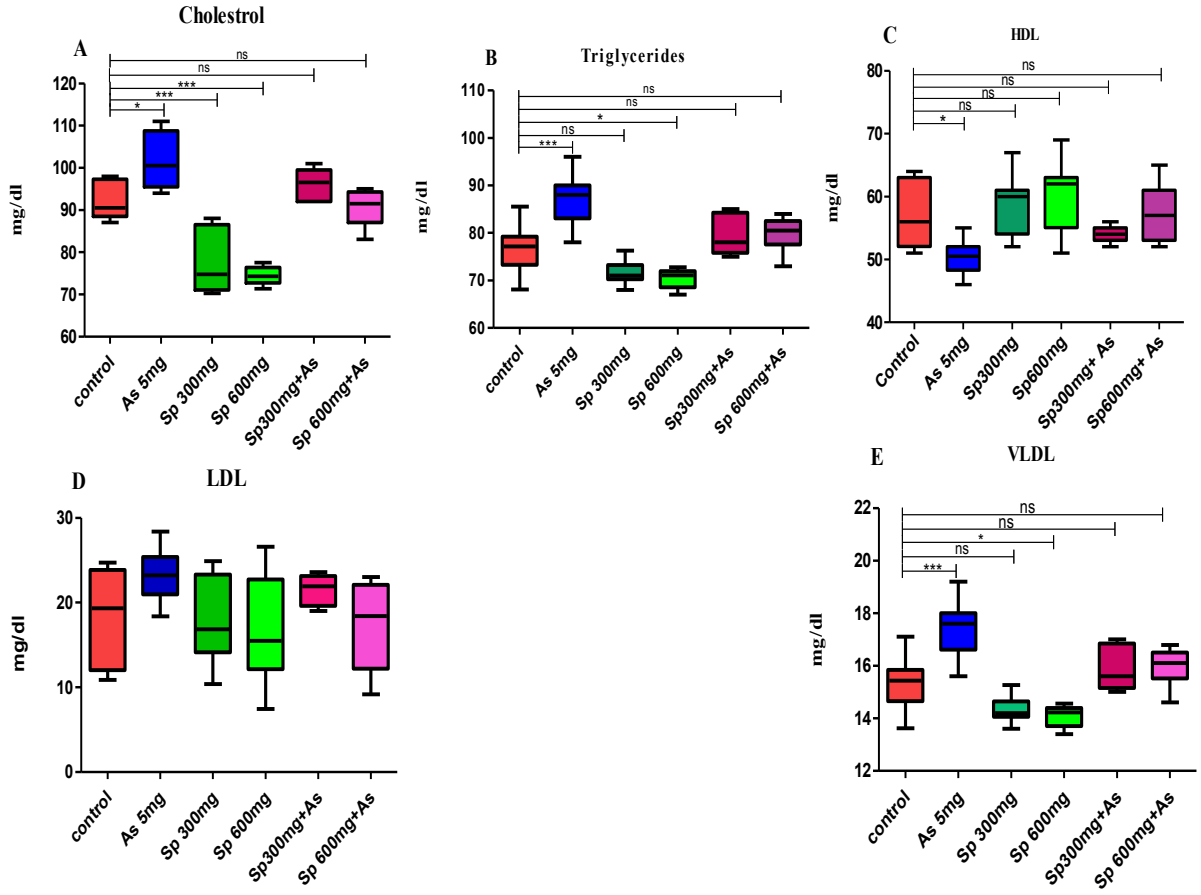

Fig. 2. **Illustrates the Lipid profile counts in both the control and experimental groups: A) cholesterol, (B) triglyceride, (C) HDL, (D) LDL, (E) VLDL.**

TABLE 2. Demonstrates the Lipid profile measurements in both the control and experimental groups.

Lipid Profile	Control	Arsenate 5mg	Spirulina 300mg	Spirulina 600mg	Sp300mg+ As 5mg	Sp600mg+As 5mg
Cholesterol (mg/dl)	92±1.83	101.7±2.74*	77.52±3.12***	74.51±1.01***	96.17±1.4	90.57±1.82
Triglyceride (mg/dl)	76.72±2.02***	86.86±2.14	71.61±0.99*	70.45±0.85	79.33±1.72	79.83±1.53
HDL (mg/dl)	56.86±1.89	50.33±1.2*	59±1.87	60.43±2.20	54±0.53	57.29±1.7
LDL (mg/dl)	18.37±2.33	23.23±1.35	17.86±2.15	16.68±2.69	21.53±0.73	17.33±2.14
VLDL (mg/dl)	15.34±0.4	17.37±0.42***	14.32±0.19*	14.09±0.17	15.87±0.35	15.97±0.31

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تأثير الطحلب الازرق المخضر (سبيرولينا) ضد الفروق في معدلات الدهون والهيماتولوجية المحفزة بواسطة الزرنيخ في نماذج الجرذان

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هدف هذه الدراسة هو التحقيق في تأثير السبيرولينا على معايير الدم وملف الدهون في الجرذان المعرضة للزرنيخ . تم تقسيم ثمانية وأربعون جرذاً إلى ست مجموعات: مجموعة الاولى تتناول نظامًا غذائيًا قياسيًا، مجموعة الثانية تتلقى 5 ملغ/كغ من الزرنيخ الصوديوم ، مجموعتين الثالثة والرابعة تتلقيان 300 و600 ملغ/كغ من السبيرولينا، ومجموعتين تجمعان بين الزرنيخ والسبيرولينا بنفس الجرعات. بعد 28 يومًا، تم جمع عينات الدم لإجراء اختبارات لمعايير الدم وملف الدهون. أظهرت النتائج أن الجرذان المعرضة للأزرنيخ شهدت انخفاضًا كبيرًا في خلايا الدم الحمراء(RBCs) ، والهيموجلوبين(HGB) ، ونسبة حجم الكريات الدموية المعبأة (PCV) ، ومتوسط حجم الكرية الدموية(MCV) ، ومتوسط تركيز هيموجلوبين الكرية الدموية(MCH) ، بينما زاد عدد خلايا الدم البيضاء (WBCs) ومتوسط تركيز هيموجلوبين الكرية الدموية (MCHC) بشكل ملحوظ. ومع ذلك، استعادة تناول 300 ملغ و 600 ملغ من السبيرولينا جزئياً هذه المعايير إلى مستويات طبيعية. علاوة على ذلك، أظهرت إضافة Sp انخفاضًا ملحوظًا في مستويات الكوليسترول والجليسريدات وبروتين منخفض الكثافة للكوليسترول (VLDL) في المصل، دون تغييرات ملحوظة في بروتين عالي الكثافة للكوليسترول (HDL) وبروتين منخفض الكثافة للكوليسترول .(LDL) تسلط هذه النتائج الضوء على إمكانية السبيرولينا في التخفيف من التغيرات المسببة بواسطة الزرنيخ في معايير الدم وملف الدهون، مما يشير إلى دورها في مواجهة التأثيرات الضارة لتعرض الزرنيخ على هذه العلامات الصحية