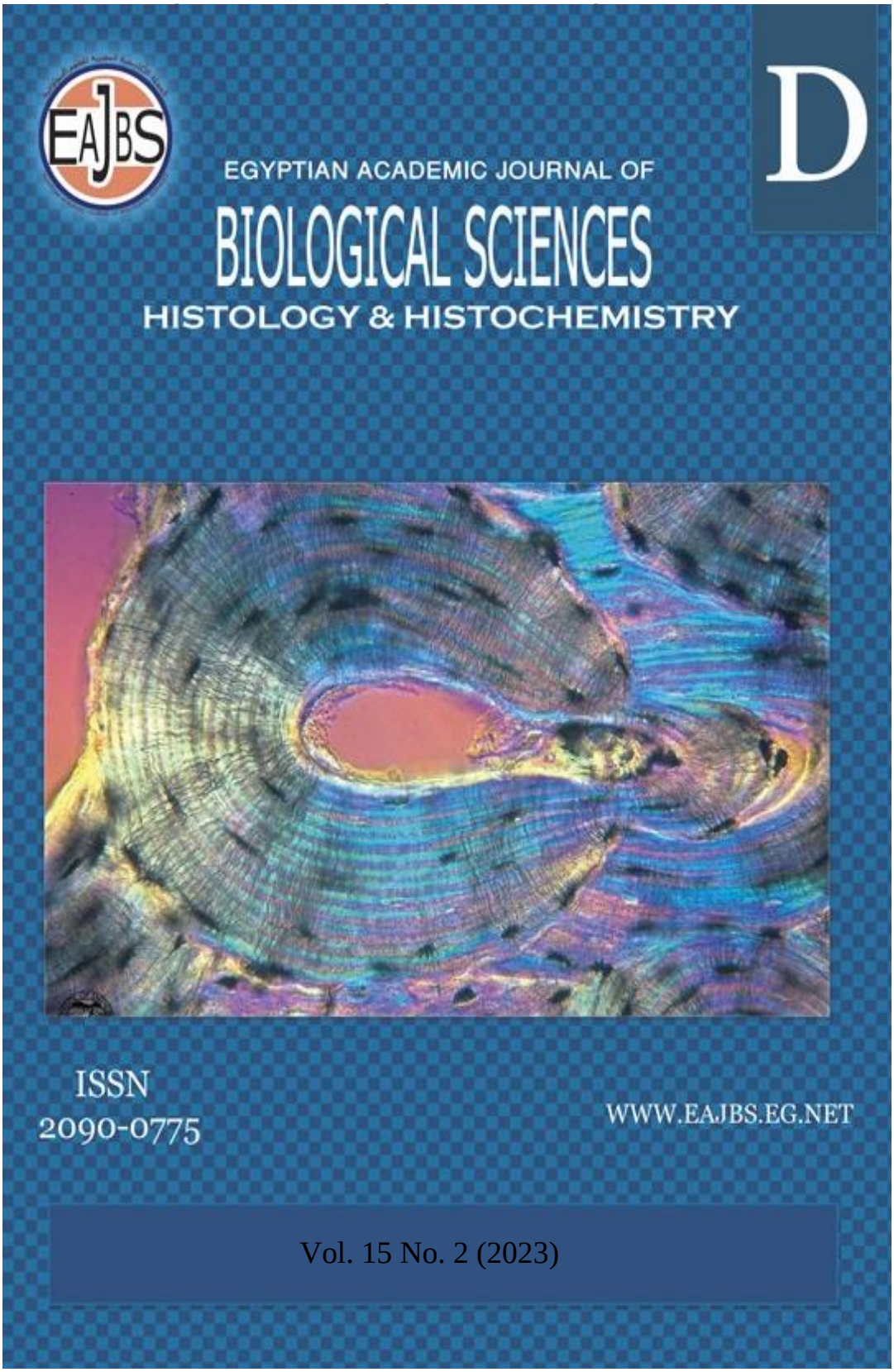

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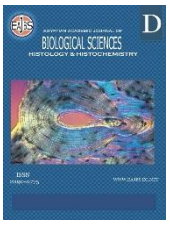

Amelioration of Sodium Arsenate-Induced Stimulation of Enzyme Activities in the Plasma and Liver, and Liver Histopathology in Rat Models by Spirulina (*Arthrospora platensis)*

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ABSTRACT

Background: Arsenic (As) contributes to metabolic disorders, including diabetes, and disrupts the functions of multiple systems, leading to associated diseases and dysfunctions. This study aims to investigate the effects of Arsenic on plasma and liver enzymes, assess liver tissue histopathological changes, and explore spirulina's potential protective role against As-induced liver damage. **Materials and Methods:** Female Wistar rats were divided into six groups, including control, sodium arsenate-only (5mg/kg body weight), Sp-only (300mg and 600mg), and combined As and Sp treatment groups. Plasma and liver samples were collected after four weeks of treatment for enzyme analysis, and part of the liver tissues were examined histologically. **Results:** Arsenic exposure significantly increased aspartate aminotransferase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), and lactate dehydrogenase (LDH) enzyme levels in plasma and liver. However, Sp supplementation at different doses showed a reduction in enzyme levels, although not statistically significant. Histological examination revealed liver damage in the As-exposed group, including congestion, leukocyte infiltration, and endothelium detachment. Sp supplementation partially attenuated these changes. **Conclusion:** This study enhances understanding of As's toxic effects and suggests that Sp supplementation may offer some protection against As-induced liver damage. **Recommendation:** To improve the findings, additional parameters such as oxidative stress markers, inflammatory markers, and liver function tests should be incorporated. These measurements will provide a more comprehensive understanding of the effects of arsenate exposure.

INTRODUCTION

Globally, the toxic effects of arsenic (As) are well-documented in humans and animals. Arsenic is considered the second most significant contributor to heavy metal poisoning, following lead (Pb) (IARC, 1987). Arsenic, a chemical element, can be found in small amounts in various places including the atmosphere, soil, and water (Gupta *et al*., 2005; Rana *et al.,* 2010). When As compounds accumulate in the environment due to natural or human-related causes, they become a significant issue for both environmental and occupational health (Gupta *et al*., 2005; Rana *et al.,* 2010).

Numerous researchers have reported the presence of as contamination and the resulting adverse health effects in individuals. It is hypothesized that various health issues, such as skin cancer, conjunctivitis, melanosis, hyperkeratosis, renal dysfunctions, hepatic and respiratory disorders, as well as hematological alterations, are commonly caused by as poisoning (Smith *et al*., 2000). According to compelling epidemiological data, arsenic is widely recognized as a confirmed cause of cancer in humans (Loprieno, 1975).

Traditional medicinal plants have been employed for a considerable period to combat life-threatening ailments, and these plants have exhibited antioxidant properties (Gargouri *et al.,* 2016). Spirulina (SP), a type of blue-green algae belonging to the Cyanobacteria family, is abundant in bioactive compounds such as proteins, lipids, carbohydrates, trace elements, pigments (phycocyanin, β-carotene), riboflavin, tocopherol, and α-linoleic acid (Yusuf *et al.,* 2016). Spirulina holds the distinction of being the largest natural source of protein worldwide and is regarded as a significant medicinal herb (Tietze, 2004). The two most commonly utilized species of this blue-green algae as nutritional supplements are spirulina platensis (*S. Platensis*) and spirulina maxima (*S. maxima*) (Sixabela *et al*., 2011; Tefera *et al*., 2016). Spirulina, arsenic recognized by the United Nations World Health Organization (WHO), has gained recent popularity as a nutritious food supplement, often referred to as "the best for tomorrow" (Anita, *et. al*. 2010). It offers numerous benefits to various bodily systems, with a particular emphasis on the immune system. Spirulina is a plentiful source of various minerals, including potassium, calcium, chromium, copper, iron, magnesium, manganese, phosphorus, selenium, sodium, and zinc (Tokusoglu & Uunal, 2003). The administration of Sp has been discovered to mitigate the cardiac damage resulting from chemotherapy (Khan *et al.*, 2005), reduce the severity of strokes, and enhance the recovery of motor function after a stroke (Wang *et al.,* 2005). Additionally, it has shown the ability to reverse age-related declines in memory and learning. Sp has also demonstrated effectiveness in preventing and treating hay fever by stimulating immunological activities (Chen *et al.*, 2005).

Based on the aforementioned points, the objective of this study is to investigate the impact of as on enzyme activities in both plasma and the liver, analyze histopathological changes in liver tissue, and explore the potential protective effects of Sp in the presence of as exposure.

MATERIALS AND METHODS

Chemicals:

a- Sodium Arsenate (Na_2HAsO_4). It was obtained from Sigma-Aldrich (MilliporeSigma) – America, in the form of white powder.

b- The best-grade *Spirulina platensis* powder was acquired from Natura Vitalis, a company based in the Netherlands.

Animal Maintenance and Treatment Regimen:

Female rats (*Rattus norvegicus*), aged 10-12 weeks and weighing approximately 205.2±8.22g (n=48), were bred in animal house at the Biology Department, Faculty of Science, University of Zakho. These rats were housed in standard polypropylene cages with four rats per cage and were maintained at a room temperature of 24°C with a 12/12-hour light-dark cycle. They had unrestricted access to a regular diet of pelletized food and distill water. Before the experiment, proper care was provided to the animals in accordance with the applicable regulations. The rats were acclimatized for one month at a temperature range of 21-24°C before the commencement of the experiments. An expert with certificate number AEC-020

conducted the testing on the rats involved in the study.

At the start of the study (week 1), the rats were randomly divided into six groups, each consisting of eight animals. Over a period of four weeks, the rats in each group were treated as follows: Group 1: Received distilled water. Group 2: Administered oral intubations of sodium arsenate at a dose of 5mg/kg body weight (BW). Group 3: Administered oral intubations of spirulina at a dose of 300mg/kg (BW). Group 4: Administered oral intubations of spirulina at a dose of 600mg/kg (BW). Group 5: Administered oral intubations containing 5mg/kg sodium arsenate and 300mg/kg spirulina and Group 6: Administered oral intubations containing 5mg/kg sodium arsenate and 600mg/kg spirulina.

1. Blood Plasma Collection and Processing:

Following a four-week period of arsenic exposure, Blood samples were collected from all animals by puncturing the heart via cardiac puncture to obtain the blood samples. After obtaining the collected blood samples, they were allowed to coagulate at room temperature in the laboratory for a period of 30 min. Following that, the blood underwent centrifugation at 4,000 rpm for 5 min to separate the plasma. The samples obtained were preserved at -20°C until they were prepared for chemical analysis.

2. Liver Tissue Processing for Homogenization:

The rats were euthanized using Diethyl ether. Afterward, the abdominal and thoracic cavities were immediately opened, and the targeted organ (liver) was extracted and rinsed with distilled water. The enzyme activities in the liver were assessed using slide kits. For histological examination, the remaining liver tissue was preserved in 10% neutral-buffered formalin for embedding and subsequent review.

3. Quantification of Enzyme Activities in the Liver:

The liver samples were rinsed in a cold saline solution and then dried before being weighed in 0.15 M Tris-Hcl Buffer with a pH of 7.4. A 10% (w/v) tissue homogenate was prepared in the same buffer for the assessment of AST, ALT, and LDH enzymes. Another portion of the tissue was washed with cold saline and treated with 0.2 M Carbonate-Bicarbonate Buffer at a pH of 10.5 for ALP assessment in the liver (Saeed & Al-Habbib, 1990). A 10% (w/v) liver homogenate was obtained in the 0.2 M Carbonate-Bicarbonate Buffer. The enzyme activities present in the supernatant were analyzed using FUJIFILM (DRI_CHEM NX500- Czech Republic) along with slide reagent kits, following the guidelines provided by the manufacturer (Al Sulivany, 2023).

4. Histopathological Investigations:

The liver samples were preserved in a 10% formalin solution containing a neutral buffer. The tissue processing was performed internally, and sections were sliced to a thickness of 5 µm using a microtome. These subsections were then subjected to treatment with hematoxylin and eosin (H&E) staining and observed under a light microscope. This process was conducted following the methodology outlined in (Ramandi *et al.*, 2017).

Statistical Analysis:

The data obtained from the experimental outcomes were analyzed using GraphPad Prism viewer mode 9 for Windows. One-way analysis of variance (ANOVA) was conducted to compare the control group with the experimental groups. Subsequently, Tukey's test for multiple comparisons was applied after ANOVA to determine the significance between different groups. Statistical significance was considered at $p < 0.05$ values.

RESULTS

1. Assessment of Enzyme Activities in Plasma:

Figure 1**,** displays the mean and standard error of the mean level of AST(U/L), ALT(U/L), ALP (U/L), and LDH (U/L) in the plasma of rats exposed to 5mg/ kg/ body weight of arsenic with or without Sp. It is clear from the results

that adding arsenic to food considerably enhanced ($p<0.0001$) the levels of these enzyme activities. On the other hand, the inclusion of Sp in the diet at various quantities (300mg and 600mg) inhibits the levels of these enzyme activities but statistically insignificant when compared with the control group. Notably, the levels of these enzymes were markedly reduced in rats' feds with a combination of 5mg arsenic with 300 mg and 600 mg of Sp as compared with rats' feds control diet alone.

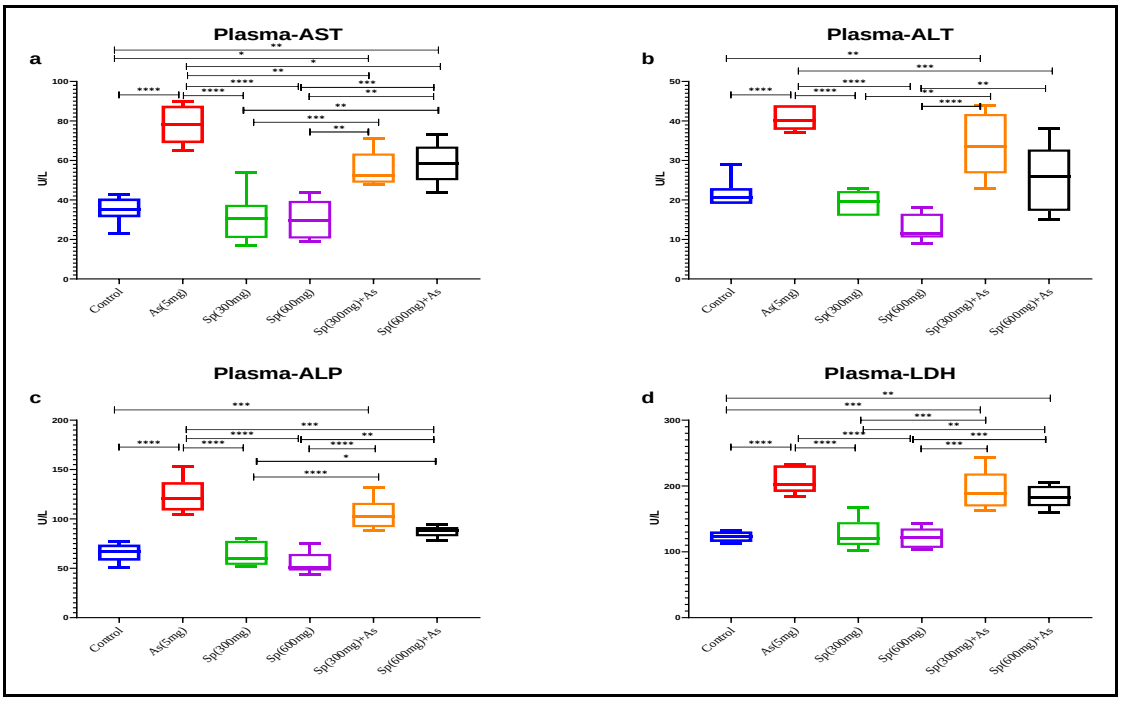

Fig. 1: Investigation of Plasma Enzyme Activity: (a) Aspartate Aminotransferase, (b) Alanine Transaminase, (c) Alkaline Phosphatase, and (d) Lactate Dehydrogenase in Rats Models Exposed to 5mg/kg Arsenate, with and without 300mg and 600mg Spirulina.

2. Assessment of Enzyme Activities in the Liver:

The data regarded liver enzyme activities, which include the AST (69.5±4.49), ALT (33.33±2,56), ALP (76.1±5.91), and LDH (214.7±11.69). Statistically, compared to the rats in the control group, the rats fed a diet plus arsenic had considerably higher levels of these enzymes in contrast to the control group (Fig. 2). The liver extract AST, ALT, ALP, and LDH levels in the rata fed 300mg and 600mg Sp were lower than those in the control groups. The presence of both Sp 300mg and 600mg inhibits arsenic toxicity to a great extent when compared with the control groups including AST (34.67±2.78), ALT (16.83±1.52), ALP (40.17±3.74), and LDH (183.5±7.34) respectively.

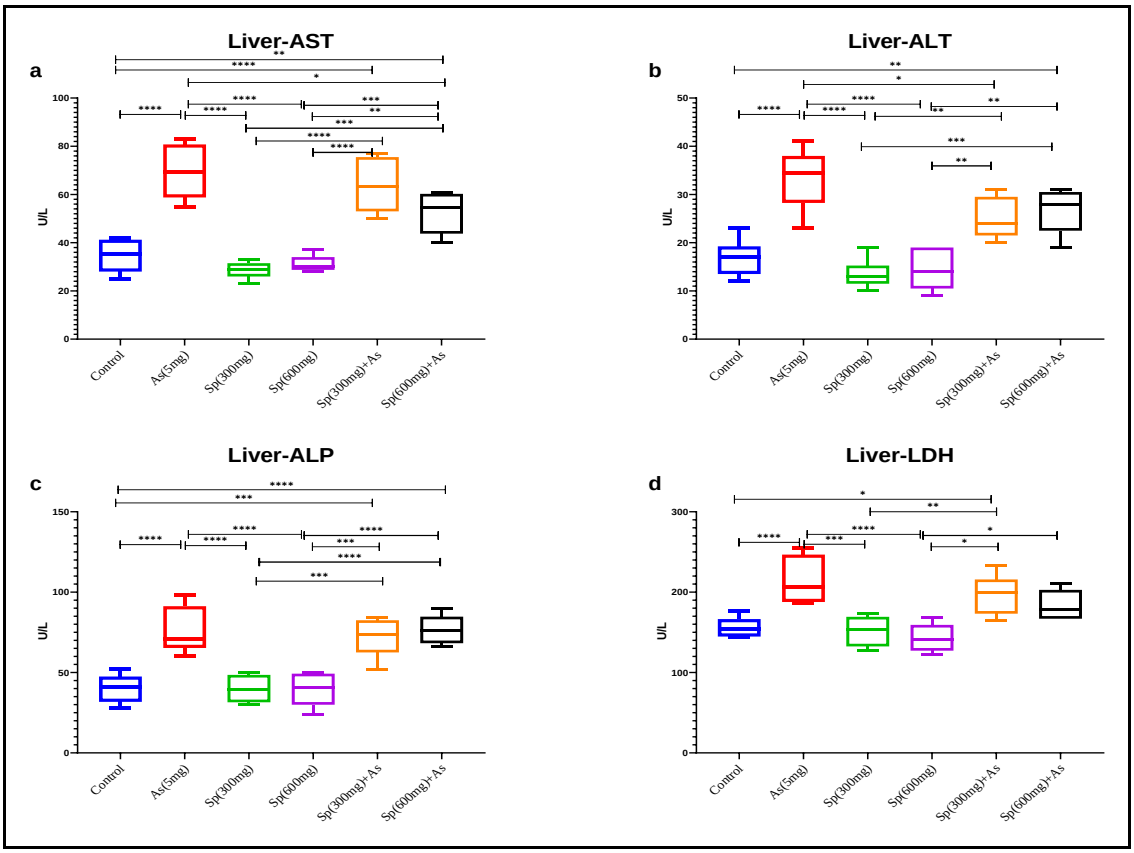

Fig. 2: Investigation of liver Enzyme Activity: (a) Aspartate Aminotransferase, (b) Alanine Transaminase, (c) Alkaline Phosphatase, and (d) Lactate Dehydrogenase) in Rats Models Exposed to 5mg/kg Arsenate, with and without 300mg and 600mg Spirulina.

3. Microscopic Examination of Liver Tissue:

In the control group, microscopic evaluations of liver-stained sections revealed the presence of normal hepatocytes, as well as a healthy sinusoidal and portal region (Fig. 3a). Administration of 5 mg/kg body weight of As to the rat resulted in noticeable alterations in the liver structure. These changes included the presence of central vein congestion, infiltration of leukocytes, erythrocyte diapedesis within expanded sinuses, as well as detachment and destruction of the endothelium (Fig. 3b). In rats treated with 300 and 600 mg/kg BW Sp hepatocytes were still normal with obvious nuclei, but cloudy degeneration (CD) of a hole lobule, mild congestion of veins and focal infiltration was remarked (Fig. 3c & d). In the combination between As and Sp at doses of 300 and 600 mg/kg BW, the microscopic examination showed reduced CD to the peripheral zone of the lobule at dose 300 to mild CD at dose 600 of SP. In addition to leukocytes central infiltration, and mild congestion, Besides that megalokaryocytes, and hepatocytes proliferation in both doses of Sp were remarked (Fig. 3e & f).

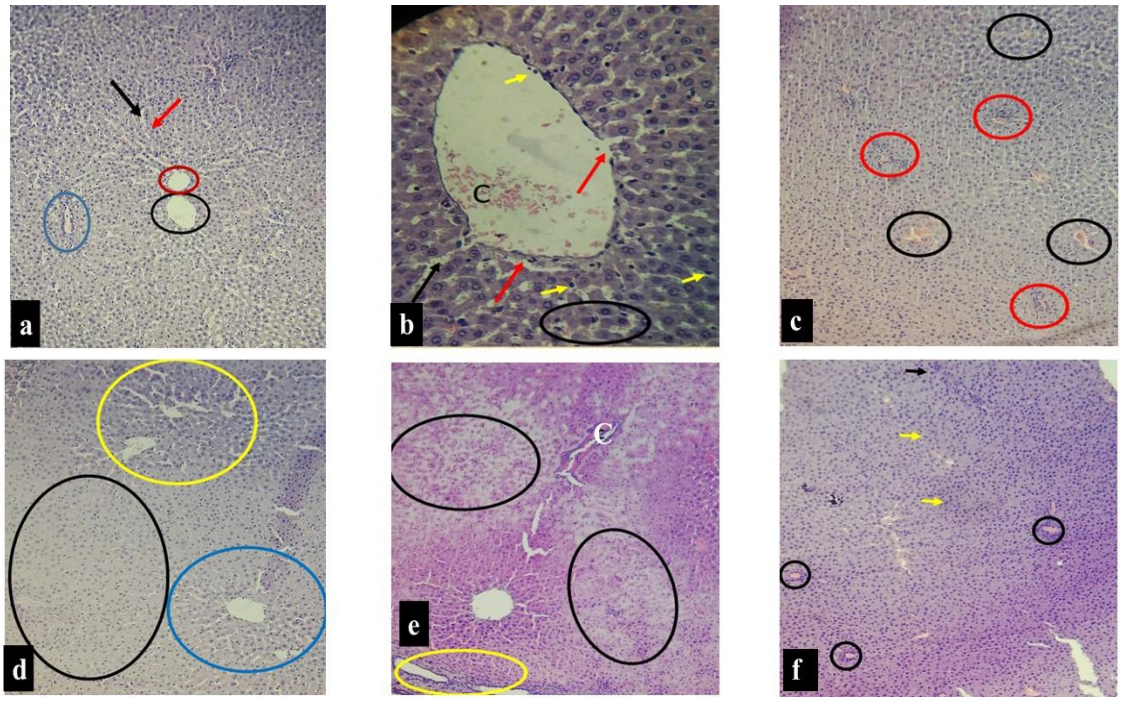

Fig. 3: reveals, (a): Portal vein (black circle), bile duct (red circle), portal artery (blue circle), sinus (black arrow), hepatic cords and hepatocytes. (b): Central vein congestion (C), Sinusoidal wide (black arrow), Endothelium detachment and destruction (red arrow), Leukocytes infiltration (yellow arrow) and RBCs Diapedesis (black circle). (c): Mild congestion (black circles), Focal filtration (red circles). (d): Mild cloudy degeneration (black circle), mild sinusoidal wide (yellow circle) and normal hepatocyte (blue circle). (e): Peripheral cloudy degenerations (black circles), Mild congestion (C), Leukocytes central infiltration (yellow circle). (f): Mild cloudy degeneration (black circle), mild sinusoidal wide (yellow circle) and normal hepatocyte (blue circle).

DISCUSSION

Increased consumption of arsenic has been demonstrated to disrupt several bodily functions and organs by disturbing different biochemical and physiological processes (Khan *et al.*, 2006; Anwar-Mohamed *et al.*, 2021). Several studies have consistently demonstrated that exposure to As, regardless of the specific inorganic salt form, significantly increases the production of free radicals (Firdausa *et al.*, 2018). The precise mechanism through which As exerts its toxic effects is not yet fully understood. However, it is believed that the generation of reactive oxygen species (ROS) by arsenic plays a significant role in this process (Shi *et al.*, 2004). Various *in vitro* and *in vivo* studies have utilized antioxidants from natural sources to investigate their potential in countering metal-induced toxicity, and the results have shown promising effects (Firdausa *et al*., 2018; Gyasi *et al.*, 2012). Aspartate aminotransferase AST and ALT are intracellular enzymes primarily present in the liver, and their elevated levels serve as reliable indicators of hepatic disease. These enzymes reside within hepatocytes and play crucial roles in various metabolic pathways (Johnston, 1999). Additionally, ALP is a significant enzyme involved in detoxification, metabolism, and the synthesis of essential macromolecules to support critical bodily functions. Any disruption in the activity of these enzymes can lead to impaired liver function, as documented in existing literature (Kavitha *et al.*, 2010; Gyasi *et al.*, 2012; Sarker *et al.*, 2012).

The current investigation's findings indicated that As-treated rats exhibited higher levels of AST, ALT, ALP, and LDH in both plasma and liver

samples compared to the control group. Multiple researchers have documented an increase in the activity of ALT and AST in various animal species when exposed to arsenic intoxication. In a study by Humtsoe *et al.* (2007), the effects of Sodium arsenate (Na_2 $HAsO_4$ $.7H_2O$) at sub-lethal concentrations of 96 mg/L and 144 mg/L were investigated on different enzymes in juvenile *Labeo rohita*. According to Roy's study (2002), the liver plays a crucial role in eliminating foreign substances (xenobiotics) in animals. Karatas and Kalay in 2002 further noted that various organic and inorganic chemicals can negatively impact the structural integrity of hepatic cell organelles and membrane transports, resulting in changes to metabolic pathways. However, this aligns with the results of Ola-Davies and Akinrinde (2016), who found that administering arsenic (2.5 mg) to the rats for seven days led to oxidative stress-related damage to hepatocellular membranes, causing the leakage of liver transaminases into the extracellular space, ultimately leading to elevated levels of these enzymes in the blood.

In a prior investigation, mice were exposed to various doses of arsenic (0, 5, 10, 20 mg/kg/day), resulting in a notable accumulation of arsenic in their livers. Other studies have also indicated that exposure to different concentrations of arsenic can lead to its accumulation in various organs of the body, including the kidneys, eyes, blood, and particularly the liver (Maiti and Chatterjee, 2001). Additionally, Mazumder (2005) suggested that the liver is more susceptible to arsenic accumulation compared to the brain due to the presence of the blood-brain barrier.

Previous studies have reported that elevated levels of arsenic (>10 μg/L) have been associated with apoptosis, oxidative DNA damage, and necrosis. Furthermore, cases of liver damage resulting from acute arsenic exposure have been documented (Jomova *et al.*, 2011).

Rats' feds Sp at levels (300 and 600 mg) resulted in a substantial drop in the ratio of AST, ALT, ALP, and LDH in the plasma and liver as compared to the group given As. These findings align with the results reported by Bashandy *et al.* (2011), who observed that Sp exhibits potent antioxidant activity and stimulates the activity of enzymes involved in scavenging free radicals. According to Wu *et al.* (2005), the protective effects of Sp could be attributed to its capacity to neutralize oxidation-initiating agents that are generated during the oxidation of proteins and lipids. Luxia *et al.* (1996)27 reported that β-carotene found in Sp may contribute to the reduction of cell damage, particularly damage to DNA molecules, thereby aiding in the regeneration of impaired liver cells. Furthermore, the antioxidant protective effects of Sp can be attributed to the presence of Phycocyanin (C-phycocyanin and allophycocyanin) as suggested by Bhat and Madyastha (2001). Moreover, the simultaneous administration of As and Sp to rats at doses of 300 and 600 mg/kg body weight resulted in a partial reduction, as evidenced by the restoration of enzyme activities in the serum and liver to their normal levels. Nonetheless, Sayed *et al.* (2015) also observed a comparable decrease in enzyme levels when Sp was present. They found that the co-administration of Sp significantly reversed the levels of AST and ALT, bringing them close to the values observed in the control group. The precise mechanism underlying the protective effects of Sp in tissue recovery is not completely understood.

However, it is recognized that Sp is a rich source of various nutrients such as protein, amino acids, iron, β-carotene, phycocyanin, γ-linolenic acid, and vitamins B1, B2, B3, B6, and B12, as well as essential fatty acids. Spirulina's micronutrients and anti-oxidant components play a crucial role in the treatment of chronic arsenic poisoning as highlighted by Korany *et al.* (2019). Various substances such as ascorbic acid,

Vitamin A, zinc, iron, Sp, lipoic acid, ascorbic acid, and α-tocopherol have shown a significant ameliorating effect against chronic arsenic poisoning. Previous research has shown that alpha-lipoic acid, ascorbic acid, and alpha-tocopherol have a combined effect, working synergistically to reduce tissue arsenic levels. As indicated by Hasan *et al.* (2015), it can therefore be argued that applying a mix of vitamins, minerals, antioxidants, and other micronutrients may serve as a unique treatment method for the prevention of arsenicosis.

These were consistent with the current findings when Gopisetty and his colleagues (2006) documented that 5mg/kg BW daily delivered caused Kupffer cell activation, severe sinusoidal dilation and congestion, infiltration of leukocytes, and degeneration of hepatocytes (Gopisetty *et al*.,2006; Koranny *et al*., 2019). And same findings were stated by Noman *et al.*, (2015) plus renal tubular degeneration and intratubular necrosis. Besides that, while using Sp as an amelioration arsenic affects organs' tissues in current outcomes comes agreement to Khan *et al.*, (2005) state that Sp has antioxidant properties that decrease liver toxicities (Khan *et al.,* 2005). Sp also stimulates antiapoptotic genes to express bcl-2 (Liu and Zhang 2002), and it contains gamma-linolenic acid, which has antioxidant properties and reduces heavy metal toxicity in the liver (Saxena and Kumar 2004), as well as stabilizes membrane functions in various tissues (Upasani and Balaraman 2003).

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