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The Protective Role of Melatonin against Sodium Arsenite-Induced Hepato-Toxicity in Male Rabbits: Biochemical and Histopathological Study

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Abstract:

Background: Sodium arsenite (Sa) is a potent environmental pollutant known to induce severe organ toxicity through oxidative stress. Melatonin (Me) is a powerful antioxidant that may mitigate these effects. **Aim:** This study was conducted to evaluate the protective role of melatonin against hepatic damage induced by sodium arsenite in male New Zealand White rabbits. **Materials and Methods:** Twenty rabbits were randomly divided into four groups: Control, Melatonin (10 mg/kg), Sodium Arsenite (5 mg/kg), and a combination group (Me+Sa) for a period of 12 weeks. **Results:** Exposure to Sa led to a significant ($P < 0.05$) increase in liver enzyme activities (AST, ALT, and ALP) and plasma bilirubin, accompanied by a significant decrease in total protein and albumin levels. Histopathological examination of the Sa group revealed extensive fatty degeneration, fibrous encapsulation, and tissue degradation. [5]Conversely, concurrent administration of melatonin with arsenic significantly ameliorated these biochemical alterations and preserved the normal hepatic architecture. **Conclusion:** The results indicate that melatonin effectively attenuates arsenic-induced hepatotoxicity by stabilizing cellular membranes and maintaining the structural integrity of the liver, likely due to its potent antioxidant properties.

Keywords: Rabbits; Sodium Arsenite; Melatonin; Hepatotoxicity; Liver Enzymes; Histopathology.

Introduction

Environmental exposure to heavy metals remains a major public health concern worldwide due to their persistence, bioaccumulation, and potential to induce serious organ toxicity[1]. Among these metals, arsenic is considered one of the most hazardous environmental pollutants, frequently contaminating drinking water, soil, and food chains [2]. Sodium arsenite, an inorganic and highly toxic form of arsenic, has been widely reported to induce severe biochemical and structural damage in vital organs, particularly the liver and kidneys, which play central roles in detoxification and excretion processes[3]. The hepatotoxic effects of sodium arsenite are primarily mediated through the generation of excessive reactive oxygen species (ROS), leading to oxidative stress, lipid peroxidation, protein oxidation, and DNA damage[4]. These oxidative events disrupt cellular redox homeostasis, impair mitochondrial function, and activate inflammatory and apoptotic pathways, ultimately resulting in tissue injury and functional deterioration of hepato-renal systems. Experimental studies have demonstrated that arsenic exposure significantly alters liver enzymes, kidney function markers, antioxidant defense systems, and histological architecture in animal models [5]. Melatonin (N-acetyl-5-methoxytryptamine) is an endogenous indoleamine primarily secreted by the pineal gland and is well recognized for its potent antioxidant, anti-inflammatory, and anti-apoptotic properties[6]. Unlike classical antioxidants, melatonin exhibits both direct free radical scavenging activity and indirect antioxidant effects by enhancing the expression and activity of endogenous antioxidant enzymes such as superoxide dismutase, catalase, and glutathione peroxidase. Moreover, melatonin has the unique ability to cross biological membranes and accumulate within subcellular compartments, including mitochondria and nuclei, making it particularly effective in protecting tissues against oxidative damage [7]. Recent experimental evidence suggests that melatonin exerts significant protective effects against heavy metal-induced toxicity by attenuating oxidative stress, modulating inflammatory responses, and preserving normal tissue architecture. Its role in mitigating arsenic-induced liver and kidney injury has gained increasing attention [8]; however, data concerning its protective efficacy in rabbit models, particularly with combined biochemical and histopathological evaluation, remain limited[9-19] . Therefore, the present study was designed to investigate the protective role of melatonin against sodium arsenite-induced hepato-renal toxicity in male rabbits through comprehensive biochemical assessments and detailed histopathological examination.

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Materials and methods

Sodium arsenite "$NaAsO_2$" was obtained from the Department of Chemistry, Faculty of Science. Melatonin was purchased from a local pharmaceutical source (El-Bayda, Libya). Twenty mature male New Zealand White rabbits (6 months old; 2089 ±97.66 g) were used. Animals were housed individually and provided with a standard pellet diet and water *ad libitum*. After acclimatization, rabbits were randomly assigned to four groups (n=5/group) for 12 weeks: Group I (Control): Received no treatment. Group II (Melatonin): Administered melatonin (10 mg/kg BW) daily via oral gavage. Group III (Arsenic): Administered sodium arsenite (5 mg/kg BW) daily via oral gavage. Group IV (Arsenic + Melatonin): Received both sodium arsenite (5 mg/kg BW) and melatonin (10 mg/kg BW) daily. At the end of the 12th week, blood samples were collected for hematological and biochemical profiling. Biochemistry: Plasma was separated by centrifugation (860 xg for 20 min). Liver function markers "AST, "ALT", "ALP", Bilirubin" were determined spectrophotometrically using standard diagnostic kits. Following sacrifice, liver tissues were immediately excised, weighed, and fixed in 10% neutral buffered formalin. The samples were processed using the paraffin embedding technique, sectioned at 5 µm thickness, and stained with Hematoxylin and Eosin "H&E" for microscopic evaluation. Statistical Analysis: Data were analyzed using one-way "ANOVA". Mean differences were compared using Duncan's Multiple Range Test at a significance level of $P < 0.05$.

Results

The administration of Sodium Arsenite "Sa" induced significant hepatic injury, as evidenced by a marked elevation in the activities of plasma enzymes: "AST" (51.63 ±1.884 U/L), "ALT" (56.46 ±3.154 U/L), and "ALP" (55.73 ±1.976 IU/L) compared to the control group. These elevated levels, indicated by the superscript 'a', suggest leakage of these enzymes from the hepatocytes into the bloodstream due to arsenic-induced membrane damage and cellular necrosis. Conversely, the Melatonin "Me" treated group exhibited the lowest enzymatic activities. Most notably, the concurrent administration of melatonin with sodium arsenite "Me+Sa" significantly mitigated these toxic effects, restoring "AST", "ALT", and "ALP" levels to values statistically comparable to the control group (superscript 'b'). This demonstrates the potent hepatoprotective role of melatonin in stabilizing hepatic cell membranes against arsenic toxicity. Regarding synthetic liver function, the Sa group showed a significant decrease in Total Protein (6.52 ±0.190 g/dl) and a decline in Albumin (3.66 ±0.207 mg/dl). This reduction reflects a decline in the liver's biosynthetic capacity or increased protein catabolism under oxidative stress caused by arsenic. The Melatonin group "Me" showed a significant increase in both Total Protein (8.34 ±0.061g/dl) and Albumin (5.03 ±0.148g/dl), reaching the highest values among all groups (superscript 'a'). In the Me+Sa group, melatonin successfully countered the arsenic-induced hypoproteinemia, maintaining protein levels within the normal physiological range. Plasma Bilirubin levels remained relatively stable in the Sa group (1.59 ±0.054 mg/dl) compared to the control, although a slight increase was observed. However, the Melatonin (Me) group showed a significant reduction in bilirubin (1.24 ±0.040 mg/dl), suggesting enhanced biliary clearance or improved antioxidant status in the liver.

Table 1: Changes in liver function markers indicators in rabbits treated with Sodium Arsenite "Sa" and Melatonin "Me".

"Enzyme "	"Experimental groups"			
	"Control "	"Me"	"Sa"	"Me+Sa"
"AST" (U/L)	42.22 ± 0.768^{b}	31.49 ± 2.140^{c}	51.63 ± 1.884^{a}	42.73 ± 0.991^{b}
"ALT" (U/L)	43.30 ± 1.109^{b}	32.08 ± 1.611^{c}	56.46 ± 3.154^{a}	44.54 ± 1.51^{b}
"ALP"(IU/L)	44.41 ± 2.415^{b}	35.94 ± 1.840^{c}	55.73 ± 1.976^{a}	46.79 ± 1.607^{b}
"Bilirubin" (mg/dl)	1.45 ± 0.027^{a}	1.24 ± 0.040^{b}	1.59 ± 0.054^{a}	1.44 ± 0.052^{a}
"Total protein" (g/dl)	7.12 ± 0.160^{bc}	8.34 ± 0.061^{a}	6.52 ± 0.190^{c}	7.25 ± 0.306^{b}
"Albumin" (mg/dl)	3.96 ± 0.068^{b}	5.03 ± 0.148^{a}	3.66 ± 0.207^{b}	3.86 ± 0.123^{b}

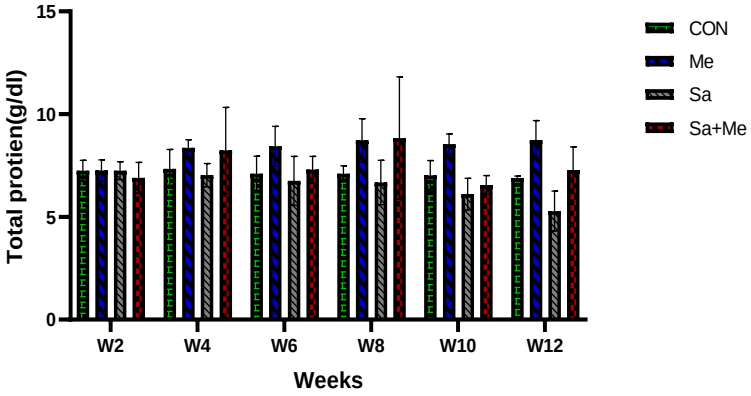

Figure 1: Effect of experimental treatments on plasma albumin levels.

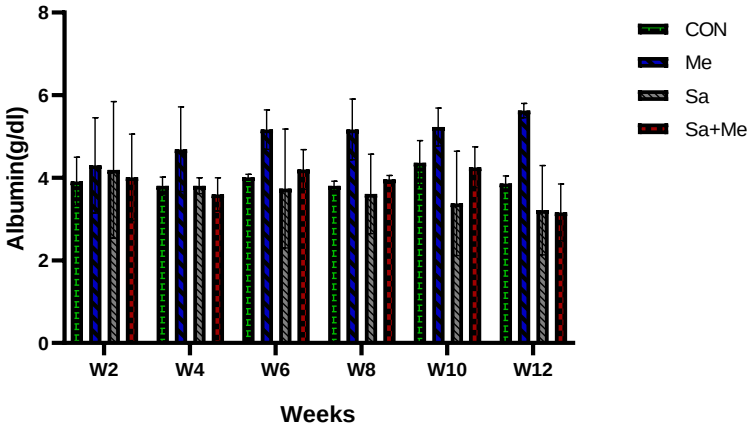

Figure 2: Variations in plasma total Protein concentrations in male rabbits treated with Melatonin and Sodium Arsenite.

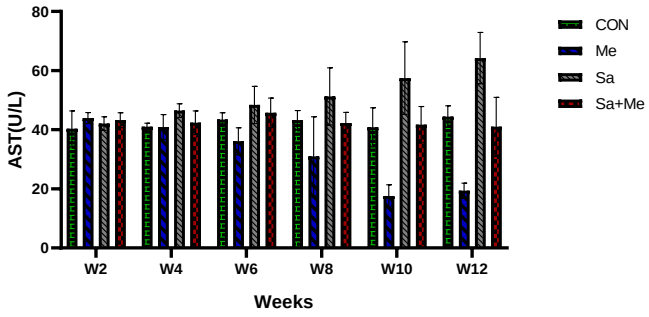

Figure 3: Periodic changes in plasma asparatate transaminase "AST" levels among experimental groups .

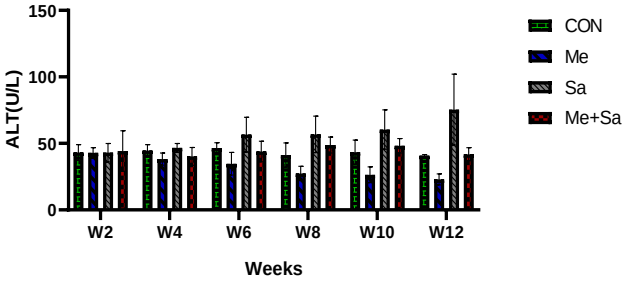

Figure 4: Effect of Melatonin and Sodium Arsenite on plasma alanine transaminase "ALT" levels in male rabbits .

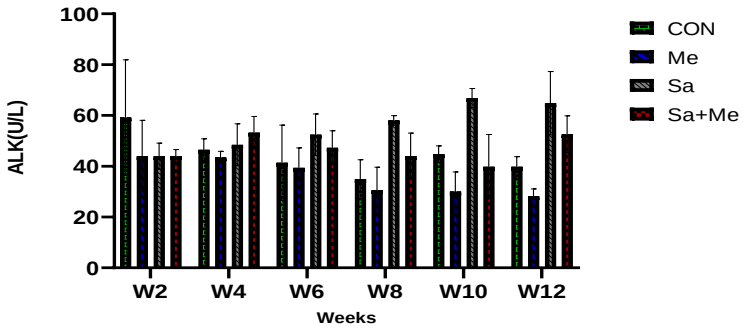

Figure 5: Impact of Melatonin and Sodium Arsenite treatments on plasma alkaline Phosphatase "ALP" activities.

The biochemical profile reveals that Sodium Arsenite "Sa" significantly increased the activities of plasma "AST", "ALT", and "ALP". These elevations are hallmark indicators of hepatocyte membrane damage. This enzymatic leakage is further explained by the histopathological findings in the Sa group, which showed extensive replacement of normal liver architecture with fatty tissue (steatosis) and the formation of thick fibrous capsules surrounding areas of degradation. The presence of these empty spaces in the tissue slides confirms the cellular necrosis that led to the surge in liver enzymes seen in the data. In contrast, the Melatonin + Sodium Arsenite (Me+Sa) group demonstrated a remarkable preservation of liver integrity. Biochemically, melatonin reduced enzyme activities to levels statistically similar to the control group. This is visually supported by the histopathological sections of the Arsenic/Melatonin group, which showed normal liver tissue architecture despite arsenic exposure. This correlation suggests that melatonin's antioxidant capacity effectively neutralizes arsenic-induced reactive oxygen species, thereby preventing lipid peroxidation and subsequent tissue fibrosis. The significant decrease in Total Protein and Albumin in the Sa-treated rabbits indicates a failure in the liver's biosynthetic capacity, likely due to the massive loss of functional hepatocytes replaced by fat and fibrous tissue. The recovery of these protein levels in the Me+Sa group aligns with the restoration of normal tissue structure, proving that melatonin not only prevents damage but also maintains the vital metabolic functions of the liver.

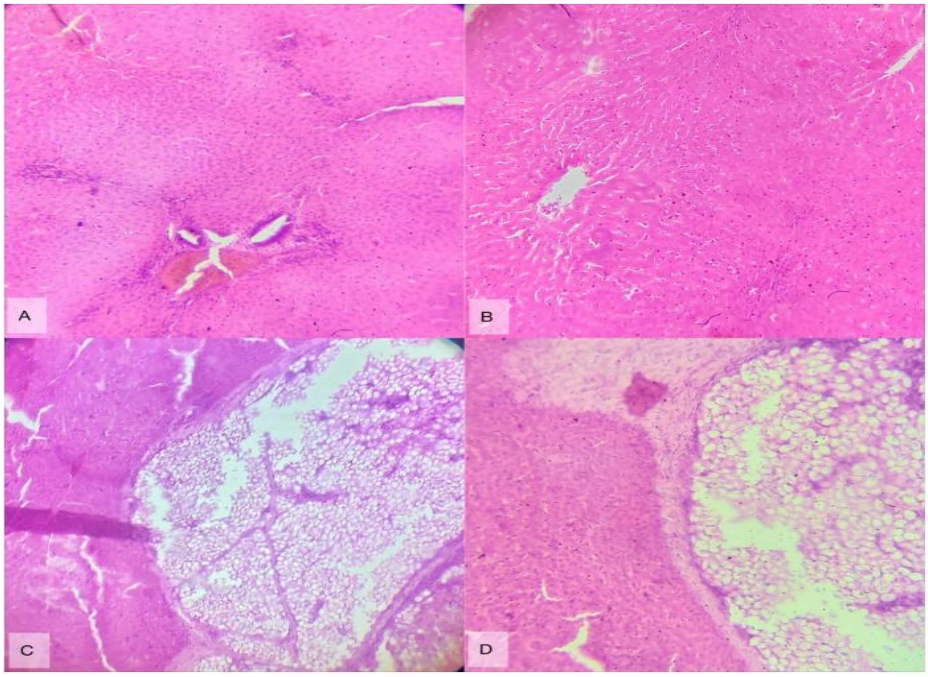

Figure 1:" Haematoxyline and Eosin stained liver tissue. A. A slide taken from Melatonin group showing normal liver tissue (magnification x20). B. A slide taken from Arsenic/ Melatonin group showing normal liver tissue (magnification x20). C. A slide taken from Arsenic group showing large areas of change of the normal liver tissue to a fat tissue. These areas are surrounded by thick fibrous capsules and containing areas of degradation leaving empty spaces(magnification x20). D. A larger magnification for the same C slide (magnification x40)".

Discussion

The present study demonstrates the potent toxicological impact of Sodium Arsenite (Sa) on the physiological and structural integrity of male rabbits, and highlights the significant protective role of Melatonin (Me). Exposure to Sodium Arsenite triggered substantial hepatic damage, characterized by a significant elevation in plasma AST, ALT, and ALP activities [21-30]. These enzymes are reliable biomarkers of hepatocyte membrane permeability; their leakage

into the systemic circulation suggests cellular necrosis and membrane lipid peroxidation [31-40]. These biochemical disturbances were visually confirmed by histopathological findings in the Sa group, which showed the replacement of normal hepatic architecture with fatty tissue and fibrous capsules. The decline in Total Protein and Albumin levels in the Sa group further reflects impaired hepatic synthetic function, likely due to the massive loss of functional hepatocytes [41-44]. However, co-treatment with Melatonin effectively mitigated these effects. Melatonin's ability to stabilize hepatic membranes and maintain protein levels within normal ranges is attributed to its powerful antioxidant properties, which neutralize arsenic-induced free radicals, thereby preserving the hepatic tissue as seen in the normal histology of the Me+Sa group[45-48].

In conclusion: Sodium arsenite induced severe hepatotoxicity, evidenced by elevated liver enzymes and significant histological damage, including fatty changes and fibrous encapsulation. Melatonin supplementation effectively attenuated these toxic effects, restoring biochemical parameters to near-control levels and preserving the liver's structural integrity. These results highlight melatonin's potent antioxidant role as a protective agent against arsenic-induced liver damage in rabbits.

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