Indian J. Vet. Pathol., 50(1) : 48-56, 2026: DOI: https://doi.org/10.56093/pykd1c30

RESEARCH ARTICLE

Hepatoprotective effect of microalgal *Spirulina* extract fortified with finger millet and *Moringa* leaves on arsenic induced hepatotoxicity in Wistar rats

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Received: 31.10.25, Accepted: 18.11.25

ABSTRACT

The present study was conducted to assess the hepatoprotective effects of microalgal *Spirulina* extract fortified with finger millet and *Moringa* leaves (MSFM) in male Wistar rat model of sodium arsenite induced hepatotoxicity. The study consisted of six groups (Group I to VI), comprising of six rats in each group. Group I (normal control) rats received normal saline orally daily for 60 days. All the rats in Group II (disease control) to VI rats received sodium arsenite @ 10mg/kg body weight orally daily for 60 days. In addition to sodium arsenite, Group III (reference control) rats received Telmisartan @ 10mg/kg body weight orally for 60 days; while Group IV, V and VI rats received MSFM@100mg/kg, @200mg/kg and @400mg/kg body weight orally daily for 60 days, respectively. Sodium arsenite exposure in Group II rats resulted in significant alterations in body weight, hematological, biochemical, antioxidant, lipid peroxidation and pathomorphological parameters of liver compared to the normal control rat. Telmisartan administration partially alleviated these changes. MSFM supplementation demonstrated a dose-dependent protective effect, evident through marked improvements in hematological and serological parameters, antioxidant status, reduction of lipid peroxidation and histopathological restoration when compared to Group II rats. Among the treatment groups, MSFM at 400 mg/kg bodyweight offered the highest degree of protection, surpassing Telmisartan, while 200 mg/kg provided moderate protection. These findings highlight the potential of *Spirulina*, *Moringa* and finger millet based MSFM formulation as a natural therapeutic strategy for mitigating sodium arsenite induced hepatotoxicity.

Key words: Arsenite, Finger millet, hepatotoxicity, microalgal *Spirulina* extract, *Moringa*

INTRODUCTION

Water is vital for all living organisms, yet its quality and availability are increasingly threatened by growing populations and rising demand for clean water in domestic and economic activities. However, river systems are often heavily polluted with heavy metals from domestic, industrial, mining, and agricultural effluents[1].

Heavy metals are major environmental pollutants due to their high toxicity, persistence, and bio-accumulative nature. Although some occur naturally, human activities have significantly increased their environmental presence. Interaction with air, water, and soil enhances their toxicity, leading to entry into the food chain and subsequent exposure in humans and animals[2]. Chronic exposure disrupts essential biochemical processes and damages vital organs such as the liver, heart, brain, and kidneys. Their non-biodegradable nature further contributes to long-term health and environmental risks[3].

Amongst the heavy metal toxicity, arsenic toxicity especially has been a significant concern in areas where the ground water level of arsenic is much high[4]. Long-term consumption of arsenic contaminated water can lead to arsenicosis, a form of chronic arsenic poisoning affecting both humans and animals. To safeguard public health, the World Health Organization has set the maximum allowable limit for arsenic in drinking water at 0.01 mg/L. Water with arsenic concentrations above this level is deemed unsafe[5].

Recent findings support that oxidative stress, through elevated reactive oxygen species and increases lipid peroxidation, disrupts normal tissue respiration, and stimulates the release of

How to cite this article: Golla H., Shilpa V.T., Girish B.C., Anjan Kumar K.R., Ramesh D., Rajashailesha N.M. and Pramod S.N. Hepatoprotective effect of microalgal *Spirulina* extract fortified with finger millet and *Moringa* leaves on arsenic induced hepatotoxicity in Wistar rats. Indian J. Vet. Pathol., 50(1) : 48-56.

pro-inflammatory cytokines, playing a key role in the development of arsenic-induced organ toxicity[6].

Spirulina is a microscopic and filamentous cyanobacterium that belongs to family *Oscillatoriaceae* and has a long history of use as food and food supplement. Multiple studies have demonstrated that *Spirulina* possesses a wide range of health-promoting properties, including anti-inflammatory, anticancer, antibacterial, antioxidant, anti-anemic potential and immune-modulatory effects[7].

Moringa's antioxidant and anti-inflammatory effects are attributed to its rich bioactive composition, including phenolic acids, flavonoids, alkaloids, phytosterols, vitamins, and minerals, supporting its traditional use against heavy metal toxicity[8]. Similarly, finger millet exhibits strong antioxidant potential due to its high levels of enzymatic (catalase, superoxide dismutase, glutathione peroxidase) and non-enzymatic (glutathione, vitamins E and C) antioxidants[9].

Spirulina, *Moringa* leaves, and finger millet are recognized for their exceptional phytonutrient, vitamin, and mineral composition, offering high-energy nutrition with a low glycemic index. These super foods possess strong antioxidant, anti-inflammatory, and immune-enhancing properties that contribute to overall health improvement**.** Fortification of microalgal *Spirulina* extract with *Moringa* leaves and finger millet is expected to enhance its therapeutic potential by developing a synergistic, nutrient-dense formulation with improved functional and protective properties. Hence, the present study was designed to assess the hepato-protective effects of *Spirulina* extract fortified with finger millet and *Moringa* leaves against arsenic-induced toxicity in Wistar rats.

MATERIALS AND METHODS

Animals: The research was carried out on adult healthy male Wistar albino rats. Adult male Wistar rats, each weighing between 150 to 200 grams, were obtained from the Chromed Biosciences Private limited Labs Plot No. C- 38, KIADB, Industrial area, Hirehalli, Mydala, Tumkur, Karnataka-572168 (Reg. No. 2171/PO/RcBiBt/S/22/CCSEA). All the rats were acclimatized to standard laboratory conditions at Small Animal House facility, Hassan for seven days prior to initiation of the experiment and maintained at 25±2°C housing temperature and relative humidity of 50 to 70 per cent and to laboratory conditions of 12-hour light/dark cycle throughout the study period and provided with regular standard pellet diet along with free access to deionized drinking water *ad libitum* throughout the course of the experiment. All the protocols were adhered as per the guidelines of the Committee for Control and Supervision of Experiments on Animals for care and use of laboratory animals and were approved by the Institutional Animal Ethics Committee at the Veterinary College, Hassan (HVC/IAEC/11/2025).

Preparation of test item and mode of administration: Sodium arsenite was obtained from Jai Maruthi Scientific Bangalore, manufactured by NICE Chemicals Private Limited, Kochi. It was administered orally at a dose of 10 mg/kg body weight, in normal saline, daily for 60 days. MSFM in powder form was received from Department of Studies in Food Technology, Davangere University, and Karnataka. It was used at the dose rates of 100, 200, and 400 mg/kg body weight. Telmisartan procured from Intas Pharmaceutical Limited, Ahmedabad, India was administered orally daily at a dose of 10 mg/kg body weight.

Experimental protocol: The study consisted of six groups (Group I to VI), comprising of six rats in each group. Group I (normal control) rats received normal saline orally daily for 60 days. All the rats in Group II (disease control) to VI rats received sodium arsenite@10mg/kg body weight orally daily for 60 days. In addition to sodium arsenite, Group III (reference control) rats received Telmisartan@10mg/kg body weight orally for 60 days; while Groups IV, V and VI rats received MSFM@100mg/kg, @200mg/kg and @400mg/kg body weight orally daily for 60 days, respectively.

Parameters assessed: Body weight (g) were assessed every week. All animals were examined twice daily for any signs of clinical abnormalities or symptoms. At the end of the study, all rats were ethically euthanized by administering an intramuscular overdose of Ketamine and Xylazine. Blood samples were drawn from the retro-orbital plexus and collected into EDTA vials for hematological analysis and serum vials for biochemical evaluation.

Estimation of antioxidant enzymes: The representative tissue samples from liver were dissected and washed with Normal saline to remove any tissue debris and blood clots. The collected liver samples were homogenized in a solution of ice-cold 0.1M Phosphate buffered saline (pH 7.4) at 4ºC and centrifuged for 10 minutes at 15,000 rpm. The supernatants were stored at -80ºC for analysis of superoxide dismutase, catalase, and thiobarbituric acid reactive substances.

Histopathological analysis: Liver tissue from rats of all the groups was subjected to histopathological studies. The tissue was fixed using 10 per cent Neutral Buffered Formalin solution and sections were prepared using paraffin blocks and stained with hematoxylin and eosin stain and Masson's Trichrome stain[10].

Histopathological scoring for liver: The liver samples were examined in random microscopic areas semi-quantitatively under high power fields and the number of changes were assessed by the counting twenty non overlapped fields for the same slide of each animal. The extent of damage and the severity of lesions in the liver were assessed semi-quantitatively[11] with slight modification (Table 1).

Statistical analysis: Statistical analysis of the data collected for various parameters was done using one-way ANOVA with Post-hoc test (Tukey's test) [12] using the software SPSS, version-16.0.

Table 1. Histopathological scoring system for liver

Parameters	Score 0	Score 1	Score 2	Score 3	Score 4
Vacuolar degeneration	Absent	Minimal (1-10 %)	Mild (11-30 %)	Moderate (31-60 %)	Severe (61-100 %)
Sinusoidal dilatation	Absent	Minimal (1-10 %)	Mild (11-30 %)	Moderate (31-60 %)	Severe (61-100 %)
Bile duct epithelium proliferation	Absent	Minimal (1-4 bile duct showing epithelial hyperplasia)	Mild (4-8 bile duct showing epithelial hyperplasia)	(8-12 bile duct showing epithelial hyperplasia)	(>12 bile duct showing epithelial hyperplasia)
New bile duct formation	Absent	Minimal (1-5 number)	Mild (5-10 number)	Moderate (10-15 number)	Severe (>15 number)
Inflammatory cell infiltration	Absent	Minimal (1-10 %)	Mild (11-30 %)	Moderate (31-60 %)	Severe (61-100 %)
Central and portal vein dilatation and congestion	Absent	Minimal (1-5 number)	Mild (5-10 number)	Moderate (10-15 number)	Severe (>15 number)
Thickening of blood vessels	Absent	Minimal (0-1 number)	Mild (2-4 number)	Moderate (4-6 number)	Severe (>8 number)

RESULTS

General observation: Group I rats remained healthy and active throughout the experiment. Group II rats exhibited clinical signs such as reduced feed intake, reduced body weight, ruffled hairs, dehydration, restlessness and were difficult to handle. The rats of Group III to VI manifested similar clinical signs as that of disease control rats, but with reduced intensity and frequency.

Body weight: The mean body weights in grams with standard error of mean at different time intervals of 0 day and 1st, 2nd, 3rd, 4th, 5th, 6th, 7th and 8th week of the experiment have been presented in Table 2. Group I rats remained healthy and active throughout the period of experiment and demonstrated steady and progressive enhancement in their body weight over the course of experiment. Body weight of Group II rats were decreased significantly from 3rd week to 8th week in comparison to Group I rats. Group III rats showed a significant and consistent increase in body weight from the 4th to 8th week compared to Group II. MSFM-treated rats (Groups IV to VI) exhibited a consistent and significant ($p<0.05$) increase in body weight throughout the study compared to Group II. Also, Group VI showed improvement comparable to Group I normal control rats throughout the experiment period.

Hematological parameters: The mean values of hematological parameters with standard error of mean on 60th day of the experiment is presented in Table 3. The

Table 2. The mean (± SE) body weight (g) values of rats of different experimental groups in the study at weekly interval

Groups	Day 0	1st Week	2nd Week	3rd Week	4th Week	5th Week	6th Week	7th Week	8th Week
Group I (NC)	170.83 ±2.83	181.67 ± 2.23^{a}	200.17 ± 3.08	223.17 ± 2.09	243.67 ± 3.13	268.50 ±3.86	276.33 ± 3.79	282.83 ± 4.04	294.50 ± 3.35
Group II (DC)	172.67 ± 3.90	179.17 ±3.72	191.33 ± 2.51^{a}	202.00 ± 2.91^{b}	212.00 ± 3.30	221.00 ± 3.13	229.00 ± 4.62	240.83 ± 4.44	250.33 ± 2.99
Group III (RC)	171.17 ± 4.61	180.50 ±4.57	193.17 ± 3.84^{a}	212.33 ± 3.26^{abc}	233.33 ± 1.78	255.50 ± 1.45	264.83 ± 1.92^{a}	273.83 ± 1.08^{a}	277.67 ± 1.15
Group IV (MSFM@100)	169.67 ± 2.38	180.17 ±1.82	191.17 ± 0.95^{a}	207.00 ± 1.95^{bc}	232.00 ± 1.64	254.00 ± 1.37	265.67 ± 1.38^{a}	275.17 ± 1.51^{a}	278.00 ± 1.72
Group V (MSFM@200)	169.50 ± 4.21	181.50 ±2.16	195.33 ± 2.29^{a}	212.33 ± 3.42^{abc}	240.00 ± 0.89^{ac}	260.50 ± 1.65^{ac}	269.67 ± 1.67^{a}	280.33 ± 1.93^{a}	282.50 ± 2.23
Group VI (MSFM@400)	170.67 ± 4.52	182.00 ±2.14	198.50 ± 2.04^{a}	216.50 ± 4.46^{ab}	246.50 ± 1.12^{a}	263.83 ± 3.61^{ac}	275.33 ± 3.61^{a}	278.50 ± 4.36^{a}	286.50 ± 4.18^{ac}

One-way ANOVA with Tukey's post hoc test (SPSS)
Mean ± SE values within a column with different superscripts differ significantly at $p < 0.05$ (n=6)

Table 3. The mean (±SE) values of various haematological parameters of rats in different groups on final day (60th day) of the study

GROUPS	TEC (106/µL)	TLC (103/µL)	Hb (g/dL)	PCV (%)	MCV (fL)	MCH (pg)	MCHC (%)
Group I (NC)	7.33±0.16^{a}	8.35±0.08^{a}	14.63±0.41^{a}	41.88±0.71^{aa}	51.51±1.36^{a}	17.01±0.53^{c}	32.76±0.83^{a}
Group II (DC)	5.18±0.21^{b}	5.30±0.38^{b}	10.36±0.45^{d}	30.38±0.74^{c}	63.75±1.64^{b}	25.13±1.44^{a}	26.20±0.88^{b}
Group III (RC)	6.98±0.13^{ac}	7.01±0.32^{c}	12.64±0.47^{bc}	36.71±0.47^{ab}	53.25±1.58^{a}	21.63±1.08^{ab}	31.04±0.72^{a}
Group IV (MSFM@100)	6.57±0.22^{c}	7.40±0.18^{ac}	11.52±0.32^{c}	36.05±1.02^{b}	53.82±1.18^{a}	22.48±1.03^{ab}	30.16±0.52^{a}
Group V (MSFM@200)	7.01±0.08^{ac}	7.64±0.22^{ac}	12.99±0.45^{abc}	39.38±1.80^{ab}	52.75 ±1.08^{a}	20.92 ± 1.14^{abc}	31.26± 0.85^{a}
Group VI (MSFM@400)	7.24±0.13^{ac}	7.85±0.31^{ac}	14.09±0.30^{ab}	40.66±1.33^{ab}	51.76 ± 0.71^{a}	18.67±0.69^{bc}	33.06 ± 0.66^{a}

One-way ANOVA with Tukey's post hoc test (SPSS)
Mean ± SE values within a column with different superscripts differ significantly at p < 0.05 (n=6)

mean values of all the hematological parameters such as Total erythrocyte count (TEC), Haemoglobin (Hb), Packed Cell Volume (PCV), Total leucocyte count (TLC) and Mean Corpuscular Haemoglobin concentration (MCHC) of Group II rats were significantly (p<0.05) decreased when compared to Group I rats, whereas Mean corpuscular volume (MCV) and Mean Corpuscular Haemoglobin (MCH) were significantly (p<0.05) elevated in Group II rats compared to Group I. Group III rats exhibited significantly(p<0.05) higher mean values of TEC, Hb, PCV, TLC, and MCHC, along with a significantly (p<0.05) lower MCV compared to Group II, while the mean MCH value remained comparable. The MSFM-treated groups (Group IV to VI) showed significant (p<0.05) improvement in TEC, TLC, and PCV values relative to Group II. Hb and MCHC levels were also significantly elevated in Groups IV to VI and were comparable to those of the Group I. The mean MCH value showed a significant (p<0.05) reduction in Group VI and was relatively lower in Groups IV and V compared to Group II, while MCV values were significantly (p<0.05) decreased across all MSFM-treated groups.

Table 4. The mean (± SE) values of various serum biochemical parameters of rats different groups on the final day (60th day) of the study

GROUPS	ALT (IU/L)	ALP (IU/L)
Group I (NC)	41.88 ± 0.42^{d}	107.54 ± 1.08^{b}
Group II (DC)	71.22 ± 1.16^{a}	131.04 ± 2.49^{a}
Group III (RC)	48.56 ± 1.51^{c}	109.93 ± 1.86^{b}
Group IV (MSFM@100)	57.65 ± 1.83^{b}	124.85 ± 1.33^{a}
Group V (MSFM@200)	49.04 ± 1.59^{c}	111.52 ± 1.20^{b}
Group VI (MSFM@400)	46.24 ± 1.53^{cd}	108.57 ± 1.29^{b}

One-way ANOVA with Tukey's post hoc test (SPSS)
Mean ± SE values within a column with different superscripts differ significantly at p < 0.05 (n=6)

Serum Biochemistry: The mean values with standard error of mean on 60th day of the experiment is presented in Table 4.

Alanine transaminase (ALT): There was a significant (p<0.05) increase in serum ALT levels of Group II rats by 70.05% than Group I rats. The mean values of serum ALT levels of Group III to VI rats were significantly decreased (p<0.05) than Group II rats.

Table 5. The mean (± SE) values of TBARS of rats in different groups on the final day (60th day) of the study.

Groups	TBARS (nmol MDA/g of Liver tissue)
Group I	7.17±0.08^{b}
Group II	16.76±0.64^{a}
Group III	11.39±0.37^{c}
Group IV (MSFM@100)	12.32±0.29^{c}
Group V (MSFM@200)	10.72±0.35^{c}
Group VI (MSFM@400)	7.50±0.26^{b}

One-way ANOVA with Tukey's post hoc test (SPSS)
Mean ± SE values within a column with different superscripts differ significantly at p < 0.05 (n=6)

Table 6. The mean (± SE) values of Catalase and Superoxide dismutase enzyme activity of rats in different groups on the final day (60th day) of the study.

Groups	Superoxide dismutase (U/mg of protein)	Catalase (µM of H_2O_2 decomposed /min)
Group I (NC)	8.50±0.32^{a}	47.67±0.35^{a}
Group II (DC)	5.55±0.21^{b}	33.75±0.45^{d}
Group III (RC)	6.89±0.37^{ab}	39.12±0.35^{c}
Group IV(MSFM@100)	7.64±0.29^{ab}	39.62±0.37^{c}
Group V(MSFM@200)	8.21±0.30^{a}	43.70±0.55^{b}
Group VI(MSFM@400)	8.37±0.18^{a}	46.70±0.97^{a}

One-way ANOVA with Tukey's post hoc test (SPSS)
Mean ± SE values within a column with different superscripts differ significantly at p < 0.05 (n=6)

Table 7. The mean (±SE) HP score of liver of rats in different groups on final day (60th day) of the study

Groups	Cellular swelling to vacuolar degeneration	Sinusoidal dilatation	Bile duct epithelium proliferation	New bile duct formation	Inflammatory cell infiltration	Central and portal vein dilatation and congestion	Thickening of blood vessels
Group I	0.37±0.08^{a}	0.27±0.02^{a}	0	0.10±0.05^{a}	0.23±0.02^{a}	0.07±0.02^{a}	0
Group II (DC)	3.55±0.02^{b}	3.23±0.15^{b}	2.52±0.17^{b}	3.42±0.20^{b}	2.90±0.09^{b}	3.05±0.05^{b}	2.74±0.04^{b}
Group III (RC)	2.97±0.07^{c}	2.83±0.14^{bc}	2.15±0.05^{c}	3.02±0.07^{bc}	2.57±0.75^{bc}	2.62±0.04^{c}	2.00±0.09^{c}
Group IV (MSFM@100)	2.92±0.11^{c}	2.85±0.20^{bc}	1.10±0.04^{d}	2.70±0.19^{c}	2.62±0.10^{bc}	2.57±0.07^{c}	1.92±0.62^{c}
Group V (MSFM@200)	2.63±0.02^{cd}	2.41±0.22^{c}	1.10±0.04^{d}	2.75±0.02^{c}	2.52±0.11^{bc}	2.02±0.04^{d}	1.22±0.04^{d}
Group VI (MSFM@400)	2.38±0.07^{d}	2.16±0.21^{c}	1.02±0.02^{d}	2.57±0.06^{c}	2.27±0.08^{c}	1.62±0.06^{e}	1.05±0.02^{d}

One-way ANOVA with Tukey's post hoc test (SPSS)
Mean ± SE values within a column with different superscripts differ significantly at $p < 0.05$ (n=6)

Alkaline phosphatase (ALP): There was a significant (p<0.05) increase in serum ALP levels of Group II rats by 21.84% than Group I rats. The mean values of serum ALP levels of Group III to VI rats were significantly decreased (p<0.05) than Group II rats.

Lipid peroxidation assay as an oxidative stress marker: The mean values of liver tissue Thiobarbituric acid reactive substances (TBARS) in nmol MDA/g of tissue levels with standard error of mean on 60th day of the experiment have been presented in Table 5. There was a significant (p<0.05) increase in TBARS levels of Group II rats in comparison to Group I rats. Among the treatment groups (III to V), there was no significant difference in mean values. The decrease in the values in Group III to VI were statistically significant (p<0.05) in comparison to Group II rats and values of Group VI rats were comparable to that of Group I rats.

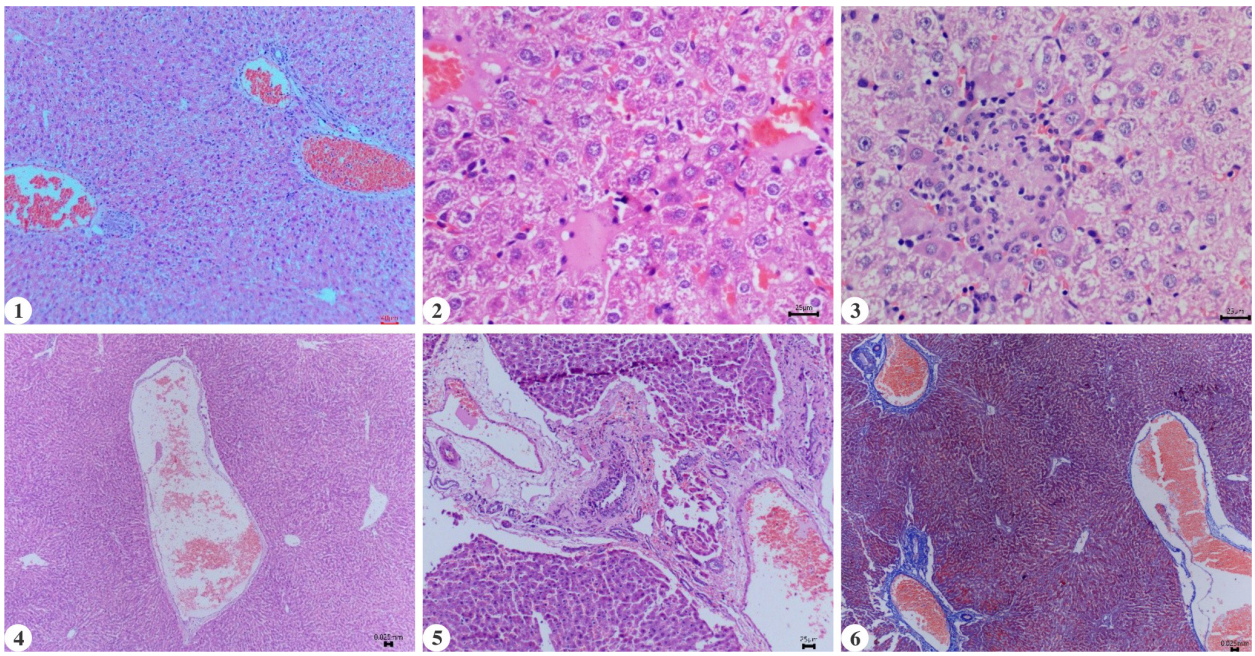

Fig. 1. Section of liver from Group II disease control rat showing vacuolar degeneration in hepatocytes along with dilated and congested portal veins H&E X 100; **Fig. 2.** Section of liver from disease control (Group II) rat showing degenerated hepatocytes with oedema and haemorrhage H&E X 400; **Fig. 3.** Section of liver from disease control (Group II) rat showing a necrotic foci with inflammatory cell infiltration H&E X 400; **Fig. 4.** Section of liver from disease control (Group II) rat showing severe dilated central vein with thickening of wall H&E X 40; **Fig. 5.** Section of liver from disease control (Group II) rat showing dilated portal vein, proliferation of bile duct and peri-portal fibrosis H&E X 100; **Fig 6.** Section of liver from disease control (Group II) rat showing dilated central and portal vein and peri-portal fibrosis MT X 40

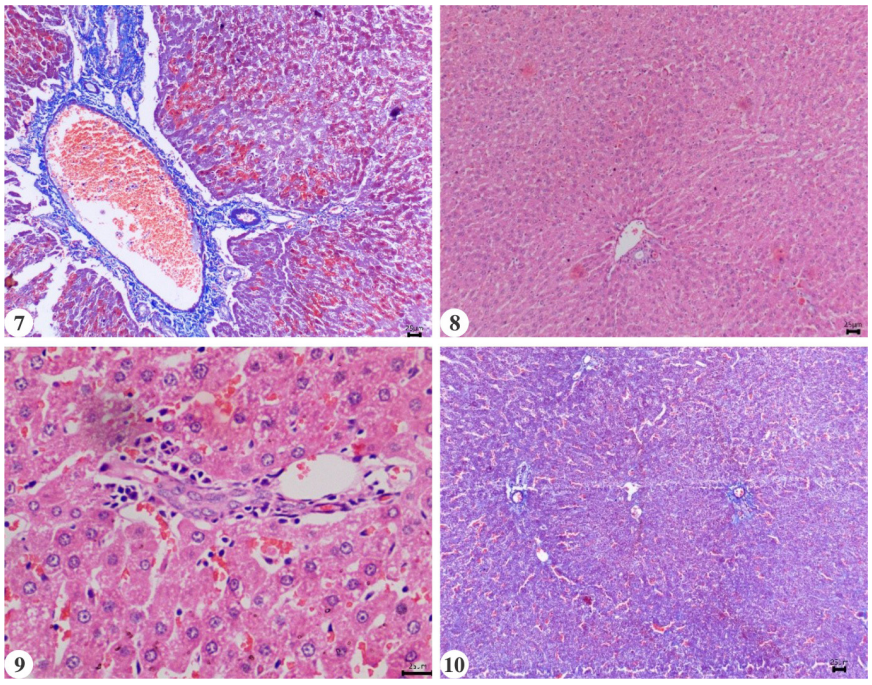

Fig. 7. Section of liver from disease control (Group II) rat showing dilated portal vein, proliferation of bile duct and peri-portal fibrosis. MT X 100; **Fig. 8**. Section of liver from Group VI (MSFM@400) rat showing improvement in hepatocytes and normal portal triad compared to Group II rats. H&E X 100; **Fig. 9.** Section of liver from Group VI (MSFM@400) rat showing improvement in hepatic architecture with minimal peri-portal inflammation compared to Group II rats. H &E X 400; **Fig. 10.** Section of liver from Group VI (MSFM@400) rat showing normal architecture of portal triad. MT X 100

Antioxidant enzymes: The mean values of liver tissues with standard error of mean on 60th day of the experiment have been presented in Table 6.

Catalase (CAT): There was a significant (p<0.05) decrease in catalase enzyme activity of Group II rats in comparison to Group I rats. All the treatment groups were significantly (p<0.05) higher than Group II rats. Group VI showed a significant (p<0.05) increase in CAT activity compared to all other treatment groups (III to V) and were comparable to that of Group I rats.

Superoxide dismutase (SOD): There was a significant (p<0.05) decrease in superoxide dismutase enzyme activity of Group II rats in comparison to Group I rats. Among the MSFM treated groups, there were no significant difference in mean values of SOD activity and values of Group V and VI were comparable to that of Group I rats.

Histopathology: The liver of the disease control group (Group II) rats showed severe loss of normal architecture of hepatocytes with degenerative changes ranging from cellular swelling to vacuolar degeneration throughout the hepatic parenchyma. Individual hepatic cell necrosis with cytoplasmic eosinophilia and pyknotic nuclei was noted in multiple areas with infiltration of inflammatory cells. Occasional fatty changes along with apoptotic cells were evident. A moderate to severe degree of congestion of central and portal vein were observed. The central vein and portal vein diameter was increased compared to normal control rats. The bile duct epithelium showed severe proliferation with new bile duct formation in few triads. The portal triad showed mild inflammation with fibroplasia (Fig 1 to 7).

The liver of treatment group rats (IV to VI) showed mild to moderate

Table 8. The mean (±SE) HP score of liver of rats in different groups on final day (60th day) of the study

Groups	Cellular swelling to vacuolar degeneration	Sinusoidal dilatation	Bile duct epithelium proliferation	New bile duct formation	Inflammatory cell infiltration	Central and portal vein dilatation and congestion	Thickening of blood vessels
Group I	0.37±0.08^{a}	0.27±0.02^{a}	0	0.10±0.05^{a}	0.23±0.02^{a}	0.07±0.02^{a}	0
Group II (DC)	3.55±0.02^{b}	3.23±0.15^{b}	2.52±0.17^{b}	3.42±0.20^{b}	2.90±0.09^{b}	3.05±0.05^{b}	2.74±0.04^{b}
Group III (RC)	2.97±0.07^{c}	2.83±0.14^{bc}	2.15±0.05^{c}	3.02±0.07^{bc}	2.57±0.75^{bc}	2.62±0.04^{c}	2.00±0.09^{c}
Group IV (MSFM@100)	2.92±0.11^{c}	2.85±0.20^{bc}	1.10±0.04^{d}	2.70±0.19^{c}	2.62±0.10^{bc}	2.57±0.07^{c}	1.92±0.62^{c}
Group V (MSFM@200)	2.63±0.02^{cd}	2.41±0.22^{c}	1.10±0.04^{d}	2.75±0.02^{c}	2.52±0.11^{bc}	2.02±0.04^{d}	1.22±0.04^{d}
Group VI (MSFM@400)	2.38±0.07^{d}	2.16±0.21^{c}	1.02±0.02^{d}	2.57±0.06^{c}	2.27±0.08^{c}	1.62±0.06^{e}	1.05±0.02^{d}

One-way ANOVA with Tukey's post hoc test (SPSS)
Mean ± SE values within a column with different superscripts differ significantly at p < 0.05 (n=6)

improvement when compared to Group II rats characterised by decreased degenerative changes in hepatocytes, reduced dilatation and congestion of veins, with mild proliferation of bile duct epithelium (Fig 8 to 10).

Histopathological score of liver

The mean HP severity score values of liver with standard error of mean are presented in Table 7. The mean HP score of Group II disease control rats for each of the recorded lesion were significantly (p <0.05) higher than mean HP score of Group I normal control rats. The mean HP score of Group III to VI were significantly (p <0.05) lower than mean HP score of Group II disease control rats.

DISCUSSION

In the present study, sodium arsenite administration resulted in reduced feed intake, decreased body weight, ruffled hair coat and mild hair loss. These clinical manifestations are consistent with earlier finding who also observed similar signs in arsenic-exposed rats[13]. Our findings of sodium arsenite induced reduction in body weight are in accordance with reports of previous workers[14-15]. The progressive loss of body weight gain in sodium arsenite administration group may be because of gradual onset of anorexia due to toxaemia[16]. Sodium arsenite induced hepatotoxicity related decrease in hematological parameters like TEC, Hb, PCV, TLC, MCHC and elevated MCV, MCH levels were similar to those previously reported[11,17]. The reduction in PCV, Hb, RBC, and MCHC levels observed in arsenic alone exposed groups may be mechanistically linked to impaired heme biosynthesis. Arsenic has been reported to inhibit the activity of aminolevulinic acid dehydratase, a critical enzyme in the porphyrin synthesis pathway, thereby disrupting heme formation and consequently diminishing erythropoiesis and hemoglobin synthesis[18]. Arsenic participates in cellular redox cycling, promoting the formation of ROS, which in turn inflict oxidative stress on erythrocytes. This oxidative damage compromises membrane integrity, leads to the generation of methemoglobin, and triggers erythrocyte lysis and cell death[19]. The significant reduction in TLC levels can be attributed to the direct cytotoxic effects of arsenic bone marrow leading to suppressed hematopoiesis and resulting in erythrocytopenia and leukopenia[20]. An elevation in MCV and MCH observed following arsenic exposure is generally suggestive of a macrocytic response associated with anemia[21]. Reduced MCHC in arsenic toxicity group results from a combination of impaired heme biosynthesis, oxidative stress-induced Hb degradation, and macrocytic anaemia, leading to a decrease in the concentration of Hb per unit volume of erythrocyte[18].

A significant elevation in the mean serum levels of ALT and ALP was recorded in the present study, which is consistent with the earlier observations[22-23]. Arsenic-induced hepatic toxicity is primarily attributed to oxidative stress resulting from excessive production of reactive oxygen species (ROS), which leads to structural and functional impairment of hepatocytes. The consequent damage to hepatocellular membranes causes the leakage of intracellular enzymes such as ALT and ALP into the bloodstream, thereby reflecting hepatic injury[24].

In the present study, a significant elevation in hepatic TBARS levels accompanied by a marked reduction in CAT and SOD enzyme activities was observed, indicating increased lipid peroxidation and oxidative stress in rats. These findings are consistent with earlier reports[15,25]. The pronounced rise in MDA levels in the arsenic-exposed group reflects enhanced lipid peroxidation resulting from excessive generation of reactive oxygen and nitrogen species. Arsenic promotes ROS production by disrupting mitochondrial electron transport, activating NADPH oxidase, promoting oxidative metabolism of its intermediates, and releasing redox-active iron from ferritin. The observed decline in enzymatic antioxidants such as SOD and CAT may be attributed to direct inhibition of their activity or depletion of essential cofactors by arsenic and its metabolites[26].

The examination of liver tissue from rats in the disease control group revealed extensive damage characterized by loss of architecture of hepatocytes with degenerative changes from cellular swelling to vacuolar degeneration throughout the hepatic parenchyma. Individual hepatic cell necrosis with cytoplasmic eosinophila and pyknotic nuclei was noted in multiple areas with infiltration of inflammatory cells. A moderate to severe degree of congestion of central and portal vein were observed. These observations were consistent with previous studies[27-28]. These histopathological alterations primarily result from sodium arsenite induced oxidative stress and inflammatory injury by production of ROS. The resultant oxidative burden leads to structural and functional damage followed by degenerative changes and necrosis of hepatocytes and inflammatory changes[29]. The observed dilatation and congestion of hepatic central and portal veins in disease group may be attributed to arsenic-induced portal hypertension, which are associated with overexpression of endothelins and vascular endothelial growth factor-B[30]. Arsenic-induced hepatic fibroplasia and fibrosis may arise from multiple mechanisms, including alterations in histone acetylation, oxidative stress, apoptosis, DNA methylation, and other epigenetic modifications. Additionally, microRNA-21 mediates abnormal hepatocyte–hepatic stellate cell interactions *via* the hypoxia inducible factor-1α/Vascular endothelial growth factor signalling pathway, further contributing to fibroplasia and fibrosis[31].

Improvement in treatment groups (Group IV to VI) may be attributed to the antioxidant, anti-inflammatory

anti-apoptotic properties of MSFM. *Spirulina* possesses a potent antioxidant profile comprising β-carotene, tocopherol, chlorophyll, phycobiliproteins such as phycocyanin, cryptoxanthin, and allophycocyanin, which collectively contribute to effective free radical scavenging and attenuation of oxidative stress[32]. Among these, phycocyanin a major biliprotein containing the tetrapyrrole pigment phyco-cyanobilin plays a central role in *Spirulina's* antioxidant potential. It not only exhibits strong free radical quenching activity but also demonstrates notable metal-binding and chelating properties, thereby aiding in the detoxification and elimination of heavy metals from the body. C-phycocyanin also inhibits phosphorylation of *p38MAPK*, thereby regulating cytokine synthesis which in turn reduces the inflammation[33].

Finger millet is a good source of phytochemicals mainly phenolic and flavonoids compounds. The polyphenols consist of hydroxybenzoic (protocatechuic, p-hydroxybenzoic) acids, hydroxyl-cinnamic (p-coumaric, ferulic, syringic) acids, flavonoids (quercetin, apigenin, catechin, epicatechin), and pro-anthocyanidins. All these can act as reducing agents (free radical terminators), metal chelators, and singlet oxygen quenchers[34]. The *Moringa* plant provides a rich and rare combination of zeatin, quercetin, β-sitosterol, caffeoylquinic acid and kaempferol which contribute to its antioxidant and anti-inflammatory property[35].

The current research showed that MSFM exhibited significant, dose-dependent protection against sodium arsenite-induced hepatotoxicity, with higher doses (200 and 400 mg/kg body weight) showing enhanced efficacy. The improvement in biochemical, antioxidant, and histopathological parameters highlights its potential as a supportive intervention for arsenic toxicity. However, further mechanistic and clinical studies are needed to confirm its therapeutic applicability.

***Financial support & sponsorship*:** None

***Conflicts of Interest*:** None

***Use of Artificial Intelligence (AI) - Assisted Technology for manuscript preparation*:** The authors confirm that there was no use of AI- assisted technology for assisting in the writing of the manuscript and no images were manipulated using AI.

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