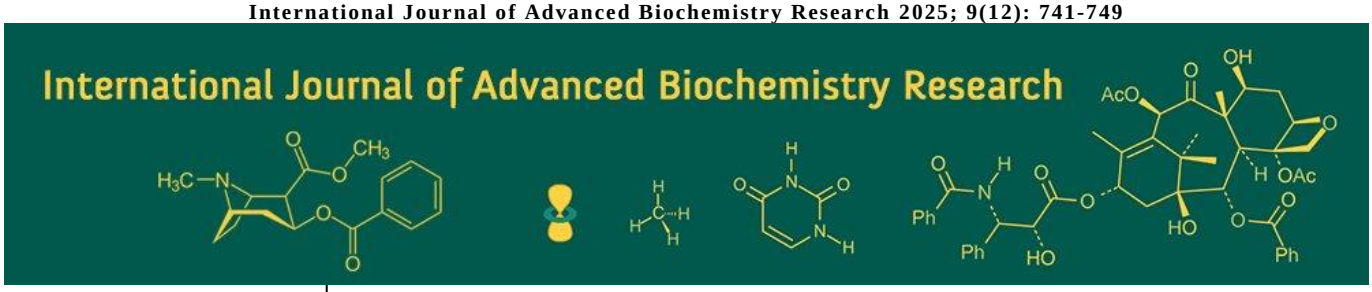

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Evaluation of the nephroprotective and antioxidant efficacy of *spirulina* extract fortified with *moringa* leaves and finger millet in arsenic-treated wistar rats

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Abstract
In the current study, a male Wistar rat model of sodium arsenite-induced nephrotoxicity was used to evaluate the nephro-protective effects of microalgal *Spirulina* extract fortified with finger millet and *Moringa* leaves (MSFM). The study consisted of Group I to VI comprising of six rats in each group. During the study period of 60 days, rats in Group I (normal control) received normal saline orally every day. While all rats in Groups II (disease control) through VI were given sodium arsenite orally at a dose of 10 mg/kg body weight. In addition to sodium arsenite, Group III (reference control) rats were given Telmisartan at 10 mg/kg body weight orally for 60 days and Group IV, V, and VI rats were given MSFM at 100 mg/kg, 200 mg/kg, and 400 mg/kg body weight orally daily for 60 days, respectively. In comparison to the normal control rats, Group II rats exposed to sodium arsenite showed notable changes in body weight, haematological, biochemical, antioxidant, lipid peroxidation, and kidney pathomorphological markers. When compared to Group II rats, MSFM supplementation showed a dose-dependent protective effect, as evidenced by notable improvements in antioxidant status, lipid peroxidation reduction, histopathological restoration, and haematological and serological markers. While 200 mg/kg supplied moderate protection, 400 mg/kg bodyweight of MSFM provided the best level of protection across the therapy groups, outperforming Telmisartan. These results demonstrate the possibility of an MSFM formulation based on finger millet, *Moringa*, and *Spirulina* as a natural therapeutic approach to lessen sodium arsenite-induced nephrotoxicity.

Keywords: Finger millet, Microalgal *Spirulina* extract, *Moringa*, Nephrotoxicity, Sodium arsenite

Introduction
Industrialization has severely polluted water bodies by releasing untreated chemical waste. Major pollutants include heavy metals, pesticides, petroleum products, and polychlorinated biphenyls. Since water is a strong solvent, these toxic substances easily dissolve and contaminate aquatic systems. Among these, heavy metal and metalloid contamination in drinking water is especially concerning. Toxic elements such as arsenic (from natural sources, smelting, and power plants), mercury (from chlor-alkali plants, lamps, and hospital waste), cadmium (from zinc smelting, batteries, e-waste, and paint sludge), and lead (from batteries, paints, smelting, and coal-based power plants) are widely present and alarming. This widespread contamination poses a serious threat to both environmental and public health (Sharma, 2017) [1].

Exposure to heavy metals in humans has been linked to serious and progressively worsening health issues, impacting vital organs such as the liver, heart, brain, and kidneys. These metals disrupt normal biological functions by interfering with essential biochemical processes. Due to their non-biodegradable nature, heavy metals persist in the body and environment, leading to long-term health risks (Hamdy *et al*., 2018) [2].

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 (Das, 2020) [3]. Arsenic can inactivate up to 200 enzymes, particularly those involved in cellular energy production, deoxyribose nucleic acid (DNA) synthesis, and repair. It interferes with adenosine triphosphate (ATP) production by inhibiting mitochondrial respiration and ATP synthesis. Additionally, arsenic contributes to oxidative stress, genotoxic damage, and disruption of

DNA repair processes by production of reactive oxygen species (ROS). It also influences epigenetic changes and activates specific signal transduction pathways, resulting in abnormal gene expression (Ratnaike, 2003) [4].

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, and immune-modulatory effects. Additionally, early research has reported promising results regarding its hypolipidemic, antidiabetic, anti-obesity, and antianemic potential (Kulshreshtha *et al*., 2008) [5].

The leaves of *Moringa oleifera*, rich in polyphenols, flavanols, phenolic acids, kaempferol, quercetin, and essential vitamins and minerals, exhibit antioxidant, free radical-chelating, and anti-inflammatory properties, which help reduce hepatic and renal damage and support the normalization of related biomarkers (Razis *et al*., 2014) [6].

Finger millet possesses strong antioxidant activity due to its rich phenolic and flavonoid content, along with enzymatic and non-enzymatic antioxidants such as catalase and vitamins E and C. Phenolic acids, flavonoids, and tannins in the seed coat act as radical scavengers and metal chelators, while its anti-inflammatory effects mediated through inhibition of enzymes like A5-lipoxygenase and xanthine oxidase contribute to reduced hepatic enzyme and renal biomarkers levels (Kumar *et al*., 2021) [7].

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 nephro-protective effects of *Spirulina* extract fortified with finger millet and *Moringa* leaves against arsenic-induced nephro-toxicity in Wistar rats.

Materials and Methods

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 ℃ 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 in Hassan (HVC/IAEC/11/2025).

Preparation of test items 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:** This 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 kidney were dissected and washed with Normal saline to remove any tissue debris and blood clots. The collected kidney 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:** Kidney 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. Similarly, the standard procedure of Masson's trichome staining was employed to stain required histopathological sections (Suvarna *et al*., 2018) [8].
- **Histopathological scoring for Kidney:** The Kidney 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 kidney were assessed semi-quantitatively (Al Forkan *et al*., 2016) [9] with slight modification as follows;

Histopathological scoring system for kidney					
Parameters	**Score 0**	**Score 1**	**Score 2**	**Score 3**	**Score 4**
Tubular cells vacuolations	Absent	Minimal (1-10%)	Mild (11-30%)	Moderate (31-60%)	Severe (61-100%)
Cortical haemorrhage and congestion	Absent	Minimal (1-10%)	Mild (11-30%)	Moderate (31-60%)	Severe (61-100%)
Medullary haemorrhage and congestion	Absent	Minimal (1-10%)	Mild (11-30%)	Moderate (31-60%)	Severe (61-100%)
Mononuclear cell infiltration	Absent	Minimal (1-10%)	Mild (11-30%)	Moderate (31-60%)	Severe (61-100%)
Eosinophilic debris in the lumen of tubules	Absent	Minimal (1-10%)	Mild (11-30%)	Moderate (31-60%)	Severe (61-100%)
Thickening of blood vessels	Absent	Minimal (0-1 number)	Mild (2-4 number)	Moderate (5-7 number)	Severe (>8 number)

Statistical analysis: Statistical analysis of the data collected for various parameters was done using one-way ANOVA with Post-hoc test (Tukey's test) (Snecdecor and Cochran, 1994) using the software SPSS, version-16.0.

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 1. 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-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.
- **Hematology parameters:** The mean values of hematological parameters with standard error of mean on 60th day of the experiment is presented in Table 2. The 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.
- **Serum Biochemistry:** The mean values with standard error of mean on 60th day of the experiment is presented in Table 3.
- **Serum creatinine:** There was a significant (p<0.05) increase in serum creatinine of Group II rats in comparison to Group I rats. Among the MSFM treated groups, there were no significant difference in mean creatinine values. A significant (p<0.05) reduction in creatinine levels was observed in Groups VI when compared to Group II. However, the reductions observed in Groups III to V were not statistically significant in comparison to Group II rats.
- **Blood Urea Nitrogen (BUN):** There was a significant (p<0.05) increase in BUN of Group II rats in comparison to Group I rats. The mean values of BUN in treatment groups (III, V and VI) showed significant improvement in comparison to Group II rats. While, Group IV rats showed no improvement in comparison to Group II rats. However, Group VI rats showed decrease in the BUN values which was statistically significant in comparison to Group II rats and were comparable to that of Group I rats.
- **Lipid peroxidation assay as an oxidative stress marker:** The mean values of kidney tissue Thiobarbituric acid reactive substances (TBARS) in nmol Malondialdehyde (MDA)/g of tissue levels with standard error of mean on 60th day of the experiment have been presented in Table 4. There was a significant (p<0.05) increase in MDA levels of Group II rats in comparison to Group I rats. Among the treatment groups (III to VI), there were 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.
- **Antioxidant enzymes:** The mean values of Catalase and Superoxide dismutase enzyme activity of rats kidney tissues with standard error of mean on 60th day of the experiment have been presented in Table 5.
- **Catalase (CAT):** There was a significant (p<0.05) decrease in catalase enzyme activity of Group II rats in comparison to Group I rats. The mean values of Group III to VI were significantly (p<0.05) increased in

comparison to Group II rats. Furthermore, Group VI exhibited a significant (p<0.05) increase over Group III to V rats and was 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. The mean values of Group III to VI were significantly (p<0.05) increased in comparison to Group II rats and were comparable to that of Group I rats.

Histopathology

The kidneys of the disease control group (Group II) showed varied histopathological changes characterized by tubular degeneration ranging from moderate to severe degree. Kidney showed mild to moderate degree of congestion and haemorrhage involving both cortex and medulla. The proximal and distal convoluted tubules showed degenerative changes characterised by severe cellular swelling to vacuolation with reduced tubular lumen size in few tubules. Occasionally, few tubules showed eosinophilic debris in their lumen. The glomerular architecture was slightly altered with reduced urinary space. The degenerative changes of tubules were accompanied by loss of cilia and infiltration of inflammatory cells in moderate degree in renal interstitial area consisting mainly of mononuclear cells *viz* lymphocytes and macrophages (Fig. 1 to 6). The blood vessels in the renal parenchyma showed thickening of tunica media (Fig. 7 and 8).

The kidneys of the reference control group rats (Group III) and treatment Groups (IV to VI) showed improved tubular architecture with decreased swelling of renal tubules and reduced vacuolations of renal tubular epithelial cells when compared to Group II rats. The degree of congestion and haemorrhages were mild to minimal when compared to Group II rats (Fig. 9 to 12).

Histopathological score of kidney

The mean HP severity score of all sectioned kidney samples was assessed for lesions like tubular cell swelling to vacuolar degeneration, cortical haemorrhage and congestion, medullary haemorrhage and congestion, mononuclear cell infiltration, eosinophilic debris in the lumen of tubules and thickening of blood vessels. The mean HP severity score values of kidney with standard error of mean are presented in Table 6. 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 current investigation, exposure to sodium arsenite caused diminished feed intake, reduced body weight gain, rough hair coat, and mild hair loss. These symptoms were comparable to those previously reported in rats subjected to arsenic toxicity (Goyal, 2012) [11].

Our observation of decreased body weight following sodium arsenite exposure was consistent with the findings of earlier researchers (Dhar *et al*., 2005; El-Demerdash *et al*., 2009; Goyal, 2012; Muthumani and Prabhu, 2013) [11, 12, 13, 14]. The gradual decline in body weight observed in the sodium arsenite-treated group may be attributed to the progressive development of anorexia resulting from toxemia (Biswas *et al*., 2000) [15]. Kaltreider *et al*. (2001) [16] found that even low levels of arsenic can interfere with the glucocorticoid hormonal system. This system plays a crucial role in regulating glucose levels and managing carbohydrate, fat, and protein metabolism. Disruption of glucocorticoid function may therefore lead to metabolic disturbances, contributing to either weight loss or gain.

In the present study, sodium arsenite exposure led to significant alterations in hematological parameters, including reductions in TEC, Hb, PCV, TLC, and MCHC, along with elevations in MCV and MCH, consistent with previous reports (Kannan *et al*., 2001; Al-Forkan *et al*., 2016; Hosseinzadeh *et al*., 2018) [17, 9, 18]. The decrease in PCV, Hb, RBC, and MCHC observed in arsenic-exposed animals can be attributed, in part, to arsenic's interference with heme biosynthesis. Arsenic is known to inhibit aminolevulinic acid dehydratase, a critical enzyme in the conversion of δ-aminolevulinic acid to porphobilinogen, thereby disrupting porphyrin and heme formation. This impairment reduces hemoglobin availability and affects erythroid cell maturation, ultimately suppressing erythropoiesis and contributing to anemia (Ola-Davies and Akinrinde, 2016) [19]. Furthermore, arsenic induces oxidative stress in erythrocytes through cellular redox cycling, generating reactive oxygen species that damage the erythrocyte membrane, promote methemoglobin formation, and trigger hemolysis and cell death (Sato *et al*., 1998) [20]. Additional mechanisms, such as interference with folic acid absorption, which is essential for DNA synthesis and red blood cell production and its role as a capillary toxin that increases erythrocyte fragility, further exacerbate the hematological deficiencies observed in arsenic toxicity (Biswas *et al*., 1998) [21]. Collectively, these multifaceted effects of arsenic contribute to the marked anemia and altered hematological profile noted in exposed animals.

The marked decline in TLC levels in this study may be due to the direct cytotoxic effects of arsenic on the bone marrow, which suppress hematopoietic activity and consequently lead to erythrocytopenia and leukopenia (Sajan *et al*., 2010) [22].

The increase in MCV and MCH observed after arsenic exposure typically indicates a macrocytic response linked to anemia. The decline in MCHC levels in the arsenic-treated group may result from multiple factors, including disrupted heme biosynthesis, oxidative stress mediated haemoglobin degradation, and the development of macrocytic anemia, collectively contributing to a reduced haemoglobin concentration per erythrocyte (Ola-Davies and Akinrinde, 2016) [19].

A significant elevation in the mean serum levels of creatinine and BUN was recorded in the present study, which are consistent with the earlier observations (Yousef *et al*., 2008; Messarah *et al*., 2012; Afolabi *et al*., 2016) [23, 24, 25]. In the present study, the observed elevation in plasma urea and creatinine levels following arsenic exposure reflects impaired renal function, most likely resulting from glomerular damage and reduced filtration efficiency. Arsenic undergoes biotransformation to monomethylarsinic and dimethylarsinic acids, which are primarily excreted through glomerular filtration and partially reabsorbed by proximal tubular cells. The accumulation of these metabolites within renal tubular cells can induce pronounced oxidative stress, leading to tubular cell injury and subsequent nephrotoxicity (Sinha *et al*., 2008; Peraza *et al*., 2006; Majhi *et al*., 2011; Rizwan *et al*., 2014; Wang *et al*., 2014) [26, 27, 28, 29, 30].

In the present study, a significant elevation in kidney 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 (El-Demerdash *et al*., 2009: Zhang *et* al., 2014) [13, 31]. 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 (Hu *et al*., 2020) [32].

The kidneys of the disease control group showed varied histopathological changes characterized by tubular degeneration ranging from mild to severe. The renal tubules showed degenerative changes characterised by cellular swelling to vacuolation, eosinophilic debris in their lumen. The glomerular architecture was slightly altered with reduced urinary space. The degenerative changes of tubules were accompanied by loss of cilia and infiltration of inflammatory cells in moderate degree consisting mainly of mononuclear cells *viz* lymphocytes and macrophages. The blood vessels in the renal parenchyma showed thickening of tunica media. Comparable histopathological alterations have been documented by Liu *et al*. (2000) [33], Fahmy *et al*. (2008) [34] and Gora (2014) [35].

Improvement in treatment groups (Group IV to VI) may be attributed to the antioxidant, anti-inflammatory anti-apoptotic properties of MSFM. *Spirulina* is rich in a diverse array of antioxidants, including β-carotene, tocopherol, chlorophyll, and phycobiliproteins such as phycocyanin, cryptoxanthin, and allophycocyanin, all of which collectively enhance its free radical-scavenging capacity and mitigate oxidative stress (El-Naggar *et al*., 2018) [36]. Among these, phycocyanin a predominant biliprotein containing the tetrapyrrole chromophore phycocyanobilin plays a pivotal role in *Spirulina's* antioxidant efficacy. It exhibits strong free radical-quenching activity along with significant metal-binding and chelating abilities, facilitating the detoxification and removal of heavy metals from the body. Furthermore, C-phycocyanin modulates inflammatory responses by inhibiting the phosphorylation of *p38 MAPK*, thereby down regulating cytokine synthesis and attenuating inflammation (Aladaileh *et al*., 2020) [37].

Abd Elhalim *et al*. (2017) [38] demonstrated that *Spirulina* prevents apoptosis by up regulating *Bcl*-2 mRNA expression. This effect is mediated through increased intracellular *Bcl*-2 protein levels, which protect against oxidative stress by inhibiting mitochondrial release of cytochrome-C. As a result, *Bcl*-2 overexpression enhances the cellular capacity to regulate ROS and supports the activity of endogenous antioxidant enzymes, thereby preventing ROS-induced *Bcl*-2 depletion and reducing cell death.

According to Mundkar *et al*. (2022) [39], the anti-inflammatory properties of *Moringa* are primarily attributed to its glucosinolates. During hydrolysis, these compounds yield isothiocyanates, which interact with Keap-1 and facilitate the nuclear translocation of *Nrf*-2. Inside the nucleus, *Nrf*-2 activates the antioxidant response element, triggering a phase II antioxidant defense that suppresses *NF-κB* activation and reduces the release of pro-inflammatory cytokines such as interleukins and *TNF*-α. In addition, *Moringa* modulates apoptosis by increasing *Bcl*-2 expression while decreasing *Bax*, cytochrome-c, and caspase-3 levels, thereby protecting cells from oxidative stress-induced damage.

Finger millet is a rich source of flavonoids (quercetin, catechin, gallocatechin, epicatechin, epigallocatechin, taxifolin, vitexin, tricin, luteolin, myricetin, apigenin, kaempferol, naringenin, daidzein, procyanidin B1, orientin, isoorientin, isovitexin, saponarin, violanthin, lucenin-1) and phenolic acids (gentisic, vanillic, gallic, syringic, salicylic, protocatechuic, p-hydroxybenzoic, chlorogenic, caffeic, sinapic, p-coumaric, trans-cinnamic, and ferulic acids). These bioactive compounds exert strong antioxidant and anti-inflammatory effects (Udeh *et al*., 2017) [40].

The present study demonstrates that microalgal *Spirulina* extract fortified with finger millet and *Moringa* leaves powder provides significant, dose-dependent protection against sodium arsenite-induced nephrotoxicity, with the higher doses (200 and 400 mg/kg body weight) exhibiting greater protective effects. The observed improvements in biochemical markers, antioxidant status, and histopathological outcomes underscore its potential as a therapeutic adjunct in arsenic toxicity. Nevertheless, additional mechanistic investigations and clinical evaluations are warranted to fully establish its efficacy and translational relevance.

Table 1: 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^{a}	181.67±2.23^{a}	200.17 ±3.08^{a}	223.17 ±2.09^{a}	243.67 ±3.13^{a}	268.50±3.86^{a}	276.33 ±3.79^{a}	282.83 ±4.04^{a}	294.50 ±3.35^{a}
Group II (DC)	172.67 ±3.90^{a}	179.17±3.72^{a}	191.33 ±2.51^{a}	202.00 ±2.91^{b}	212.00 ±3.30^{b}	221.00 ±3.13^{b}	229.00 ±4.62^{b}	240.83 ±4.44^{b}	250.33 ±2.99^{b}
Group III (RC)	171.17 ±4.61^{a}	180.50 ±4.57^{a}	193.17 ±3.84^{a}	212.33±3.26^{abc}	233.33 ±1.78^{c}	255.50 ±1.45^{c}	264.83 ±1.92^{a}	273.83 ±1.08^{a}	277.67 ±1.15^{c}
Group IV (MSFM@100)	169.67 ±2.38^{a}	180.17 ±1.82^{a}	191.17 ±0.95^{a}	207.00 ±1.95^{bc}	232.00 ±1.64^{c}	254.00 ±1.37^{c}	265.67 ±1.38^{a}	275.17 ±1.51^{a}	278.00 ±1.72^{c}
Group V (MSFM@200)	169.50 ±4.21^{a}	181.50 ±2.16^{a}	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^{c}
Group VI (MSFM@400)	170.67 ±4.52^{a}	182.00 ±2.14^{a}	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 2: The mean (±SE) values of various haematological parameters of rats in different groups on final day (60th day) of the study

GROUPS	TEC (10^6/µL)	TLC (10^3/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^{a}	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)

Table 3: The mean (±SE) values of various serum biochemical parameters of rats different groups on the final day (60th day) of the study

GROUPS	CREATININE(mg/dL)	BUN(mg/dL)
Group I (NC)	0.55 ±0.01^{b}	16.37 ±0.99^{d}
Group II (DC)	0.84 ±0.04^{a}	25.76±1.37^{a}
Group III (RC)	0.67 ±0.02^{ab}	20.53±0.72^{bc}
Group IV (MSFM@100)	0.70 ±0.02^{ab}	22.84±0.45^{ab}
Group V (MSFM@200)	0.68 ±0.06^{ab}	19.91±0.62^{bcd}
Group VI (MSFM@400)	0.60 ±0.04^{b}	18.50±0.58^{cd}

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 4: 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 Kidney tissue)
Group I	7.26±0.17^{b}
Group II	17.92±0.69^{a}
Group III	11.06±0.54^{c}
Group IV (MSFM@100)	11.56±0.51^{c}
Group V (MSFM@200)	10.28±0.37^{c}
Group VI (MSFM@400)	9.59±0.27^{c}

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 5: 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	Catalase (µM of H_2O_2 decomposed/min)	Superoxide dismutase (U/mg of protein)
Group I (NC)	47.71 ±0.58^{a}	8.54 ±0.34^{a}
Group II (DC)	34.57±0.64^{c}	4.25±0.23^{b}
Group III (RC)	39.57±0.47^{b}	6.78±0.41^{a}
Group IV(MSFM@100)	7.52±0.33^{a}	7.52±0.33^{a}
Group V(MSFM@200)	8.15±0.20^{a}	43.70±0.55^{b}
Group VI(MSFM@400)	8.53±0.20^{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 6: The mean (±SE) HP score of kidney of rats in different groups on final day (60th day) of the study

Groups	Tubular cell swelling to vacuolation	Cortical haemorrhages and congestion	Medullary haemorrhages and congestion	Mononuclear cell infiltration	Eosinophilic debris in tubular lumen	Thickening of blood vessels
Group I (NC)	0.33±0.04^{a}	0.20±0.04^{a}	0.20±0.04^{a}	0.20±0.04^{a}	0.22±0.04^{a}	0.10±0.00^{a}
Group II (DC)	3.27±0.04^{b}	2.42±0.08^{b}	3.25±0.18^{b}	2.89±0.17^{b}	2.27±0.07^{b}	2.80±0.40^{b}
Group III (RC)	2.75±0.15^{bc}	1.72±0.08^{c}	2.65±0.07^{c}	2.73±0.09^{b}	2.10±0.07^{b}	2.12±0.16^{bc}
Group IV (MSFM@100)	2.55±0.08^{cd}	1.60±0.70^{c}	2.65±0.05^{c}	2.16±0.11^{c}	2.05±0.05^{b}	2.22±0.25^{bc}
Group V (MSFM@200)	2.32±0.19^{cd}	1.57±0.06^{c}	2.15±0.02^{d}	1.32±0.10^{d}	1.95±0.09^{b}	1.27±0.21^{cd}
Group VI (MSFM@400)	1.97±0.27^{d}	1.25±0.06^{d}	1.62±0.08^{e}	1.22±0.09^{d}	1.34±0.08^{c}	1.00±0.00^{ad}

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)

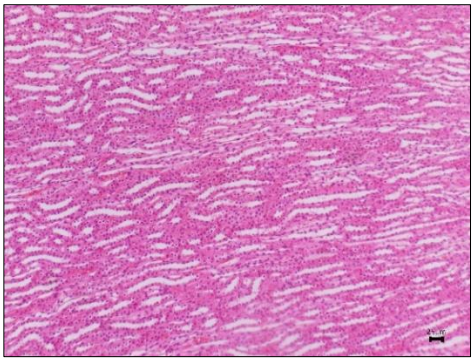

Fig 1: Section of kidney from Group I control rat showing normal architecture of medulla (H &E X 100)

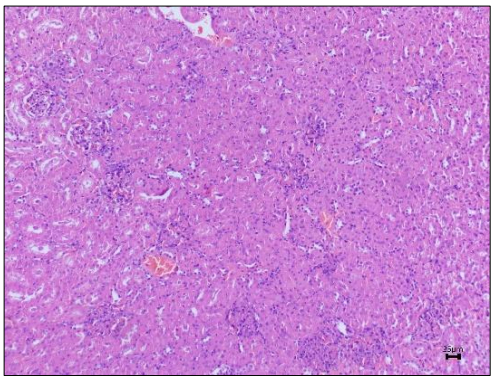

Fig 2: Section of kidney from disease control (Group II) rat showing degenerative changes in tubules with congestion and haemorrhage (H&E X 100)

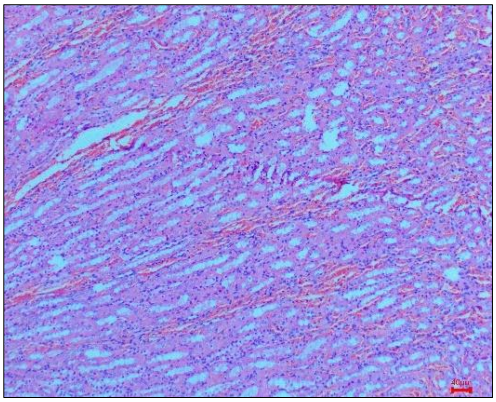

Fig 3: Section of kidney from disease control (Group II) rat showing degenerative changes in tubules with haemorrhage in medullary region (MT X 100)

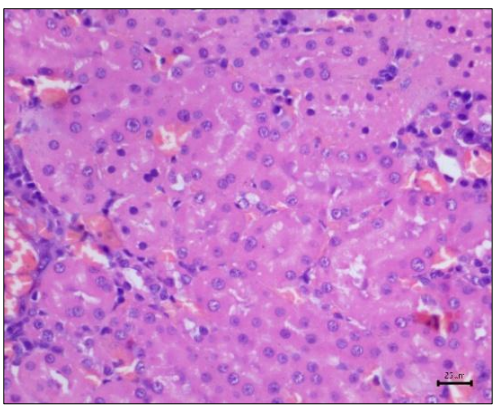

Fig 4: Section of kidney from disease control (Group II) rat showing swollen renal tubules with reduced luminal diameter (H&E X 400)

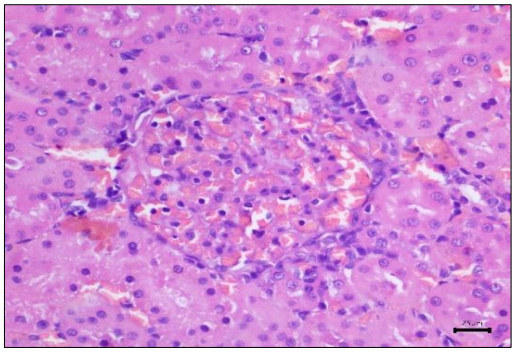

Fig 5: Section of kidney from disease control (Group II) rat showing swollen degenerated tubules and glomerulus with reduced urinary space (H&E X 400)

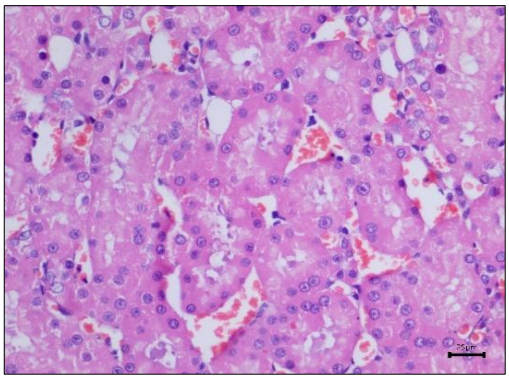

Fig 6: Section of kidney from disease control (Group II) rat showing swollen degenerating tubules with vacuole formation along with haemorrhage (H&E X 400)

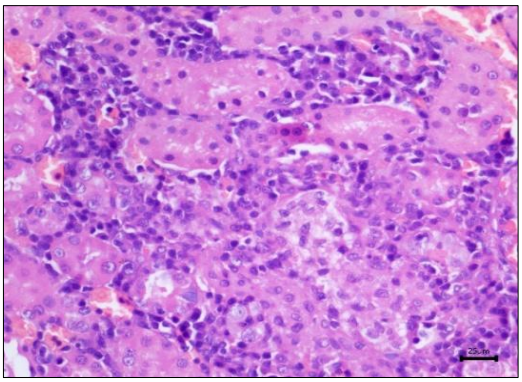

Fig 7: Section of kidney from disease control (Group II) rat showing swollen degenerated tubules with inflammatory cell infiltration in intertubular areas (H &E X 400)

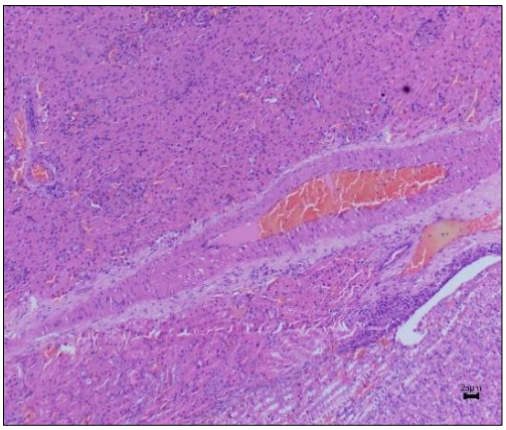

Fig 8: Section of kidney from disease control (Group II) rat showing thickened artery with periarterial fibrosis (H&E X 100)

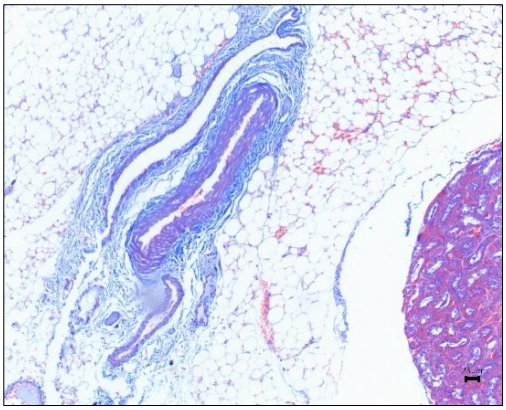

Fig 9: Section of renal artery from disease control (Group II) rat showing thickening of tunica media (MT X 100)

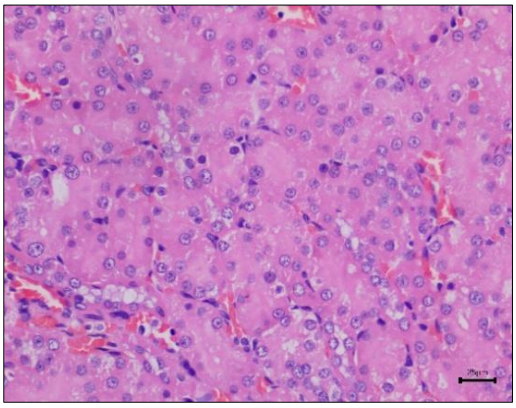

Fig 10: Section of kidney from Group III reference control rat showing mild improvement in renal swelling with minimal vacuolations when compared to Group II rats (H &E X 400)

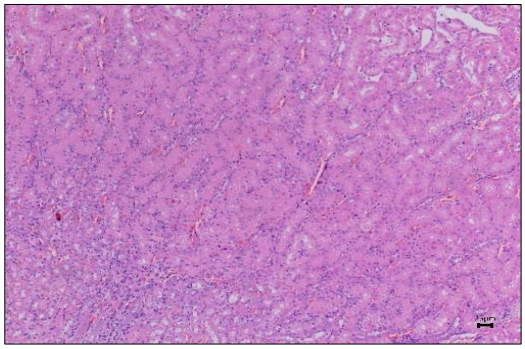

Fig 11: Section of kidney from Group V (MSFM@200) rat showing improvement in renal tubules with decreased vacuolations compared to Group II rats (H&E X 100)

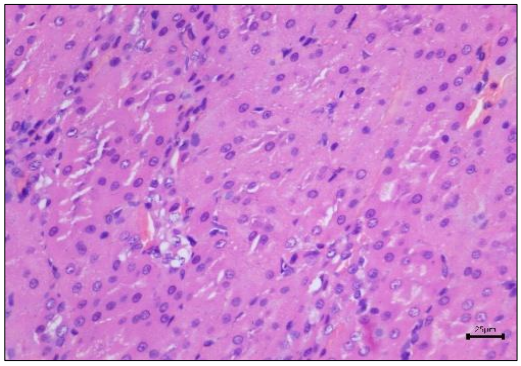

Fig 12: Section of kidney from Group VI (MSFM@400) rat showing improvement in renal swelling with minimal vacuolations when compared to Group II rats (H &E X 400)

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Conflicts of Interest
None

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