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AMELIORATIVE POTENTIAL OF *Moringa oleifera* LEAF EXTRACT AGAINST ARSENIC TOXICITY IN *Labeo rohita*

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ABSTRACT

Arsenic (As) is one of the most harmful pollutants in water bodies which accumulate in animals and bio magnify from lower trophic level to higher trophic level causing imbalance in physiological phenomenon, leading to retarded growth and mortality. Fish is an important resource of healthy protein and poly-unsaturated fatty acids for human diet, it must be free from contaminants and metal toxicants. However, the presence of various metalloids like arsenic in the aquatic environment significantly impart change in the fish meat quality making it unfit for human consumption and overall quantity of fish meat production is affected due to the presence of sufficient amount of arsenic in the water bodies. Therefore, its elimination becomes a global challenge. *Moringa oleifera* (*M. oleifera*), a medicinal plant containing several pharmacological properties, was evaluated for ameliorating adverse effects of sub-lethal concentration of arsenic (1/ 3rd of 96 h LC_{50} = 6.75 mgL^{-1}) in *Labeo rohita*. For this purpose, acclimatized individuals of *Labeo rohita* were randomly allocated to six experimental glass aquaria in triplicates. The experimental fish were exposed to arsenic alone and in a combination with 2 and 4 % *M. oleifera* leaves extract for 28 days. Results of current study revealed that immune biomarkers such as total protein, albumin and globulin contents remarkably ($p \leq 0.05$) lowered on arsenic exposure. Moreover, upon arsenic exposure red blood cell count (RBC), hemoglobin (Hb), hematocrit (Ht), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) significantly ($p \leq 0.05$) decreased, whereas white blood cells (WBC) mean corpuscular volume (MCV) and platelets significantly increased. Conversely, fish treated with 2% or 4% *M. oleifera* leaf extract showed significant improvement and normalized the immune and hematological alteration in *Labeo rohita* with respect to time and dose dependent manner. The results of present study thus concluded that arsenic induced immunological and hematological alterations were ameliorated by the *M. oleifera* leaves extract supplementation. Moreover, 2% or 4% *M. oleifera* leaf extract supplementation both ameliorate the arsenic induced toxicity but 4% *M. oleifera* leaf extract supplementation more significantly ameliorate arsenic induced toxic effect.

Key words: Hematology, Immune, Fish, Amelioration, Arsenic.

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INTRODUCTION

Arsenic, a toxic metalloid element, present in many ecosystems across the world, including China, Pakistan, Chile, India, Mexico, Argentina, Taiwan, Bangladesh, Mongolia, Japan, Poland, Vietnam, Nepal, and the United States (Ali, 2018; Basheer, 2018). Globally, arsenic contamination in water resources ranged from 0-360mgL^{-1} (Shaji *et al.,* 2021). Arsenic contamination in Pakistan groundwater resources has been documented in many areas including the Kalalanwala area near Lahore, Punjab (Farooqi *et al.,* 2007), Muzaffargar (10-906 µg L^{-1}) (Nickson *et al.,* 2005), Mailsi (11-828 µgL^{-1}) (Rasoola *et al.,* 2016) and Tharparkar (12-812 µgL^{-1}) (Brahman *et al.,* 2013). Jabeen and Javed 2011 reported arsenic contamination in water and bed sediments of Ravi River ranged from 19.20 to 21.47 mgL^{-1}, 24.92µg/g to 34.70µg/g, respectively. Arsenic levels that are too high are extremely dangerous for both the environment and human health. Because of the severe toxicity of As even at low concentration the USEPA set a limit of 150 µg L^{-1} for chronic exposure to aquatic organisms (USEPA 2002).

Fish exposed to environmental toxins often show various kinds of immune, growth, histological and blood chemistry disturbances (Ramesh *et al.,* 2014). Fish blood exhibits the early effects of arsenic poisoning as it is mostly absorbed through the large gill surface area, where there is a very weak barrier between the metal salt and blood as well as through the buccal cavity (Rabbane

et al., 2022). Acute toxicity on fish blood is also caused by many other metals like mercury, nickel and chromium, and synthetic pyrethroids such azodrin, mancozeb, cypermethrin and fenvalerate (Kumar and Banerjee, 2016).

Hematological indices like red blood corpuscles (RBCs), hemoglobin (Hb), white blood cells (WBC), packed cell volume (PCV), mean corpuscular hemoglobin concentration (MCHC) and mean corpuscular hemoglobin (MCH) have frequently been used to determine the blood ability to carry oxygen (Shah and Altindag, 2004). They also play a significant role in environmental monitoring of pollutants in aquatic ecosystems, serve as biomarkers of disease and stress (Ololade, *et al.,* 2009). Beside this, serum immune biomarkers analysis such as total protein, albumin and globulin can be used to determine the general health status and targeted toxicity in animals. These biomarkers also recommended to provide early warning signals of dangerous alterations in stressed organisms (Cataldi *et al.,* 1998).

Plant-based medicines have long been used widely and played a significant part in healthcare in all civilizations and communities. In most developing countries, traditional herbal remedies are a part of societies and the main therapeutic approach. These alternative therapies are widely accepted by societies, cost-effective, readily available, and frequently efficient (Aziz *et al.,* 2018). The moringa plant provides both nutritional and therapeutic benefits (Pareek *et al.,* 2023). It has economic significance due to its medicinal applications and nutritional advantages (Milla *et al.,* 2021). It is a member of the sub-Himalayan Moringaceae family, which is native to Afghanistan, Bangladesh, Pakistan, and India (Khan *et al.,* 2023). The active phytoconstituents alkaloids, saponins, tannins, sterols, anthraquinones, terpenoids, flavonoids and vitamins are abundant in moringa leaves, in addition to the different minerals found in them. These elements are necessary for the antioxidant properties of this plant as well as its capacity to protect against free radicals (Pareek *et al.,* 2023). The three main polyphenols found in moringa extracts are quercetin, kaempferol and chlorogenic acid, which have antiproliferative, antihypertensive, and anti-inflammatory activities (Vergara-Jimenez *et al.,* 2017). Additionally, *M. oleifera* leaf extract has been demonstrated to successfully guard against growth, hematological and immune suppression brought on by cadmium (Mallya *et al.,* 2017) and lead (Melebary and Elnaggar 2022). The aim of current research was to evaluate protective role of *M. oleifera* leaf extract against arsenic induced hematological alteration and immune suppression in *Labeo rohita*.

MATERIALS AND METHODS

Experimental Animal: *Labeo rohita* individuals (30±2g) were collected from fish pond of University of Veterinary and Animal Sciences Lahore, Pakistan and placed in cemented rectangular tanks containing flow through aerated water for acclimatization according to Hunn *et al.,* (1968). The fish were fed with commercial pelleted diet (30 % CP) up to satiation twice a day.

***Moringa oleifera* leaves extract and diet preparation:** *Moringa oleifera* leaves were collected and identified from Department of Biological Sciences, University of Veterinary and Animal Science, Lahore. The leaves were shade dried, powdered in grinding mill and then macerated with methanol for 72hr. After maceration the extract filtered and evaporated to dryness by using a rotatory evaporator (DAI HAN SCIENTIFIC WEV-100-1L) at 45℃ (Handa *et al.,* 2008). The residues were collected and stored at 4℃ until use. The chemical composition of extract was determined by GC-MS analysis according to Hameed and Sayed (2019).

Feed preparation: Fish feed ingredients were purchased from local market Pattoki, Pakistan, and analyzed for chemical composition following AOAC (2016). Following ingredients as shown in Table 1 mixed and combined in ratio to make 30% CP (NRC, 2011). Ingredients were divided into three parts. Two parts supplemented with 2% and 4% (w/w) Ahmed *et al.* (2020) *M. oleifera* leaf extract respectively, named as D_1 and D_2. third part was without *M. oleifera* supplement (D_0). All ingredients were mixed and combined to form pallets. The prepared feed was air dried and stored in air tight container at 4°C.

Experimental design: In current study 180 acclimatized individuals of *Labeo rohita* randomly selected from cemented rectangular tanks and divided into six glass aquaria (having 300L water) with 15 fish in each aquarium. These glass aquaria designated treatment name as T_1, T_2, T_3, T_4, T_5 and T_6. Treatment 1 (T_1) and 2 (T_2) were fed with (D_0) di*et al*ong with zero and 1/3rd of 96h LC_{50} of arsenic (6.75 mgL^{-1}) exposure. Treatment 3 (T_3) and 4 (T_4) were fed with D_1 and D_2 diet, respectively with zero arsenic exposure. Treatment 5 (T_5) and 6 (T_6) were fed on D_1 and D_2 diet, along with 1/3rd of 96h LC_{50} of arsenic (6.75 mgL^{-1}) exposure. Fish were fed with their respective diets up to satiation twice a day for 28 days. Whole experiment was conducted in triplicate. Aquaria water was replenished with clean and well aerated water twice a week containing the same arsenic concentration (6.75 mgL^{-1}) containing the same arsenic concentration (6.75 mgL^{-1}) to avoid from ammonia stress and maintain water quality. In each aquarium desired arsenic concentration was confirmed through absorption spectrophotometer analysis and was found significantly

similar with corresponding concentration. Current experiment was conducted under constant water temperature, *p*H, dissolved oxygen, and hardness of 30°C, 7.5, 5.5 and 150 mgL^{-1} respectively. These water quality parameters like pH, dissolved oxygen, temperature and hardness were checked by *p*H (ST 300), DO (ST 300D Ohaus, Corporation, USA) and multimeter (AD-3000, Adwa, Romania) respectively.

Table 1 showing feed ingredients composition and percentage.

Feed Ingredients (g/kg)	D_0	D_1	D_2
Fish Meal	250	250	250
Canola Meal	300	280	260
Wheat Flour	85	85	85
Rice Polish	250	250	250
Minerals[1]	20	20	20
Vitamin Premix[2]	20	20	20
Fish Oil	70	70	70
M. oleifera leaf extract	00	20	40
	Proximate composition		
Crude Ash	63.8	65.0	63.2
Crude Fat	73.8	81.5	81.6
Crude Protein	311.2	307.5	320
Moisture	82.1	83.6	85.5
Energy (kcal/kg)	3769	3816.5	3817.2

Vitamin and mineral mixture premixes cover the minerals &vitamins levels for *Labeo rohita* as recommended by (NRC, 2011). [1] Vitamins premix each 1 kg contains vit. A 580000 IU, vit. E. 720 mg, vit. D_3 8600 IU, vit. K_3 142 mg, folic acid 86 mg, B_{12} 58 mg, vit B_1 58 mg, vit B_2 34 mg, vit. B_6 34 mg, vit C 0.1 mg, vit. pantothenic acid 8 mg. [2] Mineral premix each 1 kg contains copper sulfate 3400 mg, zinc methionine 3000 mg, iron sulfate 2000 mg, manganese sulfate 65 mg, cobalt sulfate 572 mg, calcium iodide 25 mg, sodium selenite 25 mg, and calcium carbonate (as carrier substance) 1000mg.

During experimental trial, two fish were randomly selected from each aquarium (six fish per treatment) at 7th, 14th, 21st and 28th. To overcome handling stress Fish were anaesthetized with MS-222 (30 mgL^{-1}). Using 3ml sterile hypodermic micro syringe blood was collected from the caudal vein of fish. Collected blood apportioned into two groups one group poured into K_3EDTA tube for hematology and another group into gel & clot activator tubes for serum collection. Collected sera was stored at -40°C for biochemical analysis.

Immunological parameters: Serum total protein and albumin contents were measured by using their respective Bioactiva Diagnostic (Bad Homburg, Germany) kits. 1000µl of reagent was taken into the cuvette and 20µl of serum sample was also added. Obtained mixture was mixed properly and inserted into serum chemistry analyzer for results at 546nm and 37°C. Serum globulin was measured by subtracting the albumin contents from total protein of the same sample described by (Khalil and Korni, 2017).

Hematological analysis: Hematological analysis was performed by using Celltac α hematology analyzer (MEK-6500K) of Nihon Kohdens company). It is an automatic hematology analyzer and was calibrated for hematological analysis in fish according to manufacturer's recommendation before analysis. New calibration coefficient was determined by using both manual and analyzer assessed values and entered into the analyzer for calibration. After this blood from K_3EDTA tubes was aspirated through capillary in open mode and results were obtained on screen after 60 seconds

Statistical analysis: Obtained data of biochemical parameters were checked for normality and homogeneity of variance via Kolmogorov-Smirnov and Levene's tests, respectively. Data subjected to two-way repeated measure ANOVA using the GraphPad prism software (version 9.4.1 for Windows). Results were shown as mean ± SE. Differences between groups were assessed by Tukey's honest-significant difference test and effects with a probability of $p \leq 0.05$ were considered significant.

RESULTS

GC-MS profile of *M. oleifera* leaf extract: The GC-MS analysis of identified *M. oleifera* leaf extract identified the main components as well as their proportionate contribution to the overall peak area and retention time (Fig. 1 and Table 2). The data showed that there were 22 constituents in the MLE, with the majority being quercetin (42.66%), acetic acid (17.77%), Ethyl iso-allocholate (12.66%), 1-Methyle cyclopentanole (4.4%), p-Xylene (2.8%), Octadecanoic acid (2.8%), Linoleic acid (2.66%).

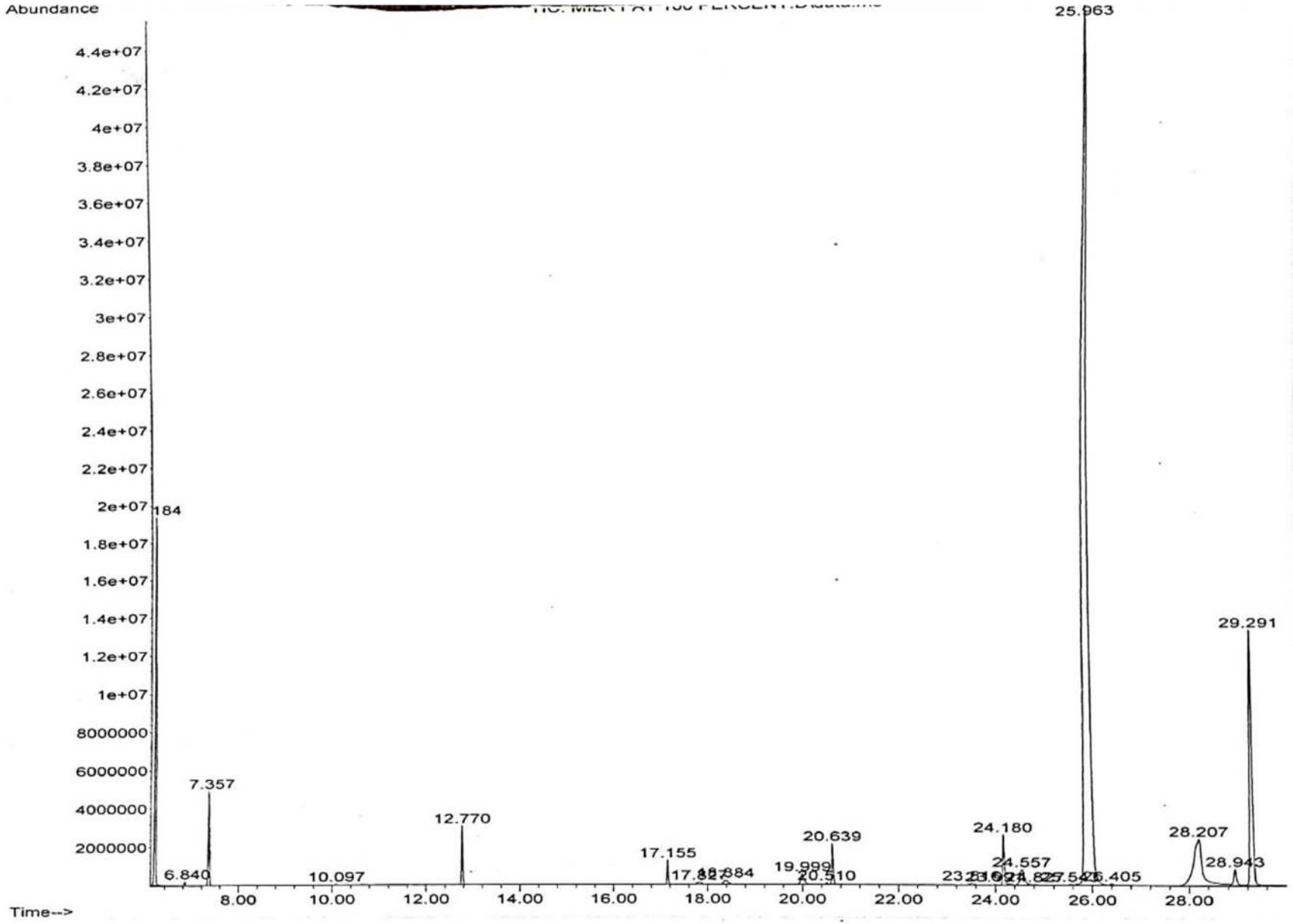

Fig. 1 GC-MS chromatogram of *M. oleifera* leaf extract.

Table 2 Peak area (%) and retention time of different compounds identified in *M. oleifera* leaf extract determined by GC-MS.

Sr.	Identified compounds	Retention time (minutes)	Peak area %	Molecular formula	Molecular weight (g/mol)
1	Acetic acid	1.84	17.77	CH_3COOH	60.05
2	1,3,5-Cycloheptatriene	6.840	1.11	C_7H_8	92
3	1-Methyle cyclopentanole	7.357	4.4	$C_6H_{12}O$	100.16
4	Ethyle benzene	10.097	0.22	C_8H_{10}	106.16
5	p-Xylene	12.770	2.8	C_8H_{10}	106.17
6	Phenyl-acetaldehyde	17.15	2.22	C_8H_8O	120.15
7	Eugenol	17.82	0.44	$C_{10}H_{12}O_2$	164
8	Galacto-heptulose	18.884	0.88	$C_7H_{14}O_7$	210.18
9	Hexadecanoic acid	19.99	2.22	$C_{16}H_{32}O_2$	256.43
10	Heptadecanoic acid, methyl ester	20.51	0.44	$C_{17}H_{34}O$	270.45
11	Linoleic acid	20.63	2.66	$C_{18}H_{36}O_2$	280.44
12	Oleic acid, methyl ester	23.51	0.22	$C_{18}H_{34}O_2$	282.46
13	Androst-5,7-dien-3-ol-17-one	23.91	0.22	$C_{19}H_{24}O_2$	284.39
14	Octadecanoic acid	24.180	2.8	$C_{18}H_{30}O_2$	284.42
15	Vitamin A	24.55	0.22	$C_{20}H_{30}O$	286.45
16	Kaempferol	24.82	1.55	$C_{15}H_{10}O_6$	286.23
17	Phytol	25.52	0.22	$C_{20}H_{40}O$	296.53
18	Quercetin	25.96	42.66	$C_{15}H_{10}O_7$	302.23
19	11-Eicosenoic acid	26.40	0.22	$C_{20}H_{38}O_2$	310.51
20	Sitosterol	28.20	2.22	$C_{29}H_{50}O$	414.71
21	Vitamin E	28.29	1.55	$C_{29}H_{50}O_2$	430.71
22	Ethyl iso-allocholate	28.94	12.66	$C_{26}H_{44}O_5$	436.33

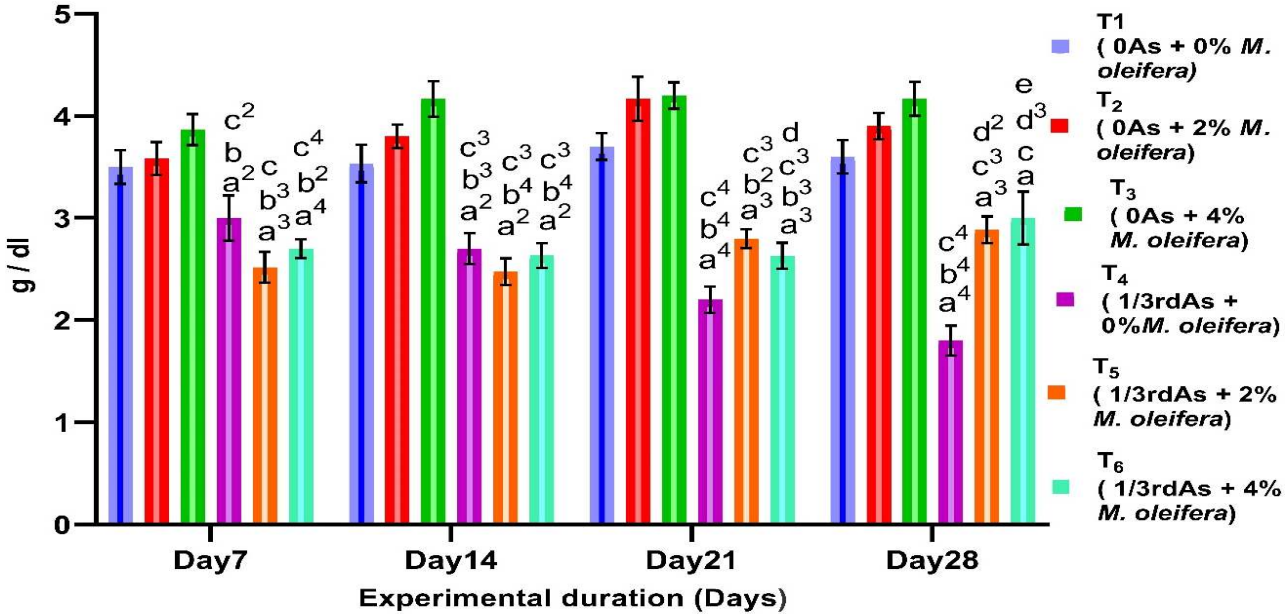

Fig. 2 Total protein contents of *Labeo rohita* exposed to arsenic (1/3rd of 96hr LC_{50}) and / or fed with *M. oleifera* leaf extract supplemented diet. Each column represents the mean ± SE. Columns with different alphabets and superscripts are statistically different at $p \leq 0.05$ [2] $p \leq 0.01$, [3] $p \leq 0.001$, [4] $p \leq 0.0001$.

[a] p value: T_1 versus T_2, T_3, T_4, T_5 and T_6.

[b] p value: T_2 versus T_3, T_4, T_5 and T_6.

[c] p value: T_3 versus T_4, T_5 and T_6.

[d] p value: T_4 versus T_5 and T_6.

[e] p value: T_5 versus and T_6.

Effect of arsenic and/or *M. oleifera* on immunity biomarkers: Serum innates immune biomarkers, such as total protein, albumin and globulin and were significantly ($p \leq 0.05$) lower in arsenic exposed group (T_4) compared to other groups as represented in (Fig. 2-4). 2 and 4 % *M. oleifera* leaf extract supplementation to arsenic exposed group T_5 and T_6 significantly restored serum immunological biomarker level compared to arsenic exposed group (T_4). But 4 % *M. oleifera* leaf extract supplementation significantly ($p \leq 0.0001$) restored serum immunological biomarker level. Moreover, albumin, globulin and total protein level were higher in *Moringa oleifera* leaf extract supplementation treatment (T_2 and T_3) than control (T_1) group.

Effects of arsenic and/or *M. oleifera* on hematological profile: The effects of arsenic and/ or *M. oleifera* leaf extract supplementation on hematological profile represented in Table. 3. The fish fed with 2 and 4 % *M. oleifera* leaf extract supplemented diet (T_2 and T_3) exhibits no significant variations in hematological indices but exhibited higher RBC, Hb, HCT as compared to control group (T_1). In contrast, fish exposed to arsenic ($1/3^{rd}$ of the 96h LC_{50}) had significantly reduced RBC, Hb concentrations, HCT, MCH, MCHC values and total leukocyte counts than other groups. However, most of these components were significantly improved in arsenic exposed group fed with 2 and 4% *M. oleifera* leaf extract supplemented diet

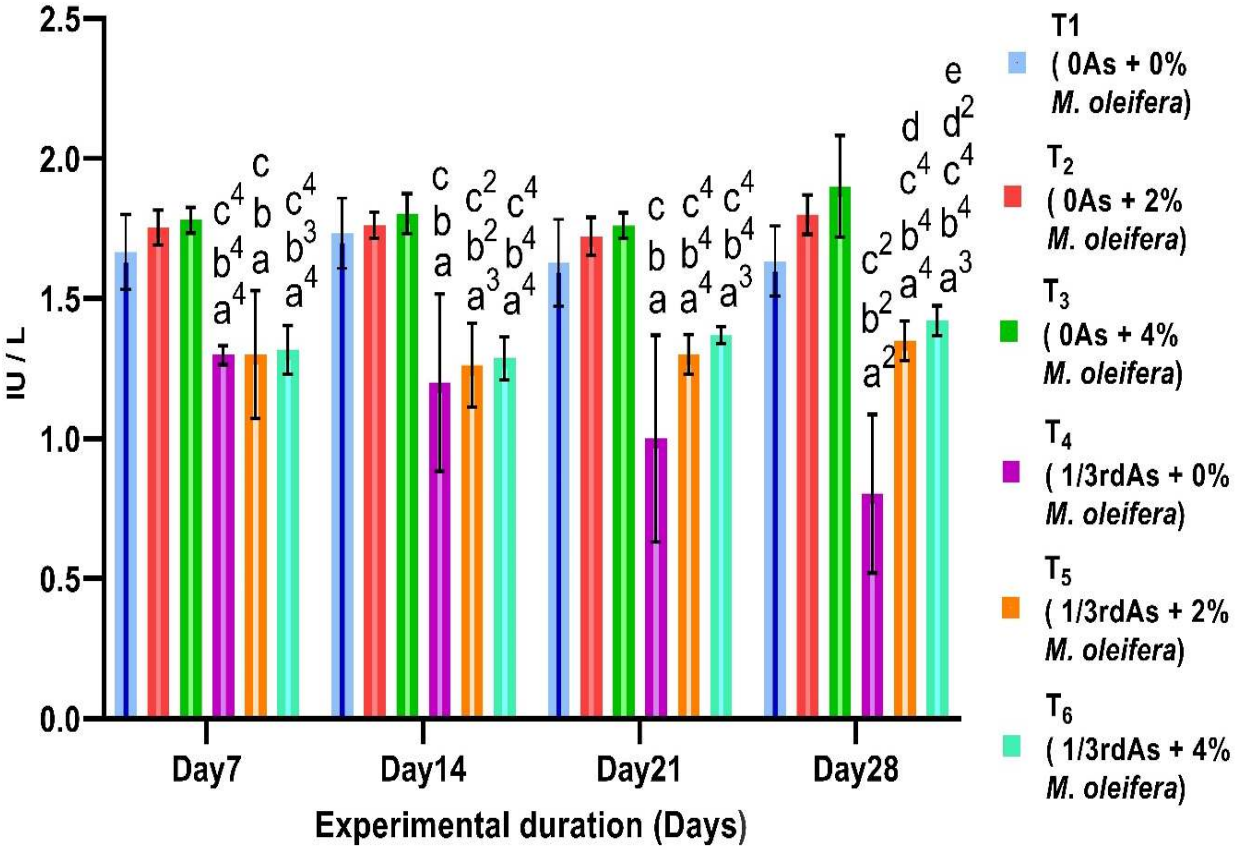

Fig 3 Albumin contents of *Labeo rohita* exposed to arsenic ($1/3^{rd}$ of 96hr LC_{50}) and / or fed with *M. oleifera* leaf extract supplemented diet. Each column represents the mean ± SE. Columns with different alphabets and superscripts are statistically different at $p \leq 0.05$ [2] $p \leq 0.01$, [3] $p \leq 0.001$, [4] $p \leq 0.0001$.

[a] **p value: T_1 versus T_2, T_3, T_4, T_5 and T_6.**
[b] **p value: T_2 versus T_3, T_4, T_5 and T_6.**
[c] **p value: T_3 versus T_4, T_5 and T_6.**
[d] **p value: T_4 versus T_5 and T_6.**
[e] **p value: T_5 versus and T_6.**

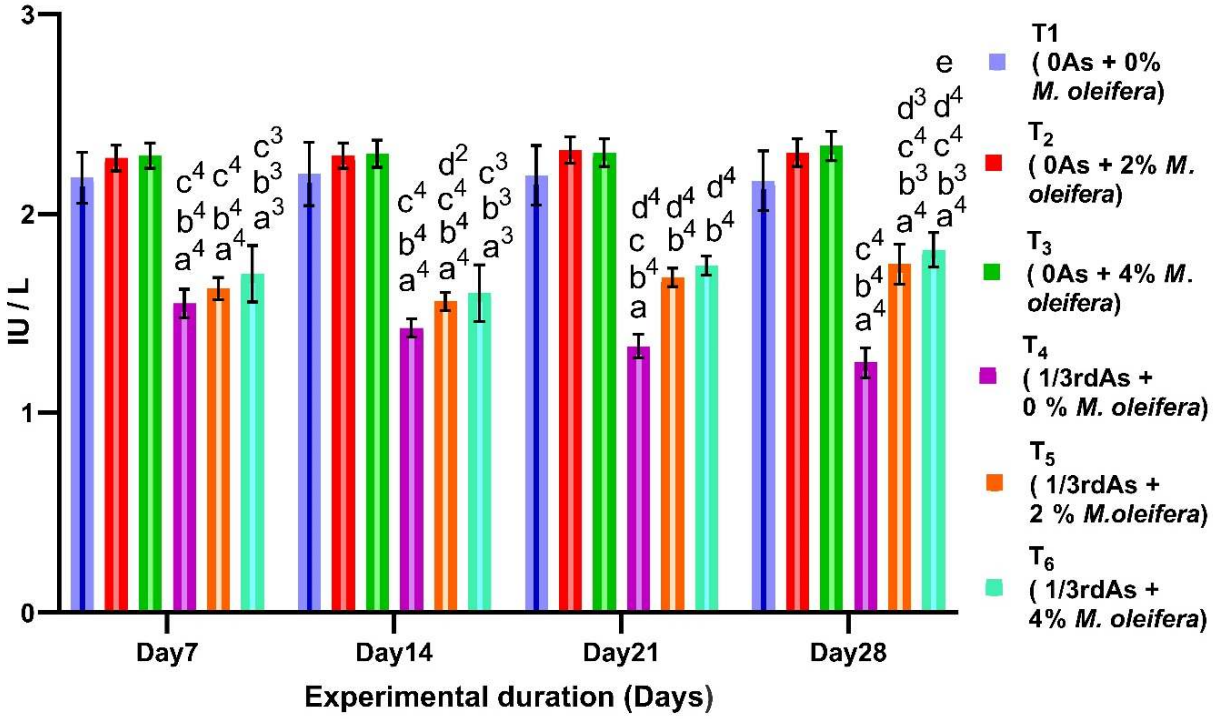

Fig. 4 Globulin contents of *Labeo rohita* exposed to arsenic (1/3rd of 96hr LC_{50}) and / or fed with *M. oleifera* leaf extract supplemented diet. Each column represents the mean ± SE. Columns with different alphabets and superscripts are statistically different at $p \leq 0.05$ [2] $p \leq 0.01$, [3] $p \leq 0.001$, [4] $p \leq 0.0001$.

[a] **p value: T_1 versus T_2, T_3, T_4, T_5 and T_6.**
[b] **p value: T_2 versus T_3, T_4, T_5 and T_6.**
[c] **p value: T_3 versus T_4, T_5 and T_6.**
[d] **p value: T_4 versus T_5 and T_6.**
[e] **p value: T_5 versus and T_6.**

Table 3. Hematological profile of *Labeo rohita* treated with arsenic and/or *M. oleifera* leaf extract supplemented diet.

Components	Duration	Experimental groups					
		T_1 (0 As + 2% *M. oleifera*)	T_2 0 As + 4% *M. oleifera*)	T_3 1/3rd As+ 0% *M. oleifera*)	T_4 1/3rd As+ 2% *M. oleifera)*	T_5 1/3rd As+ 2% *M. oleifera*)	T_6 1/3rd As+ 4% *M. oleifera*)
WBC (10^3/µl)	7th	26.02±1.45	23.02±1.15	21.02±1.15	34.06±1.85$^{b, c}$	32.25±1.50$^{b, c}$	31.25±1.16$^{a, b, c}$
	14th	26.17±1.16	22.02±1.29	21.51± 0.94	40.04±1.85$^{a, b, c}$	31.28±1.22$^{b, c, d}$	30.36±1.31$^{b, c, d}$
	21st	24.20±1.86		22.00±1.29	45.11±1.49$^{a, b, c}$	29.45±1.39$^{b, c, d}$	28.12±1.15$^{b, c, d}$
	28th	25.45±1.53	21.32±1.21	20.02±0.93	50.17±1.74$^{a, b, c}$	29.04±1.15$^{b, c, d}$	26.70±1.33$^{c, d}$
RBC (10^6/ µl)	7th	2.67±0.17	2.73±0.00	2.72±0.01	2.50±0.16	2.55±0.01$^{b, c}$	2.58±0.01$^{b, c}$
	14th	2.72 ±0.02	2.72±0.01	2.74±0.01	2.41±0.12	2.54±0.01$^{a, b, c}$	2.60±0.01$^{a, b, c}$
	21st	2.66 ± 0.03	2.75±0.01	2.76±0.01	2.27±0.12	2.59±0.01$^{b, c}$	2.62±0.01$^{b, c}$
	28th	2.69 ±0.03	2.76±0.01	2.78±0.01	2.20±0.11$^{a, b, c}$	2.60±0.01$^{b, c}$	2.65±0.01$^{b, c}$
Hemoglobin(g/dl)	7th	11.06±0.06	11.30±0.01	11.35±0.01	9.06±0.01$^{a, b, c}$	9.1783±0.01$^{a, b, c, d}$	9.30±0.01$^{a, b, c, d, e}$
	14th	11.32±0.14	11.35±0.01	11.35±0.07	8.01±0.15$^{a, b, c}$	9.27±0.01$^{a, b, c, d}$	9.38±0.01$^{a, b, c, d, e}$
	21st	10.95±0.09	11.35±0.07	11.40±0.08^{a}	6.42±0.83$^{a, b, c}$	9.40±0.01$^{a, b, c}$	9.54±0.01$^{a, b, c, e}$
	28th	11.35±0.17	11.40±0.01	11.52±0.01^{b}	6.50±0.15$^{a, b, c}$	9.58±0.01$^{a, b, c, d}$	9.77±0.01$^{a, b, c, d, e}$
HCT (%)	7th	31.00±0.30	31.50±0.22	32.42±0.17^{a}	28.39±0.15$^{a, b, c}$	28.71±0.13$^{a, b, c}$	29.01±0.28$^{a, b, c}$
	14th	30.54±0.14	32.00±0.24^{a}	33.20±0.21$^{a, b}$	27.04±0.28$^{a, b, c}$	29.01±0.28$^{a, b, c, d}$	29.30±0.27$^{a, b, c, d}$
	21st	29.51±0.14	32.83±0.18^{a}	34.00±0.28^{a}	26.45±0.15$^{a, b, c}$	28.99±0.29$^{b, c, d}$	29.32±0.27$^{b, c, d}$
	28th	30.01±0.28	33.51±0.22^{a}	34.37±0.17^{a}	25.59±0.12$^{a, b, c}$	29.32±0.27$^{b, c, d}$	29.60±0.25$^{b, c, d}$
MCV (fL)	7th	147.03 ±1.73	145±1.50	143.±1.15	162.99±1.37$^{a, b, c}$	160.08±2.08$^{a, b, c}$	157.88±1.84$^{a, b, c}$
	14th	149.16±1.57	143±0.93	141.01±1.86	172.03±1.65$^{a, b, c}$	157.41±1.33$^{a, b, c}$	155.05±1.50$^{a, b, c}$
	21st	146.08±1.65	144±1.50	139.01±0.81^{a}	183.08±1.39$^{a, b, c}$	155.26±1.5$^{a, b, c, d}$	154±1.73$^{b, c, d}$
	28th	148.01±1.63	142±1.73	137±1.50^{a}	190.01±2.43$^{a, b, c}$	153±1.87$^{b, c, d}$	151.08±1.86$^{b, c, d}$
MCH (pg)	7th	33.26±0.14	34.50±0.11^{a}	34.81±0.21^{a}	30.04±0.93$^{b, c}$	30.49±0.75$^{b, c}$	30.89±0.16$^{a, b, c}$
	14th	32.06±0.16	34.31±0.18^{a}	35.51±0.30^{a}	28.01±0.64$^{a, b, c}$	30.76±0.63$^{b, c}$	31.75±0.20$^{b, c, d}$
	21st	32.40±0.30	35.20±0.22^{a}	36.84±0.17^{a}	26.02±0.64$^{a, b, c}$	31.51±0.53$^{b, c, d}$	32.45±0.38$^{b, c, d}$
	28th	32.70±0.22	36.01±0.30^{a}	37.50±0.24^{a}	23.95±1.15$^{a, b, c}$	32.51±0.29$^{b, c, d}$	33.14±0.23$^{b, c, d}$
MCHC (g/dl)	7th	27±0.15	27.59±0.11	28±0.20^{a}	24±0.19$^{a, b, c}$	24.50±0.15$^{a, b, c}$	24.90±0.20$^{a, b, c}$
	14th	26.50±0.13	28.21±0.12^{a}	28.51±0.15^{a}	22.35±0.08$^{a, b, c}$	25.10±0.13$^{a, b, c, d}$	26.12±0.27$^{b, c, d}$
	21st	26±0.18	29±0.28^{a}	29.80±0.13^{a}	20.11±0.24$^{a, b, c}$	26±0.31$^{b, c, d}$	26.51±0.14$^{b, c, d}$
	28th	26.75±0.14	30.12±0.27^{a}	31.70±0.17$^{a, b}$	17.79±0.21$^{a, b, c}$	26.34±0.14$^{b, c, d}$	27.30±0.18$^{b, c, d, e}$
Platelets (10^3/ µl)	7th	37.30±0.37	36.81±0.17	36.49±0.28	43.74±0.15$^{a, b, c}$	43.10±0.21$^{a, b, c}$	42.74±0.13$^{a, b, c, d}$
	14th	39±0.28	37.89±0.15	36±0.57^{a}	52±0.46$^{a, b, c}$	42.73±0.15$^{a, b, c, d}$	41±0.40$^{a, b, c, d}$
	21st	40±0.46	39±0.28^{a}	37±0.23^{a}	61.03±0.93$^{a, b, c}$	41.38±0.17$^{a, b, d}$	39.61±0.18$^{a, b, d}$
	28th	42±0.57	39.50±0.28^{a}	36.76±0.20$^{a, b}$	67.01±0.81$^{a, b, c}$	39.50±0.13$^{a, c, d}$	37.24±0.20$^{b, c, d, e}$

Changes in hematological components in *Labeo rohita* exposed to arsenic (1/3rd of 96hr LC_{50}) and / or fed with *Moringa oleifera* leaf extract supplemented diet. The values are presented as the mean ± SE. Values with different alphabets are statistically different at $p \leq 0.05$.

[a] p value: T_1 versus T_2, T_3, T_4, T_5 and T_6.

[b] p value: T_2 versus T_3, T_4, T_5 and T_6.

[c] p value: T_3 versus T_4, T_5 and T_6.

[d] p value: T_4 versus T_5 and T_6

[e] p value: T_5 versus and T_6.

DISCUSSION

Because arsenic possess negative effect on non-target creatures like fish, there are now a greater number of concerns raised about its potential ecotoxicity to human beings and the environment (Briffa *et al.,* 2020). In addition to its toxicity, arsenic exposure of even sub-lethal concentrations causes its bioaccumulation and biomagnification into aquatic food chains, which poses a severe danger to the sustainability of the environment (Shahjahan *et al.,* 2022). Thus, in addition to the increasing focus on determining the potential hazards that arsenic poses to aquatic systems, there is also an urgent need to identify new therapeutic agents against arsenic toxicity, which is still a challenging issue to solve. In this context, the current study was designed to evaluate the toxic effects of arsenic in the *Labeo rohita* in terms of the hematological profile and immune biomarkers of fish, mainly in order to clarify the potential therapeutic benefits of Moringa oleifera leaf extracts on the general health status of fish.

Proteins play an important role in cell metabolism and are involved in cell physiology and architecture. In the current study, feeding fish diets supplemented with *M. oleifera* leaf extract significantly increased total protein, albumin, and globulin levels over the control diet. Previous research has shown that including plant materials or extracts in fish diets produces significantly higher levels of total protein, albumin, and globulin, suggesting increases in defensive molecules (Abdel-Wahab and El-Bahr, 2012; Abdel-Tawwab and Hamed, 2020). In addition, Nile tilapia fed diets based on pomegranate peel and moringa showed substantial increases in total protein, albumin, and globulin, according to Monir *et al.,* (2020). In the current investigation, we found that arsenic-exposed fish had low levels of total protein, albumin, and globulin, which may have been caused by proteolysis, a failure in protein synthesis brought on by hepatic and renal dysfunction (Ellis *et al.,* 1981). In arsenic-exposed fish, low level of albumin may represent low blood viscosity, whereas low levels of globulin may be due to the liver inability to synthesized sufficient globulin, an indication of weakened immunity (Zhang *et al.,* 2021). These results are in agreement with those that have been observed after toxicant exposure in several fish species (Kannan *et al.,* 2014; Singh *et al.,* 2015; Ghaffar *et al.,* 2017; Majumder and Kaviraj, 2017; Kumar *et al.,* 2019). The restored total protein level in the blood of arsenic exposed fish fed *M. oleifera* leaf extract supplemented diet demonstrates the immune stimulatory effects of *M. oleifera*. In similar research, Hamed and El-Sayed, (2018) found that Nile tilapia fed *M. oleifera* leaves extract had hepatoprotective effect, as indicated by higher serum levels of total protein and albumin than those exposed to pendimethalin without moringa supplementation. Similar findings were reported by Abdel-Tawwab *et al.,* (2020), who stated that European sea bass fed diets enriched with garlic and chitosan showed increased levels of blood total protein, albumin, and globulin.

Blood is a pathophysiological reflector of the whole organism body, and therefore, blood measurements are necessary for evaluating the structural and functional condition of fish exposed to pollutants. In current study the significant reductions in RBC count, Hb concentration, and Hct in the arsenic-exposed group may be caused by either a faster rate of erythrocyte destruction or a slower rate of RBC formation and hemoglobin synthesis (Ahmed and Fazio, 2022). The capacity of arsenic to lower blood iron concentrations, which inhibits the production of hemoglobin, or the rapid oxidation of hemoglobin to methemoglobin, among other factors, may have contributed to the decline in Hb content (Muttappa, 2015). In addition, Ambali *et al.,* (2010), Deeba *et al.,* (2017), and Quintana *et al.,* (2018) stated that, as a result of oxidative stress and rapid lipoperoxidation of erythrocyte membranes, arsenic exposure also increases RBC fragility. Furthermore, the kidney impairment brought on by arsenic may have an impact on hematopoiesis (Xing *et al.,* 2012; Zheng *et al.,* 2014). Several fish species exposed to arsenic have been documented to exhibit similar anemic circumstances in the past, including *Channa punctatus* (Jha *et al.,* 2017), *Labeo rohita* (Ghaffar *et al.,* 2016), and *Heteropneustes fossilis* (Singh and Srivastava, 2015).

M. oleifera leaf extract supplementation significantly restored arsenic-induced anemia with the help of erythrocytes formation in the current investigation. Numerous studies have shown that *M. oleifera* includes a range of amino acids, vitamins, trace elements, minerals (including iron, copper, selenium, and zinc), and phytochemical components (like saponins and flavonoids) (Meireles *et al.,* 2020). Vitamins are crucial for DNA synthesis and erythrocyte maturation, especially vitamins B6, B12, C, E, folic acid, and riboflavin (Bender and Mayes, 2006). The amino acid composition of *M. oleifera* is also necessary for the synthesis of globulin, which leads to the production of hemoglobin. Iron, one of the components used in the formation of hemoglobin, is also found in *M. oleifera* (Saini *et al*., 2014). Additionally, *M. oleifera* has been shown to strengthen and preserve the RBC membrane (Sarkar *et al.,* 2017). The results of present study thus confirmed that arsenic induced immunological and hematological alterations were ameliorated by the *Moringa oleifera* leaves extract supplementation.

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