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Phenolic extracts of Arthrospira platensis attenuates arsenic-induced haematological and renal damage by improving biochemical, apoptotic and histological profile: In Vivo and In Silico approach

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Keywords: Arthrospira platensis, LC- ESI-MS/MS, Haematological parameters, Kidney histology, Molecular docking

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Title: Phenolic extracts of *Arthrospira platensis* attenuates arsenic-induced haematological and renal damage by improving biochemical, apoptotic and histological profile: *In Vivo* and *In Silico* approach

Short Title: Phenolic extracts of *Arthrospira platensis* attenuates arsenic-induced haematological and renal damage

Abstract

Arsenic, which easily gets bio-magnified, is a potential hazardous metalloid that affects the vital organs of human beings. Considering the limitations and side effects of artificial chelating agents, this study was undertaken to evaluate the ameliorative potential of phenolic extracts of spirulina **(***Arthrospira platensis*) in *Mus musculus* model over haematological and renal degeneration caused by arsenic toxicity. In the fulfillment of the goal, we isolated the phenolic content of spirulina and the total phenolic content was estimated. The phenolic content of spirulina was screened with LC-ESI-MS/MS for the presence of active phenolic content. The phenolic extract of spirulina (100 mg/kg) was used to treat the mice model induced with arsenic toxicity (20 mg/kg). The phenolic content from spirulina showed a significant restoration of blood parameters, viz., haemoglobin (Hb), RBC, WBC, and HCT ($p < 0.05$) than arsenic induced group. Decreased weight of kidney and protein concentration, increased kidney marker enzymes, i.e., blood urea nitrogen (BUN), creatinine ($p < 0.05$) in blood serum of arsenic induced animal were the indication of reduced renal potential which significantly restored with phenolic content of spirulina co-treatment. Additionally, bioactive phenolic compounds were screened for molecular docking with most abundant proteins in affected organs by using Auto dock 4.6. The main bioactive compounds, viz., 4 Hydroxy coumarin, catechin, myricetin and kaempferol show higher binding potential with actin, albumin, pyruvate dehydrogenase and superoxide dismutase receptor proteins than arsenic trioxide. Hence, these bioactive compounds are capable of replacing arsenic molecules from screened proteins and helps in reduction of arsenic load.

Key words: *Arthrospira platensis,* LC- ESI-MS/MS, Haematological parameters, Kidney histology, Molecular docking.

Significance of the work

This study reveals the ameliorative potential of bioactive compounds of *Arthrospira platensis* against arsenic toxicity both (*in vivo* and *in silico*) that surpasses the costly conventional chelation therapy with various side effects.

A. Introduction

Arsenic (As), a non-essential trace element, is one of the most widely distributed hazardous environmental pollutants that exerts harmful effects on human health. In the 2001 priority list of hazardous substances and disease registry defined by WHO, arsenic, the "king of poison" ranked as number one. Due to its high toxicity, it is classified as a carcinogen, mutagen, and teratogen. Although WHO prescribed an arsenic limit of 10 µg/L in drinking water, as per the Indian standard drinking water specification act of 1991, the extended limit of arsenic is 50 µg/L. Arsenic toxicity causes lesions, peripheral neuropathy, skin cancer, anaemia, chronic kidney disease, etc [1].

General metal chelators like $CaNa_2EDTA$ and DMSA, which are widely used in different metal chelation processes, have various limitations. These chelating agents are restricted to extracellular fluid by physiological barriers such as cellular membranes, the blood-brain barrier, etc. whereas metals can easily cross the physiological barriers, taking shelter in cellular compartments [2]. Traditional chelating agents cause serious side effects on human health and are highly expensive. Considering the vast toxic effects of arsenic and the limitations of traditional chelating agents, this research work proposes a safe, cheap herbal medicine that ameliorates arsenic toxicity.

Today, the microalgae genus Cyanobacterium (*Arthrospira)* is widely harvested for human consumption. *A. platensis* is the most important in terms of commercial uses and is commonly known as spirulina [3]. Various research articles and the World Health Organisation (WHO) proved that this alga is non-toxic and can be safely used as a food [4,5]. Nowadays, special attention has been given to spirulina due to the high content of bioactive compounds present in it [6]. To survive in the extremely competitive environment, spirulina produces a significant number of secondary metabolites by diversifying its metabolic pathway [7]. These secondary metabolites of spirulina play an important role as antibacterial [8], antifungal [9], antiviral [10], anticancer [11], anti-parasitic [12], etc.

The present study was designed to isolate the phenolic content of spirulina (PSp) and was further analysed by LC-ESI-MS/MS and estimated. This phenolic content was further used to ameliorate the As_2O_3 induced (As) haematological and renal degeneration in *Swiss* albino mice. Simultaneously, an *in silico* study was done to understand the molecular dynamics of the chelation and antioxidant potential of PSp.

B. Materials and Methods

a) Preparation of phenolic extracts of spirulina (PSp)

The algae spirulina *(A. platensis)* was bought from a registered vendor of Vidyasagar University. For preparing the phytochemical extract, 4 gm of spirulina *(A. platensis)* powder was taken in each 20 ml of ethyl alcohol and double-distilled water. The two mixtures were then subjected to ultrasonication at 20 Hz for 30 minutes to disrupt cells. The supernatants were collected after centrifugation at 2000 × g for 15 min at 4°C and filtered using Whatman filter paper. The supernatants were collected and stored in a refrigerator at -20°C for further use [13].

b) *In vitro* detection and estimation of total polyphenolic content

The total phenolic content of spirulina was determined by the Folin-cio-calteu reagent method, according to Lallianrawna *et al.* (2013) and Keskin Sanik *et al.* (2012) [14,15]. The standard curve was plotted using different concentrations of gallic acid. The thin layer chromatography (TLC) was done for the detection of different phenolic bands by using pre-coated silica gel 60 F264 plates and choloform: methanol at 27:0.3 as the running buffer [13].

c) Screening of the phenolic contents of spirulina extracts by LC-ESI MS/MS profiling

The detection of bioactive polyphenolic contents of spirulina LC- ESI MS/MS was done. In this system, separation was carried out using High pressure liquid chromatography (HPLC, WATERS XEVO G2-XS QTof) equipped with C-18 micro column (300 µm × 150 mm). The collected phenolic content was then passed through a 0.22 µm syringe filter. Then 200 µL of the filtered sample was further dissolved in 800 µl of HPLC-grade methanol. After vortexing for 1

minute to mix well, 20 µL of this sample solution was taken for analysis. The solvents are water (solvent A) and acetonitrile (solvent B). The running conditions were for 0-6 min 95% solvent A and 5% solvent B, for 6-12 min 5% solvent A and 95% solvent B. Solvent is flowing at a rate of 40 µL/min. The source capillary was 3.51 kV, and the sampling concentration and source offset were 40 and 80, respectively. The temperatures of the source and desolvation were 100 ℃ and 300 ℃ respectively. The concentration of gas flow was 50 L/h and the desolvation of gas was 600 L/h. The total run time was 12 minutes, and the obtained data were analyzed using Masslynx v4.1 software. The bioactive compounds present in the sample were analyzed using the software Progenesis QI by waters as well as by reported literature survey.

d) Experimental animal set up

In this experiment, 12 male *Swiss albino* mice (*Mus musculus*) were used and the animals were brought from the Saha Enterprise, Regn. No.: CPCSEA REGD.NO.1328/PO/Bt/S/15/CPCSEA. Their weights ranged from 20 to 22 gm and their age was 3 weeks. They were housed in cages and fed for a 10-day adjustment period. The temperature was maintained at 25°C. All animals were kept at the animal house facility at University campus (Regn. No.: 2013/GO/Re/S/18/CPCSEA). All the protocol and standard guidelines approved by the Intuitional Animal Ethics Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) were followed.

Experimental design

The mice were divided into four different treatment groups of three mice in each:

– First group: Normal control (Normal food + Water *ad libitum*)

– Second group: Arsenic trioxide (Normal food + Water *ad libitum* + As)

– Third group: Arsenic + phenolic extract of spirulina (Normal food + Water *ad libitum* + As + PSp)

– Fourth group: Phenolic extract of spirulina (Normal food + PSp + Water *ad libitum*)

Arsenic trioxide (As_2O_3) was given at 20 mg/kg of body weight, and PSp was given at 100 mg/kg to those mice for a treatment period of 30 days. At the end of the experimental phase, all those mice were sacrificed using Ketamine anaesthesia after fasting overnight. Blood samples were collected in EDTA-anticoagulant vial which were used for blood parameters estimation. Another part of blood samples was collected in sterile, non-heparinized tubes directly from their hearts used for serum collection. These blood samples were centrifuged (10,000 RPM, 10 minutes, 4ºC) to collect blood serum. The supernatants were collected for different serum assays.

e) **Haematological parameters test**

Arsenic toxicity is associated with bone marrow damage, which in turn results in a low level of haematocrit and haemoglobin. The EDTA-anticoagulant bloods were subjected to estimate for important blood parameters like haemoglobin (Hb), RBC, WBC and haematocrit percentage (HCT) by using the Mindray BC-2800VET auto analyzer machine.

f) **Kidney protein estimation and enzyme assay**

From each sacrificed animal, a small part of the kidney was taken in a petridish. After washing with ice-cold saline, those tissues were homogenized in phosphate buffer (pH 7.4). Total protein was estimated by following Lowry's method against the BSA standard curve. For the study of kidney function, blood urea nitrogen (BUN), and creatinine were estimated spectrophotometrically. Experimental kits of Reckon were purchased from HIMEDIA.

g) Histopathological study

Kidney tissues were collected from each animal, and paraffin wax blocks were made. Tissue blocks were sectioned using a microtome. Tissue sections were collected on a grease-free slide. These tissue sections were double-stained with

Haematoxylin and Eosin (H&E) and mounted with DPX. Stained tissue sections were examined at 10x and 40x magnification under a microscope.

h) *In Silico* analysis

i. Receptor protein preparation

For performing molecular docking [16], 3D X-ray structures of proteins were obtained from the Protein Databank (RCSB PDB, https://www.rcsb.org). In preparing the receptors, the water molecules were deleted, and then polar hydrogens and Kollman charges were added to the protein molecules by using Auto Dock Tools. Finally, files were saved in PDBQT format (**Table 1**).

ii. Ligand preparation

Different ligand files of isolated bioactive compounds were downloaded from PubChem, and arsenic trioxide was downloaded from Drugbank. XML-format files were converted into PDB format by using PyMOL software. Then, using Auto Dock Tools (ADT), Gasteiger charges were added. ADT then detects the root and makes all rotatable bonds non-rotatable. Finally, files were saved in PDBQT format (**Table 2**).

Table 1. Receptor proteins affected by arsenic toxicity and sources of PDB files in study

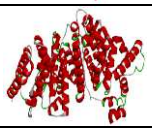
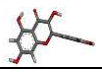
Protein	PDB ID	Affected organ by arsenic	3D Structure of protein
Actin	1J6Z	Cytoskeletal structure (17)	
Albumin	2BXI	Blood serum (18)	
Pyruvate dehydrogenase (PDH)	2BUB	Energy metabolism in all cells (19)	
Superoxide dismutase (SOD)	5YTO	Antioxidant enzyme (20)	

Table 2. Sources of ligands used in the study

Ligand	Accession no.	3D Structure of ligand
Arsenic trioxide (As)	DB01169 (21)	
Kaempferol	CID5280863 (22)	
Catechin	CID73160 (23)	

Myricetin	CID5281672 (24)	
4-hydroxycoumarin	CID54682930 (25)	

iii. Molecular Docking

The docking studies of peptides and ligands were carried out with AutoDock software 4.2.6, which is free for academic users. The Windows version of MGL Tools 1.5.7 was installed and used for parameter file preparation for docking purposes. According to the AutoDock programme, arsenic is a non-recognisable molecule. According to the standard protocol mentioned on the official webpage of AutoDock (http://autodock.scripps.edu/faqs-help/how-to/adding-new-atom-parameters-to-autodock) was followed for the parameterization of these molecules. To find all the ligand binding states, 100 conformations (GA runs) were generated. The maximum amount of energy was set at 25×10^5, the rate of crossover was 0.8, and the rate of gene mutation was 0.02 for the required search parameters of the genetic algorithm. The AutoDock tool calculates Gibbs free energy (ΔG) and inhibition constant (K_i) for every conformation in order to comprehend the efficacy of the ligand to bind with the receptor.

iv. Druggability analysis

The druggability of bioactive compounds of spirulina was done with SwissADME software. It mainly helps to analyse whether the bioactive compounds obeyed the Lipinski's rule of five [26].

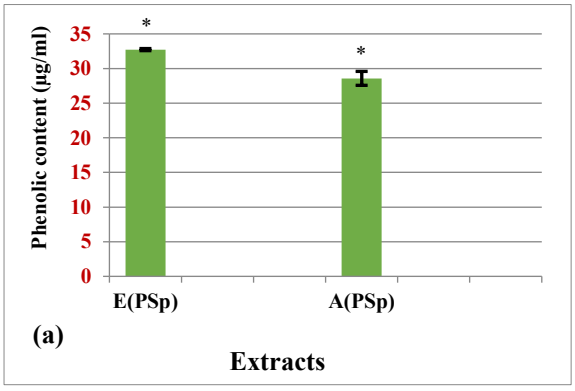
Statistical analysis

To draw statistical significance, a one-way ANOVA with descriptive statistics was performed between the collected data using SPSS software. All data were analysed at 0.05 significance levels.

A. Results and Discussion

a) Total phenolic content of spirulina (PSp)

Ethanol and aqueous extracts of spirulina were used for determination of total poly phenolic content (TPP) by Folin-cio-calteu reagent method. Ethanol extract of spirulina E(PSp) with 32.7 ± 0.150 µg/ml showed higher total poly phenolic (TPP) content than aqueous extract of spirulina A(PSp) with 28.58 ± 1 µg/ml (**Fig. a**, P <.000, one-way ANOVA). The ethanol extract of spirulina showed higher phenolic content than aqueous extract therefore, the ethanol extract of spirulina was chosen for further experiments. The thin layer chromatogram of extracted phenolic content showed different bands of phenolic compounds with R_f values ranging from 0.06 to 0.68 (**Fig. b**).



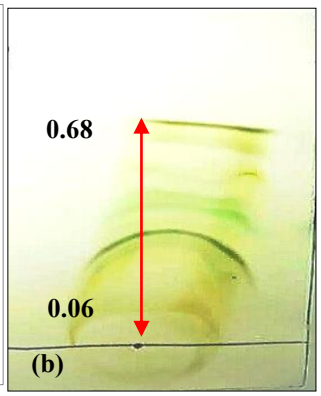


Figure 1. (a) Represents total polyphenolic content of spirulina. **(b)** Represents different bands of phenolic compounds of spirulina on TLC plate.

b) **LC-ESI MS/MS analysis of PSp**

The LC-MS result of phenolic extract of spirulina identified various phenolic contents by comparing the mass spectrum with Progenesis QI and literature study. Total eight phenolic compounds were identified. Among them four phenolic compounds viz. 4-hydroxycoumarin, catechin (27), kaempferol (28), myricetin (29,30), were the major compounds that are supposed to be present in high amount in the isolated extract (**Fig. 2**, **Table 3**).

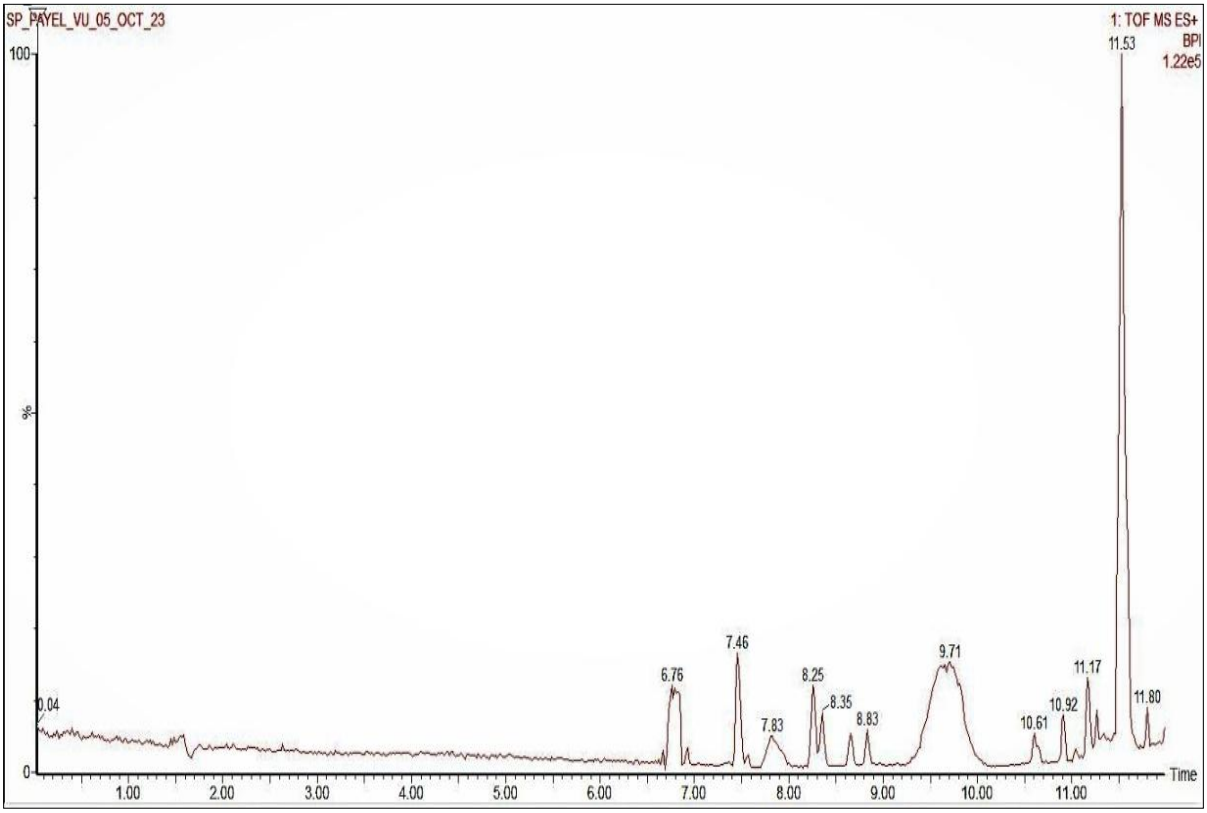


Figure 2. LC-MS (Base Peak Ionization) profile of phenolic extract of spirulina.

Table 3. Profile of phenolic extract of spirulina as obtained from LC-MS

(* Represents the phenolic compounds present in higher concentration**)**

Retention time	m/z $[M-H]^-$/ $[M-H]^+$	Peak area of extracted ion	Molecular formula	Nomenclature
9.61	119	2.64×10^3	$C_4H_6O_4$	Succinic acid
9.60	162	4.57×10^4	$C_9H_8O_3$	4-Hydroxycoumarin*
11.52	170	1.06×10^4	$C_7H_6O_5$	Gallic acid
8.31	180	1.34×10^3	$C_9H_8O_4$	Caffeic acid
6.94	280	2.47×10^3	$C_{17}H_{14}O_4$	Dimethoxyflavone
11.53	286	8.41×10^3	$C_{15}H_{10}O_6$	Kaempferol*
11.16	290	5.77×10^3	$C_{15}H_{14}O_6$	Catechin*
8.35	318	6.42×10^4	$C_{15}H_{10}O_8$	Myricetin*

c) Efficacy of phenolic extract of spirulina in recovery of haematological parameters

The Leishman-stained blood smear of treated groups showed increased blood corpuscles compared to arsenic induced group (**Fig. 3**). Reduction in important blood parameters viz. haemoglobin (Hb), RBC, WBC and HCT by 49.29%, 50.30%, 57.14%, and 49.11% respectively than normal control group, indicate arsenic toxicity directly affects the blood parameters of animals. On the contrary, with increased blood parameters haemoglobin by 48.23% and RBC by 59.02% (**Table 4,** $p < 0.000$, one-way ANOVA), WBC by 115.5% (**Table 4,** $p < 0.030$, one-way ANOVA) and HCT by 58% in 'As + PSp' group compared to arsenic induced group proved that phenolic extract of spirulina provides significant protection to blood parameters from arsenic-induced toxicity. Arsenic toxicity is associated with bone marrow damage, which in turn results in a lower level of haematocrit and haemoglobin whereas co-treatment with PSp helped in the recovery of lowered blood parameters.

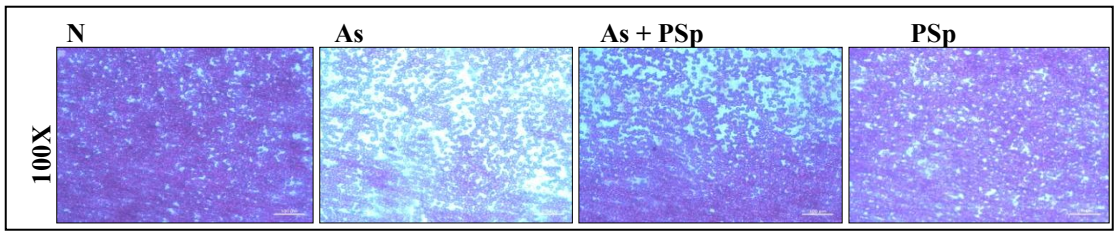


Fig. 3. Leishman-stained blood smear of all group.

Table 4. Blood parameters of different mice sets

Blood parameters	N	As	As + PSp	PSp
Haemoglobin (g/dL)	13.41 ± 0.33	6.80 ^{a*} ± 0.40	10.08 ^{b*} ± 0.16	13.20 ± 0.30
RBC (× 10^6/UL)	9.92 ± 0.52	4.93 ^{a*} ± 0.34	7.84 ^{b*} ± 0.24	10.57 ± 0.50
WBC (× 10^3/UL)	11.27 ± 2.31	4.83 ^{a*} ± 0.47	10.41 ^{b*} ± 0.74	11.93 ± 1.48
HCT (%)	41.07 ± 1.04	20.9 ^{a*} ± 1.63	33 ^{b*} ± 3.49	38.91 ± 0.28

d) Efficacy of phenolic extract of spirulina in recovery of renal parameters

On considering the kidney weights and total protein concentration of different sets of mice significant decrease by 38.70% and 51.79% respectively compared to normal control can be seen. However, arsenic induced treated with PSp shows 31.57% and 46.58% increases in kidney weight and total protein concentration compared to arsenic induced group (**Table 5**, $p < 0.001$, one-way ANOVA). Arsenic toxicity caused kidney injury which thereafter increased blood urea nitrogen (BUN) and creatinine in blood serum by 365.42% and 266.32% respectively compared to normal control mice group. On the other hand, when arsenic induced group co-treated with PSp, nominal increase of BUN and creatinine level were recorded by 100% and 103.31% respectively compared to normal control group (**Fig. 4 a, b** $p < 0.000$, one-way ANOVA). This clearly indicates ameliorative potential of phenolic content of spirulina. Previous studies also reported CKD (chronic kidney disease) due to arsenic load and ROS generation (32). H&E stained kidney section of arsenic induced mice displayed severe pathological damages viz. multiple areas of cortical interstitial haemorrhage with damaged glomerular basement membrane and disrupted tubular epithelium. Few areas of lymphocytic infiltration are present in the interstitium. 'As+ PSp' co-treated mice kidney showed normal histopathological features of glomeruli although multiple areas of haemorrhage in interstitium can be seen. Normal control and 'PSp' treated groups showed normal histopathological features of normal kidney consisting of renal cortex containing glomeruli and tubules, renal medulla containing collecting ducts [**Fig. 5 (a, e)** and (**d, h)**]. Blood urea nitrogen (BUN) is the byproduct of protein metabolism, and creatinine is a nitrogenous waste product of skeletal muscle. Both of these two nitrogenous end products then, via blood, go to the kidney where, the glomerulus filtrate

them out through urine. When the kidney fails to filtrate well, then these two parameters show a high level in blood serum. In our observation, reduced weight, protein concentration, increased BUN, and creatinine levels in blood serum were the indications of kidney damage, which were further established by the study of arsenic-induced mice histo-architecture [**Fig.5 (b, f)**]. On the contrary, co-treatment with PSp actively helps to reduce arsenic load from kidney by its antioxidant effect (31), which in turn helps in recovery of kidney tissue and the reduction of elevated BUN and creatinine levels and significant recovery can be seen in kidney histology [**Fig.5 (c, g)**].

Table 5. Relative weight of kidney and total protein concentration

('a' significant with control group, 'b' significant with arsenic treated group. Data are represented in mean ±SEM, * significant at 0.05 level)

Sets	Normal	Arsenic	Arsenic + PSp	PSp
Kidney weight (mg)	0.31 ± 0.02	0.19 ^{a*} ± 0.02	0.25 ^{b*} ± 0.01	0.31 ± 0.01
Total protein content of kidney (mg/ml)	19.77 ± 0.30	9.53 ^{a*} ± 0.20	13.97 ^{b*} ± 0.41	19.83 ± 0.12

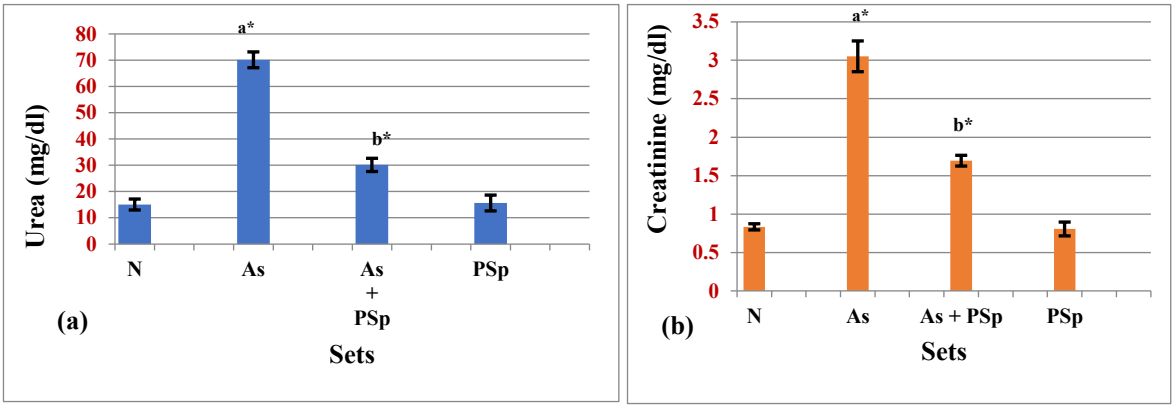
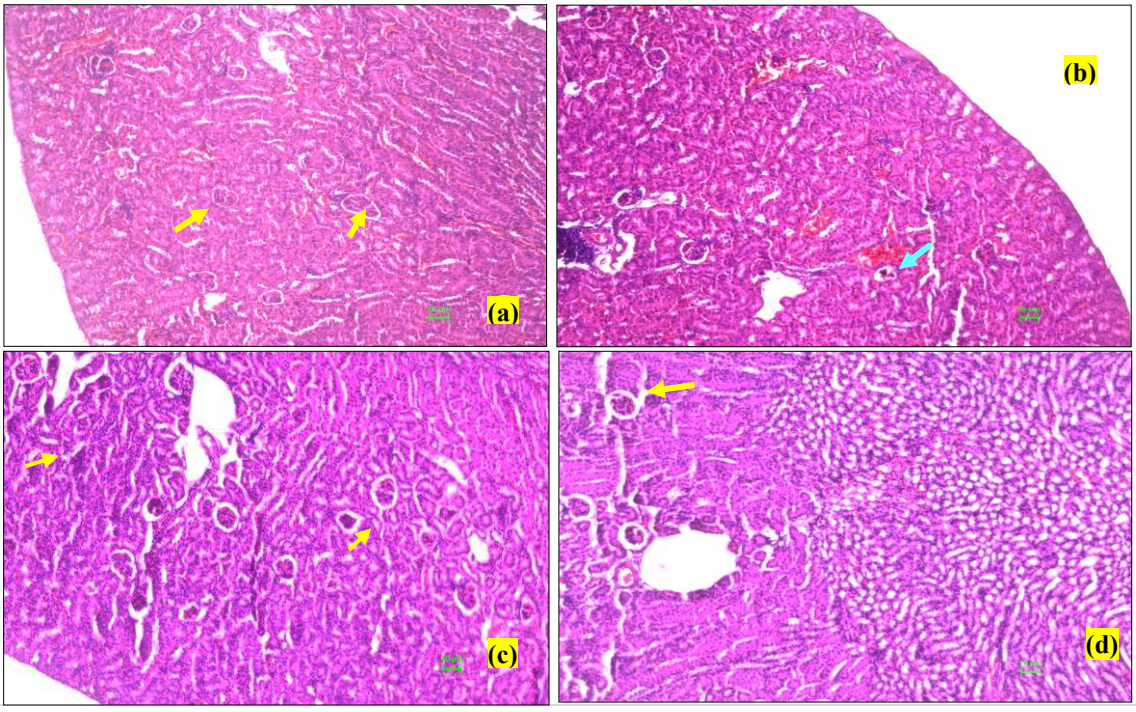


Figure 4. (a) Result of blood urea nitrogen (BUN) **(b)** Result of kidney creatinine of different mice sets. 'a' significant with control group, 'b' significant with arsenic treated group. Data are represented in mean ±SEM (* significant at 0.05 level).

(5)



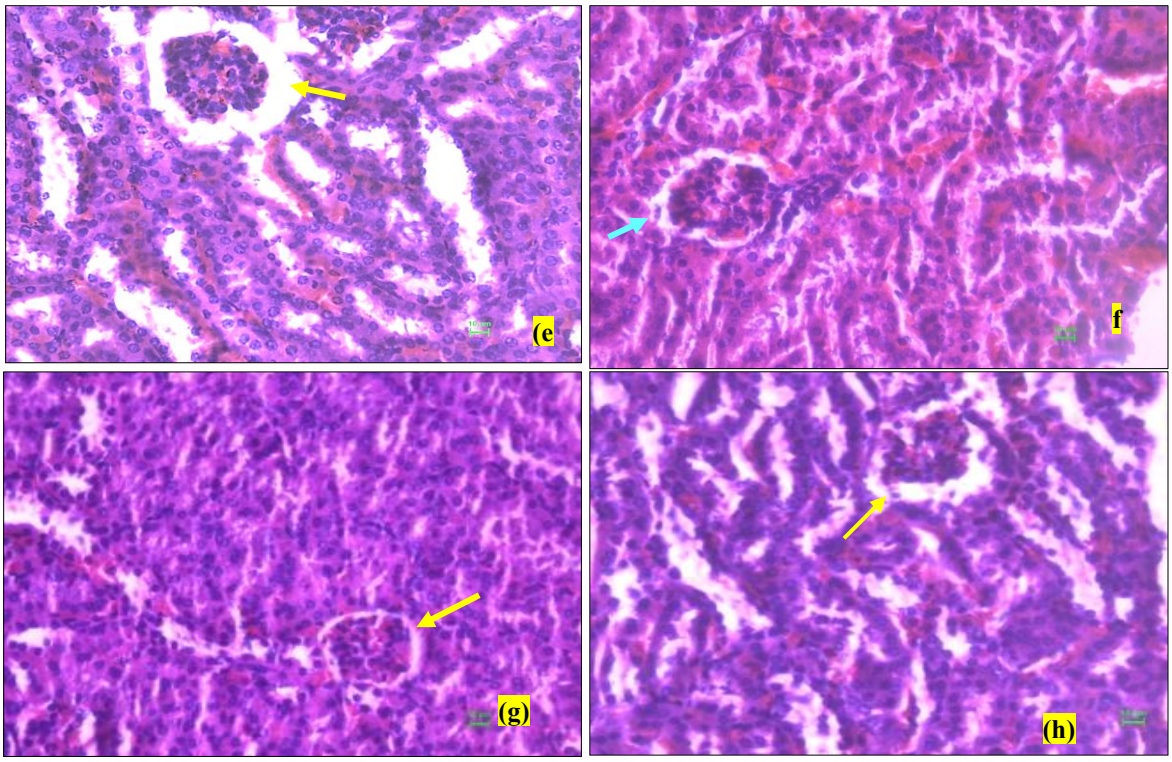


Figure 5. Histo-architecture of Haematoxylin and Eosin (H&E) stained mammalian kidney (*Mus musculus*) histological study of normal control group mice kidney in (a) 100x, (e) 400x magnification and 'PSp' group mice kidney in (d) 100x, (h) 400x magnification represent normal histoarchitecture with glomeruli (yellow arrow), tubules, renal medulla containing collecting ducts. The histology of arsenic induced group in (b) 100x and (f) 400x magnification represent interstitial haemorrhage, damaged glomerular basement membrane and tubules (cyan arrow). The histology of 'As + PSp' group in (c) 100x and (g) 400x magnification represent almost normal histopathological features with healthy glomeruli (yellow arrow).

e) *In Silico* analysis

i) Molecular docking result analysis

The output results of AutoDock are denoted by Gibbs free energy or ΔG. According to law of thermodynamic, Gibbs free energy represents the energy drug ligands need to bind with protein. Reaction equation is

$$\Delta G = - RT \text{ in } K$$

Docking score is reflecting the binding affinity of the ligand with target protein. Therefore, more negative binding score (ΔG) indicates a stronger binding affinity. K_i or inhibitory constant is the concentration of needed to achieve 50% inhibition. Lower K_i value implies higher ligand affinity for macromolecule. The results are tabulated below with details of docking score, inhibitory constant (K_i), and interacting amino acid residues.

Table 6. Docking score (ΔG) (Kcal/mol), inhibitory constant and interacting amino acid residues of ligands with different screened proteins

Receptor /protein	Arsenic trioxide (As) ligand		
	ΔG (Kcal/mol)	**Inhibitory constant (K_i)**	**Amino acid involved**
Actin	-3.62	2.24 mM	GLY 15, LEU 16, ASP 157

Albumin	-3.84	1.52 mM	ARG 348, ARG 485
PDH	-4.03	1.12 mM	TRP 124, ASN 170, ASP192
SOD	-4.46	542.45 uM	VAL 7, VAL 148

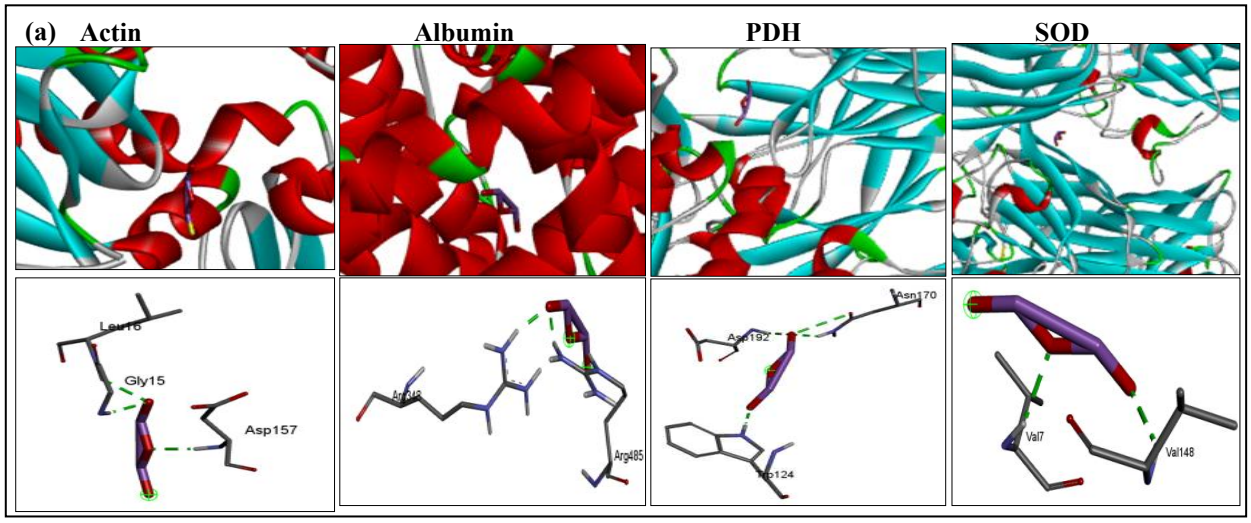
Receptor /protein	**4-hydroxy coumarin ligand**		
	ΔG (Kcal/mol)	**Inhibitory constant (K_i)**	**Amino acid involved**
Actin	-8.47	616.49 nM	TYR 133, TRP 356, LEU 346, LEU 349, MET 355, PHE 352
Albumin	-7.69	2.29 uM	TYR 150, PRO 152, ALA 254, ALA 258, LEU 283, LEU 284
PDH	-8.79	357.44 nM	LYS 512, GLN 527, ILE 529, ARG 560, ASN 562, THR 565
SOD	-7.5	3.2 nM	VAL 7, LYS 9, ASN 53

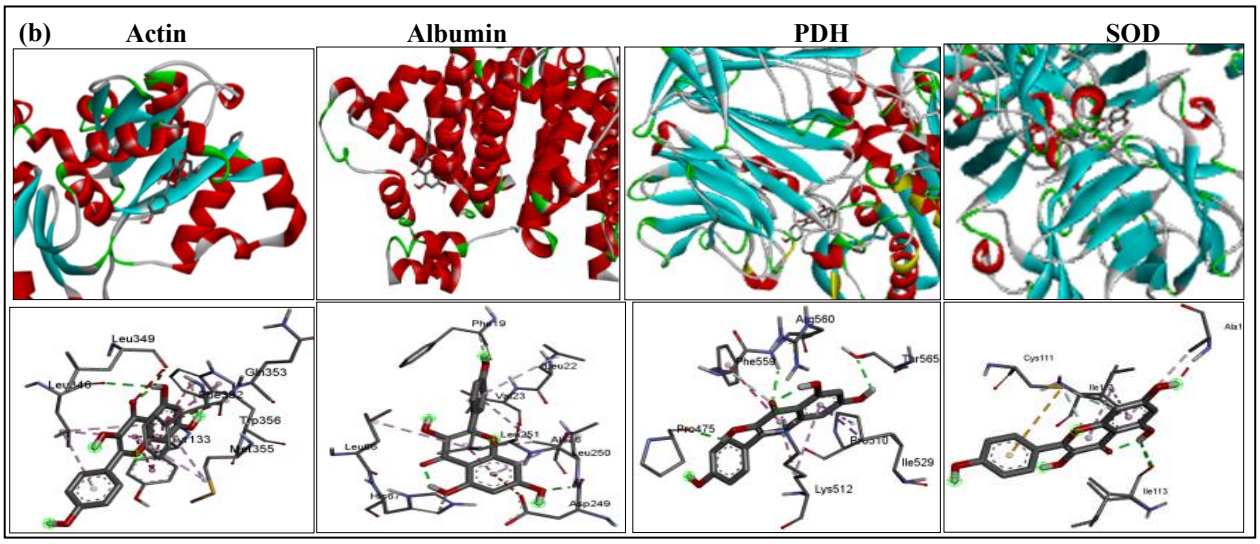
Receptor /protein	**Catechin ligand**		
	ΔG (Kcal/mol)	**Inhibitory constant (K_i)**	**Amino acid involved**
Actin	-6.78	10.72 uM	TYR 133, LEU 346, MET 355, TRP 356
Albumin	-6.69	12.51 uM	VAL 23, GLU 45, VAL 46, LEU 69, PHE 70, LYS 73
PDH	-6.96	7.90 uM	PRO 475, PRO 510, LYS 512, LIE 529, VAL 558, PHE 559, THR 565,
SOD	-5.41	108.04 uM	ALA 1, LEU 106, GLY 108, ASP 109, CYS 111, ILE 113

Receptor /protein	**Myricetin ligand**		
	ΔG (Kcal/mol)	**Inhibitory constant (K_i)**	**Amino acid involved**
Actin	-7.52	3.08 uM	ARG 116, TYR 133, VAL 134, ILE 357, GLU 361, ILE 369
Albumin	-6.63	13.77 uM	LEU 115, ARG 117, PHE 134, TYR 138, LEU 182, ARG 186
PDH	-9.13	204.11 nM	PRO 475, LYS 512, VAL 558, ARG 560, GLN 527, ASN 562 ALA 564, ILE 529, PHE 559, PRO 510
SOD	-7.91	1.58 uM	GLY 108, ASP 109, CYS 111, ILE 113, ARG 115

Receptor /protein	**Kaempferol ligand**		
	ΔG (Kcal/mol)	**Inhibitory constant (K_i)**	**Amino acid involved**

Actin	-9.65	84.56 nM	TYR 133, PHE352, LEU 346, LEU 349, TRP 356, MET 355, GLN 353
Albumin	-10.25	30.68 nM	LEU 22, VAL 23, ALA 26, PHE 19, HIS 67, LEU 66, LEU 250, LEU 251
PDH	-10.46	21.61 nM	PRO 475, PRO 510, LYS 512, ILE 529, AUG 560, PHE 559, THR 565
SOD	-8.22	938 nM	ALA 1, CYS 111, ILE 113





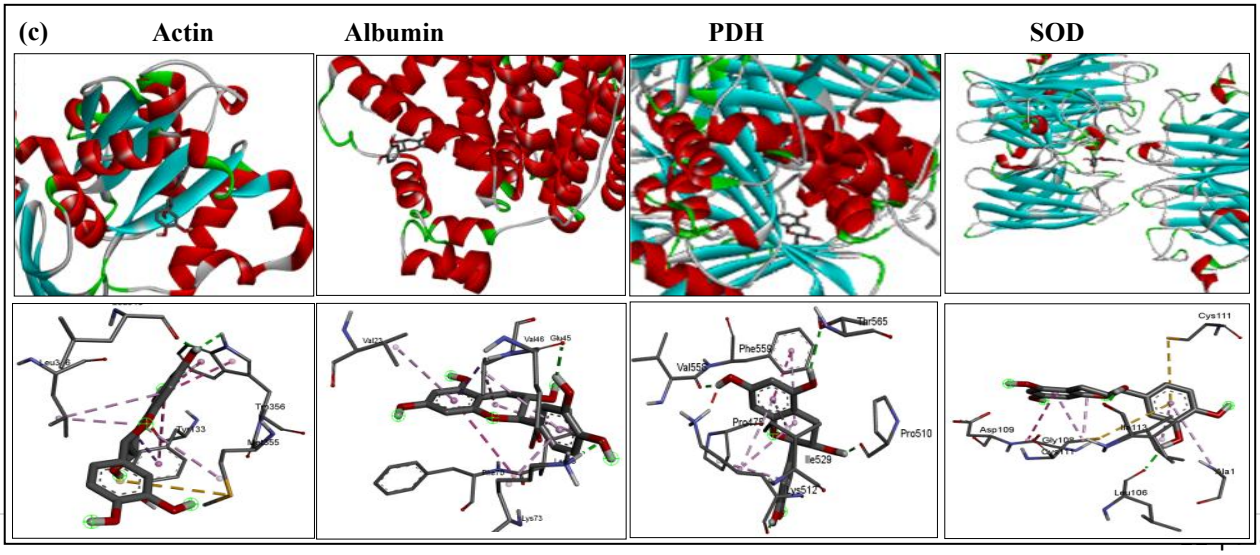


Figure 6. 3D graphics and molecular interactions among **(a)** arsenic and screened receptor proteins. **(b)** kaempferol and screened receptor proteins (**c)** catechin and screened receptor proteins.

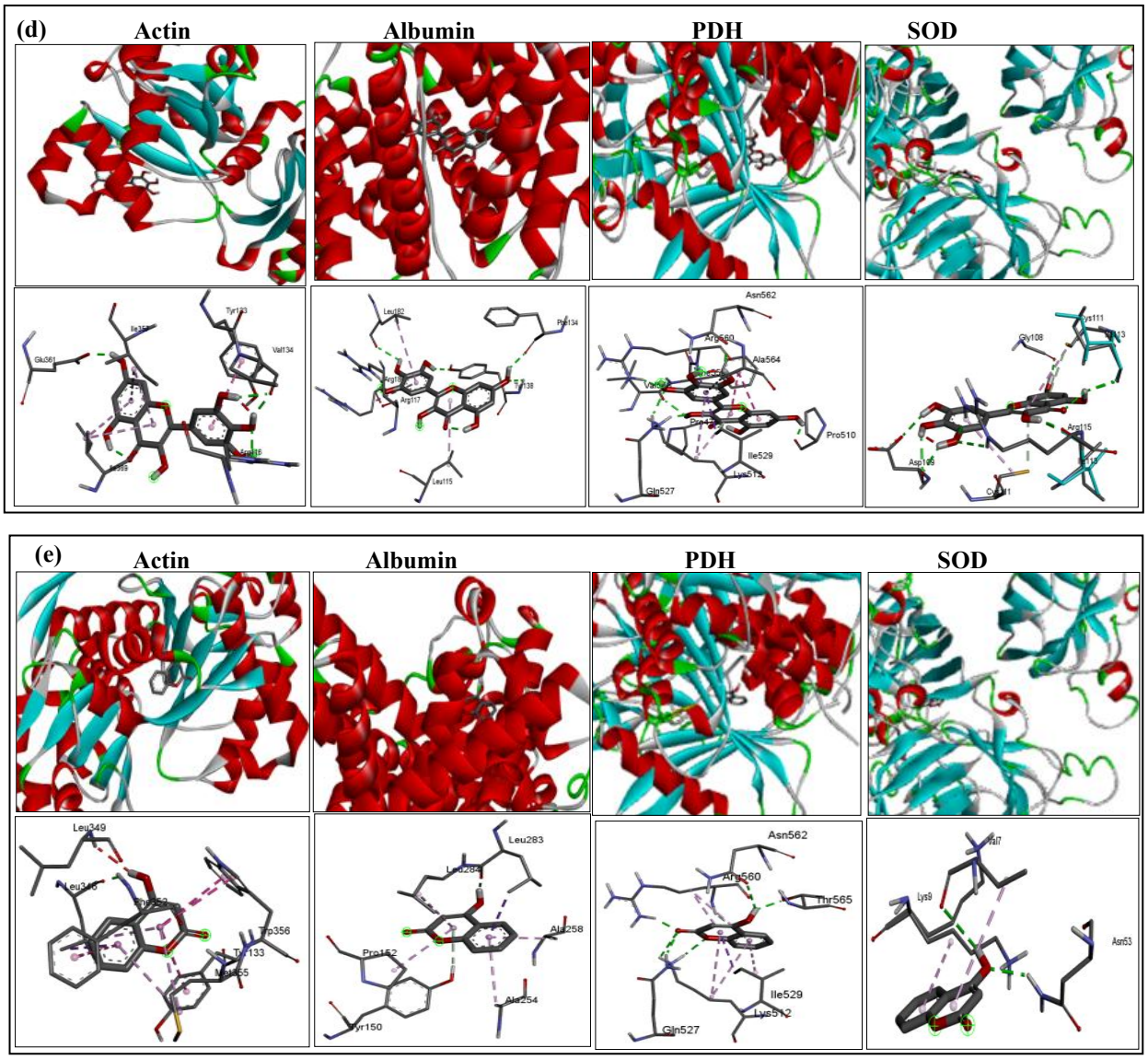


Figure 7. 3D graphics and molecular interactions among **(d)** myricetin and screened receptor proteins **(e)** 4-hydroxy coumarin and screened receptor proteins.

ii) Druggability analysis result of bioactive compounds

The bioactive compounds of PSp followed the Lipinski's Rule of five set guidelines. On evaluation of drug-likeness the SwissADME software was showing bioavailability score of 0.55 for all bioactive compounds which shows that all the bioactive compounds have the potential to be an orally active drug for human [32,33].

Table 7. SwissADME result of bioactive compounds

Ligands	Molecular Mass (≤500 Dalton)	Hydrogen Bond Donor (≤5)	Hydrogen Bond Acceptor (≤10)	No. of Rotatable Bonds (≤10)	Log p (≤5)	Molar refractivity (40-130)

Kaempferol	286.24	4	6	1	1.58	50.44
Catechin	290.27	5	6	1	0.83	74.33
4-hydroxy coumarin	162.14	1	3	0	1.43	44.51
Myricetin	318.24	6	8	1	0.79	88.06

When animals are exposed to arsenic, it binds to different enzymes and receptor proteins. Due to its high inhibitory constant (K_i) value, arsenic inhibits the normal activity of screened proteins, viz., actin, albumin, pyruvate dehydrogenase (PDH), and superoxide dismutase (Fig.6,7, table 6). When arsenic exposed animals are treated with a phenolic extract of spirulina the bioactive compounds, viz., kaempferol, catechin, 4-hydroxy coumarin, and myricetin, compete with arsenic for the active binding sites of proteins. All bioactive compounds of spirulina showed a higher docking score than arsenic for all screened proteins. The bioactive compounds easily replace arsenic from the active binding sites of screened proteins. Then released arsenic is carried away with blood to the kidney and thus excretes from the body. Among the four bioactive compounds, kaempferol showed the highest docking score, followed by 4-hydroxy coumarin, myricetin, and catechin. The lower inhibitory constant values of these bioactive compounds clearly explain that these compounds do not interfere with the enzymatic activities of screened proteins. Our *in silico* study results establish and explain the how phenolic compounds of spirulina helps in reduction of arsenic load and act as active drug. In the research work of D. Majumder *et al.* (2013), they established the potential of conventional chelators and reported meso-2,3 dimercaptosuccinic acid (DMSA) as the most potent among all [34]. Our *in silico* study results showed that the bioactive compounds of spirulina with a higher docking score are more potent than conventional chelators reported in that article. Therapeutic application of these phenolic contents derived from *Arthrospira platensis* is not yet reported in arsenic remediation. The druggability analysis confirms the potential of identified bioactive compounds as human consumable oral drug and suggests further exploration of its therapeutic use.

B. Conclusion

From the current research work, it can be concluded that the phenolic extract of spirulina provides significant protection against arsenic-induced severe haematological and renal degeneration. As a result of our research, we established that the phenolic part takes the lead role in the ameliorative potential of spirulina. The phenolic extract of the spirulina treatment does not produce any side effects and is easy to use without a prior pathological report. Although further study on human subjects is important to evaluate the potential effects of the phenolic extract of spirulina.

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