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Elucidation of the liver proteome in response to an antioxidant intake in rabbits

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Abstract

Background: Antioxidant intakes are one of the most cherished dietary approaches for the management of oxidative stress-induced liver damages. These antioxidants exist as the bioactive compounds present in plants and other natural sources functioning in varieties of ways from acting as direct scavengers of the free radicals to acting as the modifiers of genes and proteins expressions. *Chlorella vulgaris* is one of such antioxidants; it is a unicellular microalga and a rich source of polyphenols which has been reported for its capacity of reducing oxidative stress by upregulation of antioxidant genes. However, there are scarce reports on its effect on antioxidant protein expressions and functions in the liver. This situation necessitates untargeted proteomic profiling of the liver due to the antioxidant intakes as carried out in this present study. Sixteen laboratory weaner rabbits of 8 weeks old with initial average bodyweight of 1060 ± 29.42 g were randomly divided into two groups ($n = 8$ per group); the first group served as control while the second served as the treatment group were used for this study.

Results: After a period of 120 days daily consumption of 500 mg of *Chlorella vulgaris* biomass per kg bodyweight of the rabbit models, the animals were sacrificed and their livers were harvested followed by protein extraction for the untargeted proteomic profiling using LC-MS/Orbitrap Fusion Tribrid™ peptides quantifier and sequencer. Also, there was an assessment of the oxidative stress biomarkers in the liver and serum of the rabbits. Five-hundred and forty-four (544) proteins were identified out of which 204 were unique to the control, 198 were unique to the treatment group, while 142 were common to both groups of the rabbits. Antioxidant proteins commonly found in both groups were upregulated in the treatment group and were significantly associated with oxidative stress-protective activities. There was a reduction in oxidative stress biomarkers of the supplemented group as indicated by the assessment of the liver malondialdehyde concentrations ($p < 0.05$), total antioxidant capacities ($p < 0.05$), and antioxidant enzyme activities ($p < 0.05$). Similarly, these biomarkers were significantly reduced in the serum of the supplemented rabbits ($p < 0.05$).

Conclusion: The study concluded that *Chlorella vulgaris* is an antioxidant agent that could be suitable for reducing liver oxidative stress damage and it is a potential drug candidate for protecting the liver against oxidative stress damages as revealed in the rabbit models.

Keywords: Oxidative stress, *Chlorella vulgaris*, LC-MS, Malondialdehyde, Liver Proteins, Rabbits

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Background

Oxidative stress is a physiological and biochemical compromise affecting animal and human performances. It triggers damages of the liver by inducing irretrievable alteration of the lipids, proteins, and DNA contents of the liver as well as modulation of pathways associated with the control of normal biological functions of the liver [1, 2]. Therefore, to overcome these negative impacts of oxidative stress for the benefits of animals and human well-being, the intake of antioxidant-rich dietary supplements is a necessity. Microalgae are examples of such resources and previous studies have demonstrated the antioxidant efficacies of different types of microalgae among which is the microalga *Chlorella vulgaris*. The microalga *Chlorella vulgaris* is one of the most common microalgae suggested as a critical functional food and or feeds for humans and animals because of its rich protein, vitamins, and minerals [3].

According to Christaki et al. [4], microalgae generally can be considered as sources of minerals and complementary sources of protein and functional nutrients that are suitable for use in both animals and humans. *Chlorella vulgaris* as a functional food for humans, in addition to its regular nutritional composition of protein, carbohydrates, vitamins, and minerals, can provide health-promoting benefits such as immune activation, antioxidant, anticarcinogenic, and also serving as an antitoxic agent [5]. In a bid to facilitate the understanding of the mechanisms of the antioxidant properties of the microalga *Chlorella vulgaris*, its effects on patterns of gene expression in rabbit models have been investigated and it was discovered to be capable of inhibiting the progression of oxidative stress via upregulation of antioxidant genes in the liver of rabbits [6]. However, there are scarce reports on its effect on antioxidant proteins expression in the liver of rabbits; meanwhile, this is important because untargeted proteomic profiling is an approach suitable for the assessment of how effective is a gene code translated into the relevant polypeptide chains folded into functional proteins desirable for specific sub-cellular or extracellular physiological activities [7]. It remains one of the most required comparative avenues that could be used for evaluation and validation of the efficacies of supplement and nutraceutical agents including antioxidants.

For the management of liver-specific performance dysfunctions, it is highly critical to identify natural sources of an antioxidant such as microalgae to serve as dietary supplements as these could be cheap means of alleviating liver damage-related deaths across the world [1]. The alleviation of liver-associated diseases could mean the promotion of human well-being because these diseases were reported to be responsible for the cause of

approximately 2 million deaths per year across the world which are shared equally between complications of cirrhosis and viral hepatitis cum hepatocellular carcinoma, and non-alcoholic fatty liver diseases (NAFLD) [8]. However, these complications have close relationships with oxidative stress and this necessitates critical research for potential antioxidant drug candidates [9].

The microalga *Chlorella vulgaris* is an antioxidant agent with confirmed capacity of oxidative stress in inhibition at different physiological reproductive stages in rabbit models with specific oxidative stress protection for the liver [5, 10–12]. The microalga supplementation in fishes and other models was also reported and all the evaluations concluded that the microalga modulates the regulation and expression of primary antioxidant genes as mechanisms associated with its oxidative stress protection [13]. These submissions necessitate investigating the effect of the microalga on patterns of antioxidant protein expression in the liver considering its potential in the prevention and management of critical oxidative stress-induced liver diseases. This is the background on which this present study was carried out because proteins are critical to normal physiological functions since they are the biomolecules enabling genes to perform their functions in an organism. Hence, this present study hypothesized that for adequate protection against oxidative stress in the liver by *Chlorella vulgaris* it should be capable of increasing expression of proteins that are specific for the enhancement of the free radicals scavenging.

The production of these antioxidant proteins can also be used as an indicator for determining the post-translation impacts of antioxidant agents on gene expression modulation [14]. Furthermore, the process of quantifying and identification of these proteins associated with antioxidant agent supplementation could also serve as a novel approach for tracking and understanding the oxidative stress attenuation capacity of antioxidant agents such as *Chlorella vulgaris* [15]. However, to our knowledge, the effect of *Chlorella vulgaris* supplementation on the abundance of the hepatic proteome in connection with oxidative stress biomarkers in rabbits have not been reported. Therefore, the study focused on the identification and understanding of the proteins, processes, and pathways associated with oxidative stress in the liver of rabbits.

Methods

Experimental protocol and animal ethics approval

Sixteen laboratory rabbits sourced from the Laboratory Animal Facilities of Biogen Biotechnology (P) Ltd., Bangalore, India. The animals were used for this study and the approval for use of the animals and the implementation of the experimental protocols was obtained from a joint sitting of the Institutional Animal Ethics

Committee (IAEC) of National Institute of Animal Nutrition and Physiology, India, and Committee for Control and Supervision on Experiments on Animals (CPCSEA), India. The animals were divided into two experimental groups ($n = 8$ per group); the first group served as control while the second served as the treatment group. The rabbits in both groups were fed ad-libitum with commercial rabbit feed (Krishna Valley Agrotech (P) Ltd., India) as daily basal feeds. In addition to the basal feeds, rabbits in the treatment group were supplemented with 500 mg *Chlorella vulgaris* biomass per kg body weight daily for the period of 120 days. Water was also supplied ad libitum through automatic nipple drinkers, while the rabbits were housed individually in hutches.

Samples preparation and protein extraction

Sample collection and storage

The rabbits were selected one after the other for cervical dislocation to make them unconscious; the blood was collected exsanguination followed by the removal of the livers of each rabbit and kept in a cold phosphate buffer pH 7.4 then stored in a refrigerator ahead of downstream proteomics profiling at $-80\text{ }^{\circ}\text{C}$ (Thermo Fisher Scientific, India). Blood was collected for serum harvest by allowing the blood to coagulate at room temperature followed by centrifugation at 3500 rpm for 15 min (NEYA Centrifuge, India).

Quantification of the liver protein concentrations

Homogenates of each liver were prepared and the total protein concentration in each sample was determined using a commercial protein quantification kit (Arkray Healthcare (P) Ltd., India). The kit assay principle involves the reaction of protein with cupric ions in alkaline solutions to form colored chelate whose absorbance was measured at 578 nm. The assay is composed of Biuret reagent including copper sulfate (7 mmol/L), sodium hydroxide (200 mmol/L), sodium-potassium tartrate (20 mmol/L), and a surfactant. Bovine serum albumin was used as standard and the reaction mixtures were incubated at $37\text{ }^{\circ}\text{C}$ for 5 min before the absorbance measurement using a Thermo Multiskan GO microplate reader (Thermo Fisher Scientific, Finland).

Determination of oxidative stress biomarkers

Oxidative stress biomarkers including the malondialdehyde, protein carbonyl contents, total antioxidant capacity, and antioxidant enzyme activities of the liver and serum were determined using chemical assays as described in our previous studies [5].

Proteomic profiling: protein extraction, precipitation, digestion, and quantification

Protein extraction

There was protein extraction from the liver of the rabbits using the radioimmunoprecipitation assay (RIPA)

buffer made up of 25 mmol Tris HCl (pH 7.6), 150 mmol sodium chloride (NaCl), 1% nonyl phenoxypolyethoxyethanol-40 (NP-40), and 0.1% sodium dodecyl sulfate (SDS). Simply, the procedures involve washing of the liver samples earlier stored at $-80\text{ }^{\circ}\text{C}$ in phosphate buffer (pH 7.4) after being thawed overnight at $4\text{ }^{\circ}\text{C}$. Then for each of the samples, 100 mg of the liver was sampled, and lysed under liquid nitrogen after which they were homogenized for 1 min then sonicated for 80 s in 4 strokes of 15 s each at 5-s interval (Thermocube, SScooling Systems; USA). The samples after sonication were centrifuged at 10,000 rpm at $4\text{ }^{\circ}\text{C}$ for 10 min (REMI microfuge, India) and the supernatants were collected then stored at $-80\text{ }^{\circ}\text{C}$ ahead of the downstream proteomic analysis.

Protein precipitation, digestion, and desalting

There was a sampling of 20 μg protein for precipitation in acetone overnight then centrifuge at 10,000 rpm (REMI Microfuge, India). Then the precipitated protein samples were dissolved in 25 mmol ammonium bicarbonate for the analysis. The analysis commenced with the addition of 100 mmol of DTT (dithiothreitol) then incubated at $37\text{ }^{\circ}\text{C}$ for 30 min. After the incubation, there was an addition of 100 mmol IAA (Iodoacetamide) to obtain a concentration of 30 mmol in the solution and kept in dark for 30 min after which the pH of the solution was adjusted to 8 by adding 25 mmol ammonium bicarbonate. This was followed by digestion in trypsin (0.2 $\mu\text{g}/\mu\text{L}$) at a concentration of 1:50 trypsin: proteins; and after the trypsin digestion, the samples were incubated overnight at $37\text{ }^{\circ}\text{C}$. Formic acid with a final concentration of 0.1% was then added to stop the reaction. The digested protein samples were desalted using 3 \times of 100% Acetonitrile and 3 \times of 0.1% formic acid then passed through a mini-column which wets the resin and conditioned to bind peptides from the protein samples. The column used was an ion exchange mini-column while buffers including 0.1% formic acid in high-performance liquid chromatography (HPLC) water and 80% Acetonitrile + 0.1% formic acid in HPLC water were used as fundamental mobile phase solvents.

Protein sequencing and LC-MS quantification

For the peptides quantification, 1 μL of the digested samples were used for liquid chromatography-mass spectrometry (LC-MS) quantification of the peptides in the protein samples using LC Model EASY nLC 1200 (Thermo Fisher Scientific, USA), with nanoflow rate 300 nL/min and the protein sequencing was done using Orbitrap Fusion Tribrid™ peptides quantifier and sequencer.

Determination of isoelectric point and amino acids composition top 20 unique proteins in each group

The isoelectric point and amount of the amino acid in the top 20 proteins identified in each of the control and the treatment group was determined using a web-based isoelectric point calculator [16].

Statistical and bioinformatics analysis

The uniprot_rabbit_2019 database was used for the protein sequence identification; parameters including a fragment mass tolerance of 0.6 Da with a false discovery rate (FDR) of 1% at the significance threshold of $p < 0.004$ was used for the taxonomy of the proteins sequences while all possible entries of the amino acid sequence were accepted. The data obtained for oxidative stress biomarkers of the liver and serum samples were subjected to the Mann-Whitney U test at $p < 0.05$ for determining the differences between the oxidative stress biomarkers in the liver and serum of the rabbit due to the intake of the *Chlorella vulgaris*. For the functional enrichment, processes, and pathways analyses, the hepatic proteome data obtained were analyzed using the STRING database, g:profiler, and Cytoscape [17].

Results

Liver and serum oxidative stress status of the rabbits

There was a significant difference in oxidative stress status of the rabbits' liver and serum. The concentration of the liver lipid peroxidation biomarker malondialdehyde was 10.62 ± 6.20 nmol/g of the liver sample ($p < 0.05$), while the minimum and maximum lipid peroxidation biomarkers were 1.03 and 19.65 nmol/g of the liver sample, respectively. There was also a significant difference

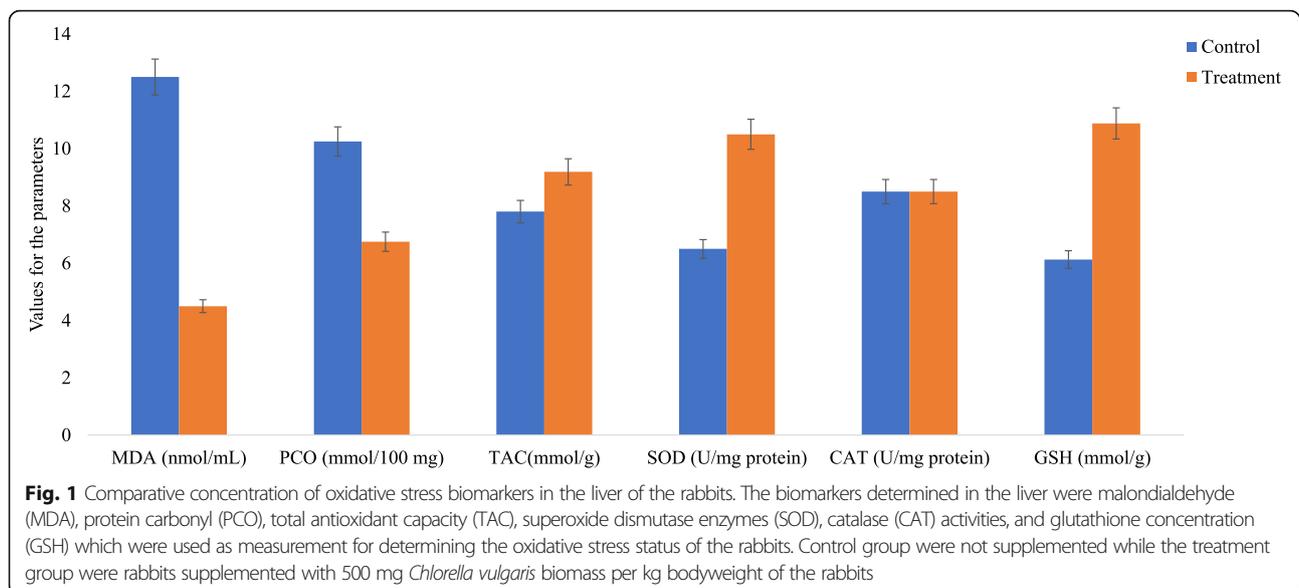
in the total antioxidant capacity, protein carbonyl content, activities of the superoxide dismutase, and the concentration of glutathione in the liver of the rabbits. The concentration of glutathione was 9.49 ± 2.39 mmol/g of the liver samples ($p < 0.05$). The supplemented rabbits had lower oxidative stress status (Figs. 1 and 2).

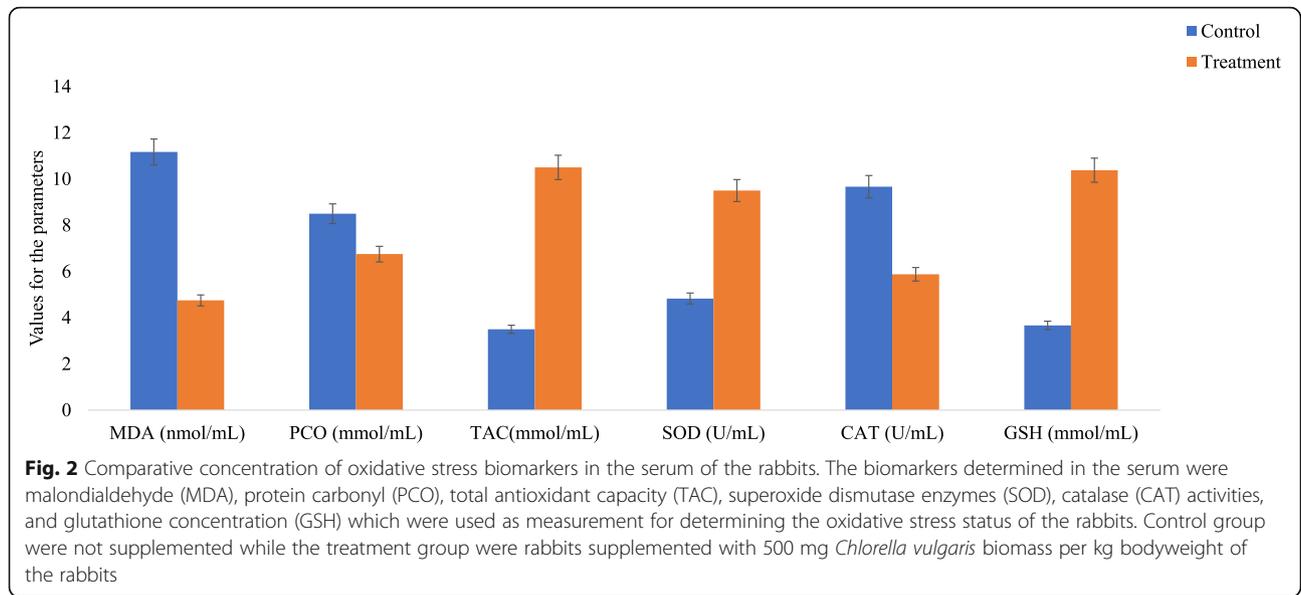
Liver proteome and protein abundance

In total, there was an identification of 544 proteins, and out of these proteins, 204 proteins were unique to the control group, 198 proteins were unique to the treatment group, while 142 proteins were common to both the control and treatment groups. The control group made up of 2, 100, 10, and 234 predicted, uncharacterized, LOC, and known proteins, respectively. Similarly, the treatment group comprised of 2, 106, 2, and 230 predicted, uncharacterized, LOC, and known proteins, respectively (Fig. 3). The topmost unique proteins in each of the groups indicated similar physicochemical features (Tables 1 and 2).

Functional enrichment of the upregulated antioxidant proteins

Antioxidant proteins including SOD1, SOD2, catalase (CAT), DHRS4, PRDX3, macrophage migration inhibitory factor (MIF), HSPD1, ALB, and glutathione S-transferase Pi 1 (GSTP1) were found in both groups but they were significantly upregulated in the treatment group compared with the control group (Fig. 4). The enrichment of these proteins revealed their involvement in biological processes and pathways relevant to oxidative stress protection as detailed in (Supplementary Table 1). Elucidation of the upregulated antioxidant proteins with a specific focus on





their roles in oxidative stress indicated that the proteins are involved in oxidoreductase activities, antioxidant protection by decomposing of hydrogen peroxide, and response to interleukin-12 complications (Fig. 5).

Discussion

In this present study, the effect of *Chlorella vulgaris* supplementation on protein expression in connection with liver oxidative stress of rabbits was investigated. It is necessary to understand this because oxidative stress is a

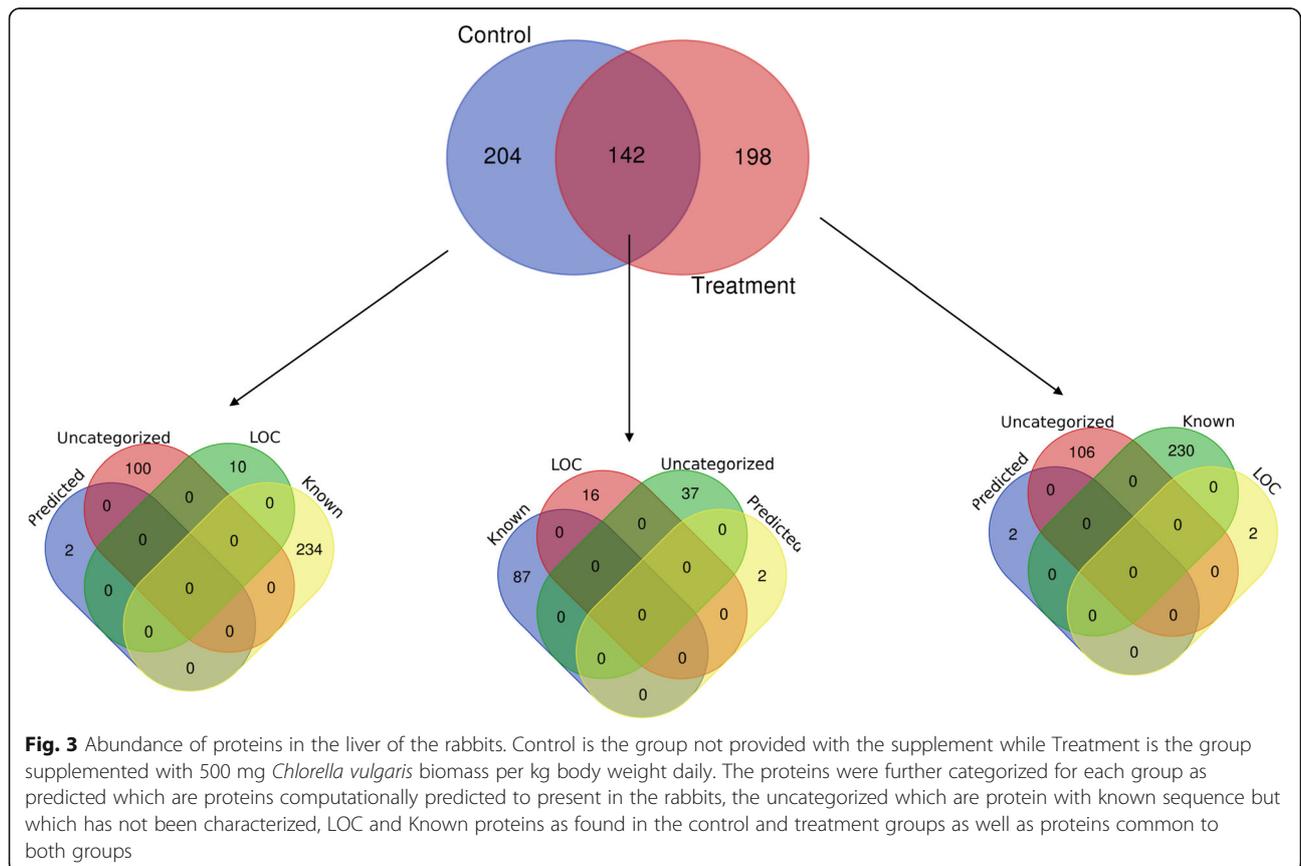
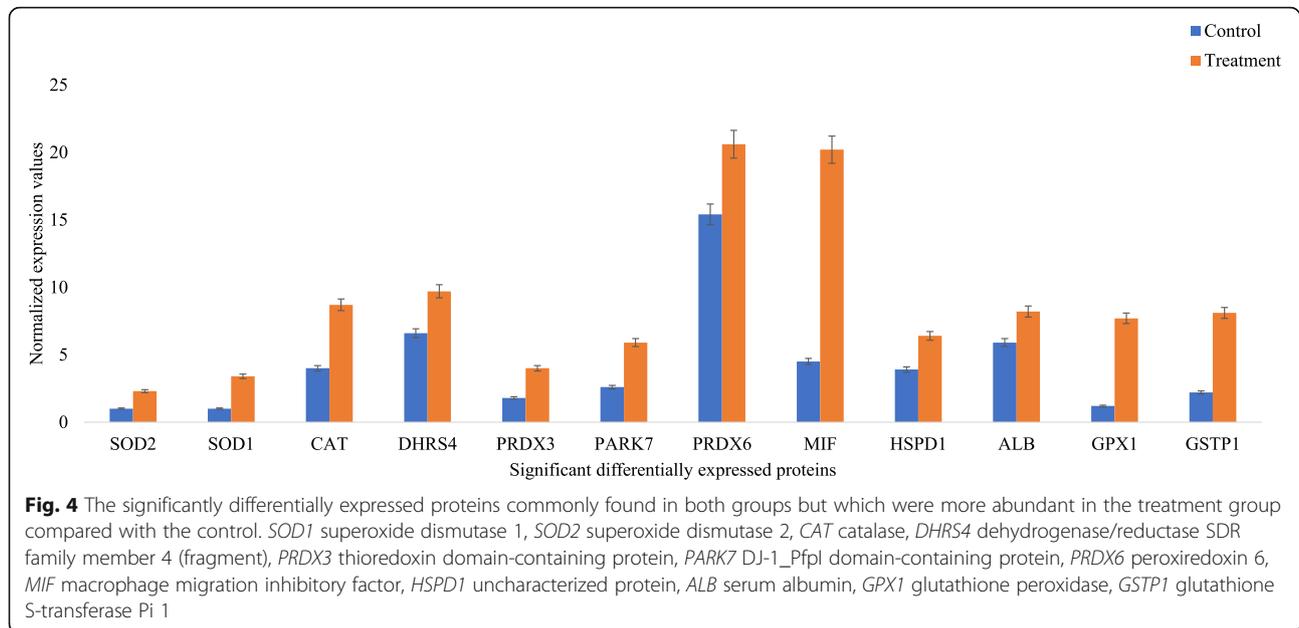


Table 1 List and physicochemical properties of topmost abundant proteins in the liver proteome of the rabbits in the control group

	Accession	Description	Mass (KDa)	Score	pI	No of amino acids
1	B8K174	Hemoglobin, beta (Predicted)	16.18	2141	7.67	147
2	B8K132	Alpha-hemoglobin (Predicted)	15.65	1042	7.68	142
3	G1TEA7	Uncharacterized protein	166.64	1017	5.89	1479
4	P12337	Liver carboxylesterase 1	62.59	912	5.92	565
5	G1SRL5	Carboxylic ester hydrolase	62.43	884	5.99	579
6	Q08862	Glutathione S-transferase Yc	25.55	665	7.91	221
7	Q08863	Glutathione S-transferase alpha I	25.73	485	8.22	223
8	G1T4A7	Uncharacterized protein	29.40	337	6.02	257
9	G1T2B1	Uncharacterized protein	25.56	320	8.53	221
10	G1T2A7	Uncharacterized protein	25.46	295	8.33	225
11	U3KNH6	Uncharacterized protein	16.56	281	7.85	221
12	Q03505	Alcohol dehydrogenase 1	40.36	267	7.46	375
13	G1T295	Epoxide hydrolase	52.72	260	6.68	455
14	Q8M117	Retinal dehydrogenase 1	54.93	240	6.51	496
15	G1SUY2	Aldedh domain-containing protein	54.83	219	6.14	521
16	G1T6W7	Catalase	59.91	199	6.69	527
17	G1SL62	Annexin	39.58	193	7.46	342
18	G1SKJ7	Glycine N-methyltransferase	33.30	185	6.66	295
19	G1SNT8	Annexin	89.35	181	5.37	786
20	P00179	Cytochrome P450 2C5	55.92	179	6.24	487

Table 2 List and physicochemical properties of topmost abundant proteins in the liver proteome of the rabbits in the treatment group

	Accession	Description	Mass (KDa)	Score	pI	No of amino acids
1	B8K174	Hemoglobin, beta (Predicted)	16.18	2804	7.67	147
2	B8K132	Alpha-hemoglobin (Predicted)	15.65	777	7.68	142
3	G1U9S2	Serum albumin	70.92	293	5.63	608
4	Q0QEN9	ATP synthase subunit beta (Fragment)	45.55	254	5.09	422
5	G1SL06	Actin, alpha skeletal muscle	42.37	248	5.03	369
6	G1SJS1	Histone H2B	13.91	248	9.76	126
7	G1T6W7	Catalase	59.91	217	6.69	527
8	P15541	Aminopeptidase N	109.65	195	5.06	966
9	G1T346	Uncharacterized protein	290.20	194	5.12	2541
10	G1T3Y8	Uncharacterized protein	56.80	185	5.58	573
11	G1SYV9	Uncharacterized protein	272.75	183	5.62	2558
12	G1SZF7	Isocitrate dehydrogenase [NADP]	51.26	176	7.9	452
13	G1SFP0	Cytosol AP domain-containing protein	56.65	175	7.74	519
14	P79226	Fructose-bisphosphate aldolase B	40.04	173	7.45	364
15	P00883	Fructose-bisphosphate aldolase A	39.77	170	7.29	364
16	G1SL62	Annexin	39.58	167	7.46	342
17	G1SDD0	Uncharacterized protein	31.53	157	7.74	280
18	G1SXX5	Uncharacterized protein	83.21	155	8.51	797
19	G1SKT4	ATP synthase subunit alpha	59.83	153	8.43	553
20	Q9GKX2	Dehydrogenase/reductase SDR family member 4 (Fragment)	27.64	152	7.71	260



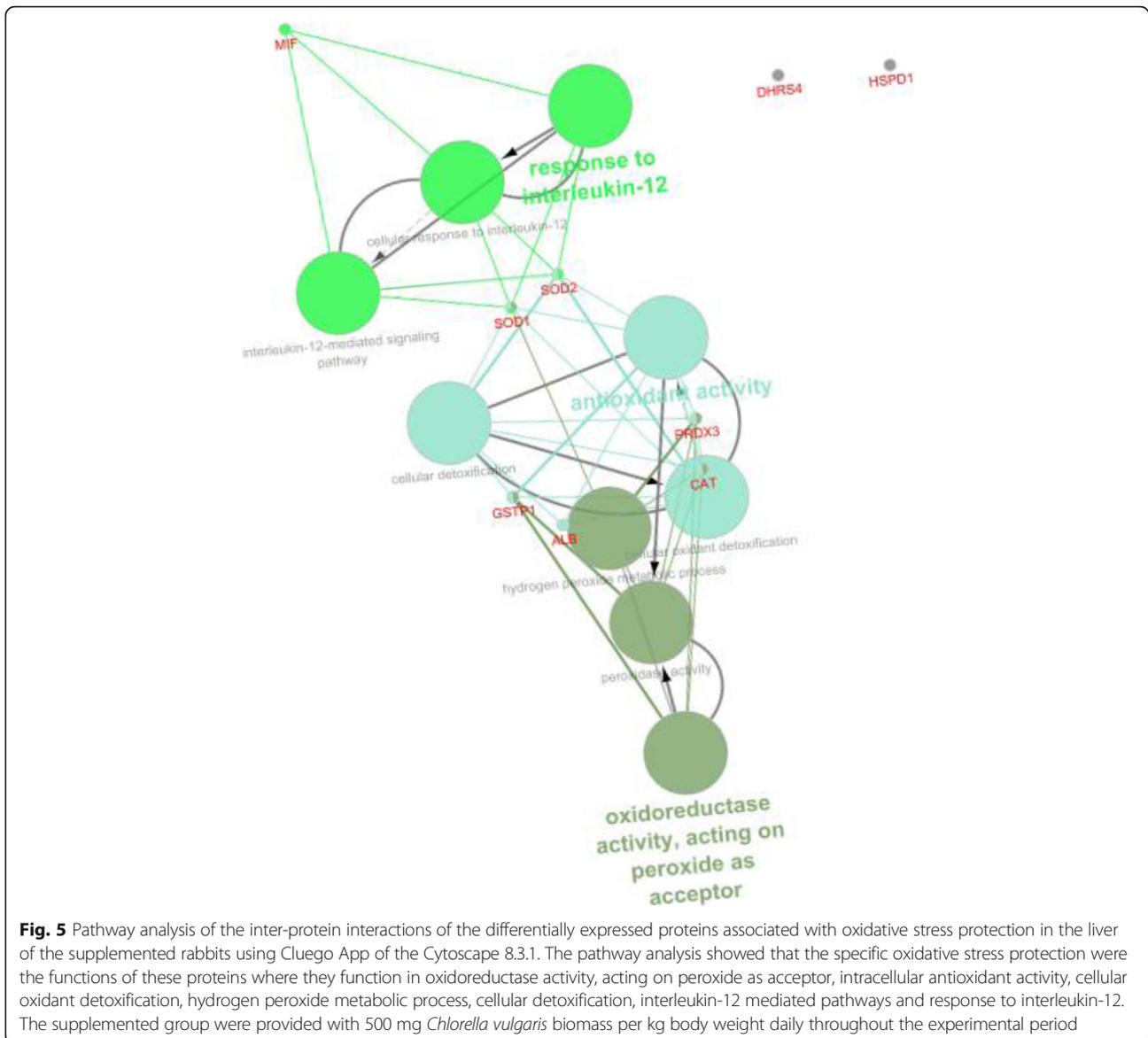
biochemical process causing the generation of reactive oxygen species, molecules, and ions formed through incomplete electron reduction reaction of oxygen and this is strongly involved in pathways affecting normal cellular functions of the liver. Oxidative stress is a critical factor responsible for the progression of liver and non-liver dysfunctional conditions such as non-alcoholic fatty liver diseases (NAFLD), male infertility, pulmonary carcinogenesis, hypoxia, and vascular malfunctions [18–22]. However, despite the ubiquitous nature of oxidative stress, the supplementation of antioxidants have been widely reported as a way-out of its complications but the exact mechanisms underlying efficacies of some of the antioxidants in oxidative stress protection in-vivo is poorly understood [23].

Although, reports from recent investigations on antioxidant agent supplementation identified that gene expression and regulation modification for cellular protection against oxidative stress in rabbits and other models are mechanisms associated with intakes of antioxidant agents including microalgae [24, 25]. Therefore, in taking a step further, this present study confirmed some of the assertions by indicating that antioxidant agents could have some post-transcription modulation impacts against oxidative stress because the supplementation of *Chlorella vulgaris* in this study led to a higher antioxidant proteins expression in the liver of the supplemented rabbits. The outcome of this present study also showed that primary antioxidant enzymes such as superoxide dismutase, catalase, and glutathione could be active across the cytoplasmic and mitochondria phases of oxidative stress protections since observations recorded showed that the proteins are more in the

treatment group versus the control. Meanwhile, this is in agreement with a previous report which stated that the increasing abundance of antioxidant proteins could be associated with the effectiveness of cytosolic and mitochondria oxidative stress protection in cells [26].

Furthermore, functional enrichment of the proteins upregulated in the supplemented rabbits indicated that in association with other proteins, they are involved in the scavenging of free radicals and their upregulation is required for reduced oxidative stress. This observation could be accepted as a confirmation that the microalga *Chlorella vulgaris* is a potential drug and nutraceutical candidate since the biological processes and pathways analysis of the proteome indicated that the supplementation of the microalga modulated cellular response of the liver to inhibit cellular oxidative stress damage basically via the hydrogen peroxide metabolism and detoxification of xenobiotics compounds. These cellular activities can be linked to prominent mechanisms involving catalytic removal of free radicals by superoxide dismutase, catalase, and peroxidase, and scavenging of reactive species by low molecular agents such as carotenoids present in the microalga *Chlorella vulgaris* [27].

The superoxide dismutase proteins were more abundant in the supplemented rabbits, and this could be regarded as the starting point of endogenous antioxidant protection mechanism whereby one superoxide radical is oxidized and the second one is reduced to eliminate continuous damage by radical oxygen. Although this mechanism usually leads to the production of hydrogen peroxide which is another reactive compound, the hydrogen peroxide produced is usually quickly acted upon by subsequent enzymatic actions. The catalase and



glutathione peroxidases carry out further actions in the cytoplasm while in the mitochondria glutathione reductase carry out the inhibition of the free radical activities; these observations agreed with submissions of [27].

The inter-protein interactions and functional enrichment analyses in this present study indicated that the supplementation of *Chlorella vulgaris* biomass as an antioxidant in rabbit led to increased activities of the antioxidant enzymes activities and increased production of their respective proteins to prevent liver oxidative stress damages and progression in a mechanism similar to impacts of ascorbate which is a common conventional antioxidant agent. In agreement with this submission, Halliwell and Gutteridge [28] suggested that antioxidant agent supplements are expected to exhibit the capability of enhancing both enzymatic and non-enzymatic

antioxidant activities. In upholding this submission, this present study demonstrated that the intake of the microalga increases the antioxidant enzymes activities and their respective proteins including the catalase (CAT), peroxidase (PRDX6), macrophage migration inhibitory factor (MIF), and glutathione S-transferase Pi 1 (GSTP1) due to their higher abundance in the supplemented group compared with the control group.

The reduction of the lipid peroxidation biomarker malondialdehyde in the liver of the supplemented rabbits could be linked with the increased expression of the antioxidant proteins because of the microalga intakes and this can be described as a mechanism of oxidative stress protection in the liver promoted by the microalga. In specific, the higher expression of the catalase protein could be primarily linked with the protection of cells

against the toxic effects of hydrogen peroxide as well as promoting the growth of immune cells including T cells and B cells; and hence, this could be described as double effects of oxidative stress protection and immune enhancement. This observation is in agreement with [29], which stated that antioxidant therapy focusing on reducing oxidative stress and enhancing activities of T cells and B cells could also lead to immune protection against oxidative stress-mediated diseases such as organ damage and comorbidities in systemic lupus erythematosus (SLE).

Similarly, increased expression of peroxiredoxin (PRDX6) in the supplemented rabbit affirm competency of the microalga supplemented to protect against oxidative stress. This is because as a protein-encoded gene and a member of the thiol-specific antioxidant protein family, it has a bifunctional enzyme with two distinct active sites involved in redox regulation of the cell [30]. The increased expression of peroxiredoxin in the supplement rabbits can reduce pro-oxidant activities of hydrogen peroxide and short-chain organic, fatty acid, and phospholipid hydroperoxides and it may also play a role in the regulation of phospholipid turnover as well as in protection against oxidative injury [7]. The microalga supplementation also increased MIF which indicates that it is a potent antioxidant agent since the gene MIF encodes a lymphokine involved in cell-mediated immunity, immunoregulation, and inflammation which plays roles in the regulation of macrophage function in host defense through the suppression of anti-inflammatory effects of glucocorticoids—a mechanism substantial for protection against environmental triggered stress at the cellular level [31].

In correlating the malondialdehyde concentration reduction with the increasing abundance of antioxidant proteins in the supplemented rabbits as observed in this study, it could be juxtaposed that supplementation of the microalga *Chlorella vulgaris* prevented attacks on lipid signaling and also led to the upregulation of its associated proteins as well as enhanced activities of the antioxidant enzymes. These submissions agreed with [32], which opined that under normal physiological conditions, there will be reduced malondialdehyde concentrations necessary for cellular stimulation, maintenance, and survival occurring through activities of antioxidant enzymes and antioxidant genes for promoting the constitutive antioxidants defense systems and protection of lipid signaling pathways as well as activation of genes expression required for upregulating antioxidants proteins needed for the formation of adequate protection and adaptation to the stress response. The underlying physiological importance of reduced malondialdehyde in association with the antioxidant protein abundance in

the liver could therefore be described as critical for the promotion of healthy living. This is because if otherwise, the cells can induce inordinate apoptosis or necrosis resulting in cell deaths and facilitation of various pathological conditions [33].

The mechanism of oxidative stress promotion of liver diseases involves the activation of stellate cells by the free radicals leading to the synthesis of collagen and the extracellular matrix [34]. These are complications associated with liver diseases such as non-alcoholic fatty liver diseases, fibrosis, and encephalopathy. This present study suggests that *Chlorella vulgaris* could serve as an antioxidant supplement suitable for the alleviation of these liver diseases. This is in agreement with a report that the microalga improved liver health through the modulation of the fasting blood sugar and lipid profile in a double-blind randomized placebo-controlled clinical trial involving 60 NAFLD patients [35, 36]. Furthermore, the present study demonstrated a possible molecular basis for the effectiveness of the microalga as an antioxidant agent suitable for the management of liver damages because of the inter-protein interaction of the significantly upregulated antioxidant proteins in the supplemented group which revealed the involvement of the proteins in more than a hundred pathways associated with oxidative stress-protective activities (Supplementary Table 1).

Conclusion

This present study in its novelty contributed that liver is an organ vulnerable to oxidative stress damages which could be a critical promoter of acute and or chronic liver diseases. The microalga *Chlorella vulgaris* is a potential candidate for antioxidant drugs that could be effectively used to reduce liver oxidative stress because it reduces the generation of oxidative stress products, enhances the activities of antioxidant enzymes, and promote the upregulation of antioxidant proteins in the liver.

Abbreviations

ICAR: Indian Council for Agricultural Research; NIAP: National Institute of Animal Nutrition and Physiology; NAFLD: Non-alcoholic fatty liver diseases; IAEC: Institutional Animal Ethics Committee; CPCSEA: Committee for Control and Supervision on Experiments on Animals; RIPA: Radioimmunoprecipitation assay; SDS: Sodium dodecyl sulfate; DTT: Dithiothreitol; IAA: Iodoacetamide; LC-MS: Liquid chromatography-mass spectrometry; HPLC: High-performance liquid chromatography; FDR: False discovery rate; CAT: Catalase; PRDX6: Peroxiredoxin; MIF: Macrophage migration inhibitory factor; GSTP1: Glutathione S-transferase Pi 1; SOD: Superoxide dismutase

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s43066-021-00118-3>.

Additional file 1. Supplementary Table 1.

Additional file 2. Protein Abundance.

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Availability of data and material

All data were reported in the manuscript and additional data are presented as supplementary materials.

Consent for publications

Not applicable.

Declarations

Authors' contributions

Conceptualization: SAB, AA. Data curation: SAB, AA. Formal analysis: SAB. Methodology: SAB, AA. Software: AA. Validation: AA, ESSA, IJR, SV, RB. Investigation: SAB. Writing—original draft: SAB. Writing—review and editing: AA, ESSA, IJR, SV, RB. All authors have read and approved the manuscript for submission.

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Ethics approval and consent to participate

Approval for use of the animals and the implementation of the experimental protocols were obtained from a joint sitting of the Institutional Animal Ethics Committee (IAEC) of National Institute of Animal Nutrition and Physiology, India, and the Committee for Control and Supervision on Experiments on Animals (CPCSEA), India. The approval reference number is 141437/GO/Re/SL/11/CPCSEA Dt.8.12.2017 Valid up to 7.12.2022.

Competing interests

The authors declare that they have no competing interests.

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