



Effects of dietary supplementation of *Chlorella vulgaris* on oxidative stress attenuation and serum biochemical profile of pregnant New Zealand White rabbits

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ABSTRACT

Oxidative stress negatively affects animals during gestation period and this condition is almost inevitable in the Tropics because of temperature elevation; therefore, objective of this study was evaluation of antioxidant effects of *Chlorella vulgaris* supplementation in pregnant rabbits. New Zealand white rabbits (40) were randomly distributed into five groups (n = 8) on day 0 of their gestation and were supplemented with 0, 200, 300, 400 and 500 mg *Chlorella vulgaris* biomass per kg body weight respectively throughout the gestation period. Blood was collected from the animals in the last week of gestation for serum oxidative stress and biochemical profile assessments. There was significant difference in serum malondialdehyde concentration, total antioxidant capacity but protein carbonyl content was not significantly different. There was also significant difference in superoxide dismutase activity, catalase activity and glutathione concentration. Furthermore, the results showed that serum biochemical profiles of the rabbits were within the normal ranges for healthy rabbits. The study therefore concluded that supplementation of *Chlorella vulgaris* significantly protects the rabbits against oxidative stress damage and has no deleterious effects on their organs function; hence, the microalga was recommended as an antioxidant supplement for pregnant rabbits.

Keywords: *Chlorella vulgaris*, Malondialdehyde, Oxidative stress, Rabbit

Microalgae contained bioactive compounds formed through biodegradation and/or biosynthesis activities of the algae components leading to the production of some modified metabolites capable of being used as functional supplements (Qin 2018). *Chlorella vulgaris* is one of the most common and commercially available microalgae, it is a rich source of antioxidants (Otlés and Pire 2001, Porse and Rudolph 2017). Oxidative stress complication is one of the most notorious unavoidable biochemical mechanisms associated with physiological dysfunctions during gestation; it is characterized by high blood pressure, excretory fluid retention as well as proteinuria (Castro-Garcia *et al.* 2018). Therefore, antioxidants supplementation during gestation period was suggested as health-promoting nutritional strategy (Idonije *et al.* 2011). Unfortunately, some common antioxidants currently used for the management of oxidative stress have proved inefficient and this situation calls for further exploitation of more natural antioxidant sources (Halliwell and Gutteridge 2015); this informed evaluation of the microalgae *Chlorella vulgaris* in this study by examining its capacity for oxidative stress attenuation in pregnant rabbits.

MATERIALS AND METHODS

Animal experimentation protocol for this research was approved by the Institutional Animal Ethics Committee (IAEC) of National Institute of Animal Nutrition and Physiology, Bengaluru; India. Forty New Zealand White rabbits obtained from Biogen Laboratory Animals Facility, Bengaluru; India were used for the study. The animals were randomly divided into five experimental groups (n = 8 per group) as Control, T1, T2, T3, and T4; each group was supplemented with 0, 200, 300 400 and 500 mg *Chlorella vulgaris* biomass per kilogram body weight of the rabbits respectively from day 0 of gestation throughout the gestation period (Table 1). During the last week of the gestation period, 2 ml blood samples were obtained through the mid-ear veins of the rabbits using disposable 2 ml syringe (Hindustan Syringes and Medical Devices Ltd, India); collected blood was centrifuged at 3,500 rpm for 15 min at 4°C (NEYA Benchtop Centrifuge; REMI India), serum obtained were kept at –80°C (Samsung, India) for downstream analysis.

Superoxide dismutase enzyme activities were determined using auto-oxidation of Benzene-1,2,3-triol in the presence of Diethylenetriaminepentaacetic acid (Guvvala *et al.* 2019). Catalase enzymes activities in the serum were determined using the serum capabilities to decompose hydrogen peroxide (H₂O₂) while reduced glutathione was determined following the protocol of Moron *et al.* (1979).

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Table 1. Quantity of *Chlorella vulgaris* biomass supplemented to animals in each experimental group

Experiment group	<i>Chlorella vulgaris</i> supplementation quantity
Control	0 mg <i>Chlorella vulgaris</i> supplementation
T1	200 mg <i>Chlorella vulgaris</i> supplement per rabbit/kg body weight
T2	300 mg <i>Chlorella vulgaris</i> supplement per rabbit/kg body weight
T3	400 mg <i>Chlorella vulgaris</i> supplement per rabbit/kg body weight
T4	500 mg <i>Chlorella vulgaris</i> supplement per rabbit/kg body weight

Serum lipid peroxidation assessment was carried out by quantification of malondialdehyde according to protocol described by Buege and Aust (1978); while protein carbonyl content was determined based on the protocol of Colombo *et al.* (2016) and overall antioxidant capacity of the serum was determined according to protocol described by Benzie and Strain (1999). The serum biochemical profile was determined using commercial kits according to manufacturers' instructions. Statistical analysis was done by subjecting all data to analysis of variance (ANOVA) using SPSS version 20.0 (IBM Corporations, USA); significant means were determined at $P < 0.05$ and the means were subjected to homogeneity test using Duncan test of the *Post hoc* tools in the software package; the results are presented as Mean $\pm$ SEM for each of the parameters determined.

RESULTS AND DISCUSSION

There were significant effects of the supplementation of the microalgae on oxidative stress products generation as well as activities of antioxidant enzymes in the pregnant rabbits' serum samples. The concentration of lipid peroxidation product malondialdehyde was 12.36 ± 1.24 nmol/mL ($P < 0.001$), protein carbonyl was 12.15 ± 5.62 mmol/mL ($P < 0.66$) and total antioxidant capacity was 41.34 ± 4.54 mmol/mL ($P < 0.002$) as presented in Table 2. The supplementation also enhanced activities of antioxidant enzymes including superoxide dismutase activity which was 6.40 ± 0.31 U/mL ($P < 0.02$), catalase activity which was 5.78 ± 0.52 ($P < 0.02$) and glutathione reduced concentration which was 6.61 ± 0.22 mmol/mL ($P < 0.001$) as presented in Table 3.

Chlorella vulgaris supplementation according to outcomes of this study led to reduction of malondialdehyde concentration in the serum of the rabbit; this is an indication that supplementation of the microalgae can prevent oxidative stress and can effectively promote health and production of animals as well as human well-being during gestation period. The reduction also suggests that the microalgae have potential to serve as animal feed supplement for management of diseased conditions where

Table 2. Effects of *Chlorella vulgaris* supplementations on serum oxidative stress biomarkers of the rabbits

Treatment	Malondialdehyde (nmol/mL)	Protein carbonyl (μ mol/mL)	Total antioxidant capacity (μ mol/mL)
Control	21.35 ± 2.61^a	30.32 ± 4.76	14.18 ± 1.56^a
T1	11.04 ± 0.32^b	9.01 ± 11.96	52.05 ± 7.73^b
T2	11.56 ± 1.60^b	7.53 ± 14.13	33.28 ± 7.75^{ab}
T3	9.32 ± 1.68^b	8.30 ± 21.81	48.42 ± 9.92^{bc}
T4	8.54 ± 1.01^b	5.62 ± 4.48	58.76 ± 3.11^c

Values are presented as Means $\pm$ SEM while means with a different superscript in the same column are significantly different ($P < 0.05$). Control, group without supplementation; T1, group supplemented with 200 mg/kg of *Chlorella vulgaris* biomass; T2, group supplemented with 300 mg/kg of *Chlorella vulgaris* biomass; T3, group supplemented with 400 mg/kg of *Chlorella vulgaris* biomass; T4, group supplemented with 500 mg/kg of *Chlorella vulgaris* biomass.

Table 3. Effects of *Chlorella vulgaris* supplementations on serum antioxidant enzymes activities of the rabbits

Treatment	Superoxide dismutase activities (U/mL)	Catalase enzyme activities (U/mL)	Glutathione reduced (μ mol/mL)
Control	4.63 ± 0.14^a	2.86 ± 0.64^a	4.45 ± 0.49^a
T1	6.59 ± 1.13^b	5.46 ± 0.92^{ab}	6.45 ± 0.10^b
T2	7.38 ± 0.39^b	7.50 ± 1.15^b	6.38 ± 0.01^b
T3	7.13 ± 0.28^b	6.37 ± 0.67^b	7.16 ± 0.02^c
T4	6.36 ± 0.10^b	6.70 ± 0.15^b	6.39 ± 0.02^b

Values are presented as Means $\pm$ SEM while means with a different superscript in the same column are significantly different ($P < 0.05$). Control, group without supplementation; T1, group supplemented with 200 mg/kg of *Chlorella vulgaris* biomass; T2, group supplemented with 300 mg/kg of *Chlorella vulgaris* biomass; T3, group supplemented with 400 mg/kg of *Chlorella vulgaris* biomass; T4, group supplemented with 500 mg/kg of *Chlorella vulgaris* biomass.

oxidative stress is reported as a pathophysiological mechanism because reduction of lipid peroxidation product malondialdehyde in the serum showed that there was inactivation of oxidative stress due to the supplementation through enhanced activities of antioxidant enzymes (Berkson 1999).

Similar reports were given as outcomes of *Chlorella vulgaris* supplementation during gestation and lactation in sheep where the supplementation of the microalgae enhanced activities of antioxidant enzymes activities, promoted the sheep health as well as their lambs' performances. Supplementation of the microalgae in pregnant and lactating mice was also reported to lower malondialdehyde concentration and improved activities of hepatic enzymes according to Singh *et al.* (1998), Skrzydlewska *et al.* (2005) and Travnicek *et al.* (2008).

The potential capacity of *Chlorella vulgaris* to inhibit oxidative stress could serve as a low cost and natural alternative to synthetic as well as expensive chemical-based antioxidant used in animal feeds and feeding management; the microalga could also serve as functional feedstuff and supplement because of its ability to react with free radicals thereby decomposing hydrogen peroxides and inhibit malondialdehyde generation as demonstrated in this study. This is also in agreement with outcomes of its supplementation in pregnant rat model where it was reported to protect against oxidative stress and improved fetal performances (Cederberg and Erikson 2003).

There was also report that supplementation of *Chlorella vulgaris* in human reduced incidence of pre-eclampsia in a population at high risk of the condition during gestation period through significant reduction in malondialdehyde concentration (Panahi *et al.* 2013). The positive effects of *Chlorella vulgaris* supplementation on activities of antioxidant enzymes as revealed in this study could be associated with reduced formation of free radicals in the rabbits' serum due to increase in activities of antioxidant enzymes which explained why the control group had higher malondialdehyde concentration and lower antioxidant enzymes activities. The bioactivities of the *Chlorella vulgaris* in reducing malondialdehyde concentration and increase enzymes activities was because of its carotenoids components; these carotenoids have de-radicalizing affinity using their double bonds to neutralize free radicals and also capable of modulating the expression of antioxidant genes and their biochemical pathways (Naguib 2000, Zuluga *et al.* 2017); these biochemical features of *Chlorella vulgaris* make it an excellent supplement for the promotion of animal performances during gestation period.

Similar to oxidative stress and antioxidant enzymes activities, serum biochemistry of the pregnant rabbits also indicated that there were significant differences in some of the serum biochemical parameters including serum alanine aminotransferase 24.49 ± 0.96 IU/L ($P < 0.03$), serum urea which was 33.30 ± 0.99 mg/dl ($P < 0.001$) and blood urea nitrogen which was 15.68 ± 0.46 mg/dl ($P < 0.001$); however, there were no significant differences in some of the biochemical parameters including creatinine 0.82 ± 0.07 mg/dl ($P < 0.70$), alkaline phosphatase which was 39.28 ± 3.18 IU/L ($P < 0.60$), direct and total bilirubin which were 1.28 ± 0.18 mg/dl ($P < 0.64$) and 2.98 ± 0.42 mg/dl ($P < 0.60$) respectively (Fig.1.).

Serum biochemical profiles of the rabbits in this study showed that the microalgae *Chlorella vulgaris* is safe for rabbit consumption because the serum biochemistry ranged within the normal levels of healthy rabbits. All the serum biochemical parameters determined in the rabbits are within the normal levels including serum alanine aminotransferase; aspartate aminotransferase; alkaline phosphatase and bilirubin; creatinine and urea in agreement with Kaneko *et al.* (2008). Serum biochemical profile as found out in this study indicated that the microalgae *Chlorella vulgaris* is tolerable and safe for rabbit consumption and it has no

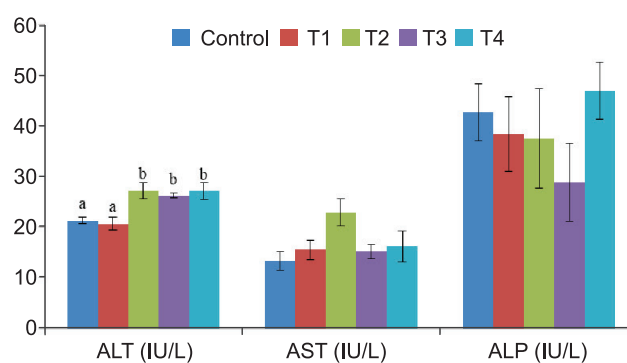


Fig.1. Effects of *Chlorella vulgaris* supplementation on serum alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) of the rabbits. The bars are presented as Means $\pm$ SEM and bars with a different superscript for the same parameters are significantly different ($P < 0.05$). Control, group without supplementation; T1, group supplemented with 200 mg/kg of *Chlorella vulgaris* biomass; T2, group supplemented with 300 mg/kg of *Chlorella vulgaris* biomass; T3, group supplemented with 400 mg/kg of *Chlorella vulgaris* biomass; T4, group supplemented with 500 mg/kg of *Chlorella vulgaris* biomass.

negative impacts on liver and renal functions as well as functionalities of all internal organs of the rabbits since all the serum values are within the normal range of healthy New Zealand White rabbits (Djerroua *et al.* 2011).

In conclusion, having find out through this study that supplementation of microalgae *Chlorella vulgaris* in rabbit during gestation prevented occurrence of oxidative stress through reduction in oxidative stress biomarker with increase antioxidant enzymes activities as well as promotion of the rabbits well-being since the consumption of the microalgae has no negative impacts on the rabbit liver and kidney functions as well as other internal organs which makes it tolerable for animals; hence, it can be concluded as a suitable antioxidant for pregnant rabbit and it is recommended as supplement for rabbits during gestation and its trial as antioxidant supplement in other food producing animals during gestation period is also warranted.

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