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IMPACT OF PRENATAL DEPRIVATION AND SPIRULINA SUPPLEMENTATION ON RATS F1 OFFSPRINGS' NEURO PSYCHOLOGICAL DEVELOPMENT

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Abstract:

It is becoming more and more common to research protein malnutrition, often referred to as protein-calorie malnutrition, as it impacts the structural proteins, enzymes, and neurotransmitters in the brain. If the mother's diet is deficient in protein, her foetus might be born with developmental abnormalities. Thus, inadequate nutrition for the expectant woman is a key contributing cause to perinatal mortality and morbidity. Recent studies have shown a robust relationship, highlighting the linear nature of this relationship, between eating habits and mental health. According to our most recent study, babies born to mothers who are deficient in protein have weak forelimb neuromuscular strength, poor habituation, low anxiety, delayed reflex ontogeny, and decreased cognitive functioning. Protein malnutrition, or PMN, is a global problem that mostly affects populations in Asia and Africa. It adversely affects the hippocampal circuitry's structural and physiological components. Very little is known about the effects of maternal nutritional supplements on neurons and glial cells during malnutrition, despite mounting evidence that PMN induces changes in the nervous system. Here, we aimed to investigate the protective effects of maternal supplementation with Spirulina against PMN-induced oxidative stress, reactive gliosis, and neuronal damage in the hippocampus of F1 offspring. Twenty-four healthy, three-month-old Sprague Dawley females (n = 24) were put on diets rich in protein (LP; 8% protein) and low in protein (NC; 20% protein) fifteen days prior to conception. Based on the quantity of sperulina supplementation (400 mg/kg/b.wt. orally throughout the gestation and lactation period), the females in the NC and LP groups were divided into two groups: the low protein group (LP SPI) and the normal control group (NC SPI). In the present study, F1 progeny births were used. The current work expands on

earlier discoveries of enhanced neurobehavioral and cognitive capacities in protein-deprived rats supplemented with spirulina by integrating neurochemical and morphometric examination of glial cells and neurons. As a consequence, several of the neuropathological changes linked to PMN were partially prevented. These changes included decreased reactive gliosis and apoptotic cell population, lessened oxidative brain damage, enhanced dendritic branch complexity with fewer injured neurons, and more mushroom-shaped spine density. The results suggest that when mothers supplement with Spirulina, cellular changes in the hippocampus areas caused by PMN are partially reversed, and this suggests the possibility of using Spirulina in malnutrition therapeutic efforts. It is predicted that repeated exposure to different early-life stressors will affect a person's behavioural development and increase their chance of acquiring neuropsychiatric disorders. The present study was designed to mimic such circumstances in a rat model in order to examine behavioural deficiencies between adolescence and adulthood. Female Wistar rats (n = 32; 140–150 gm) were given a low protein (LP; 8% protein) or control (20% protein) diet 15 days before to conception, and the diet was adhered to throughout the experiment. Eight experimental groups were formed by intraperitoneal injection of either lipopolysaccharide (LPS—bacterial endotoxin; 0.3 mg/kg body weight; PND 3 and 5) or DLT+LPS (LP—pyrethroid insecticide; 0.7 mg/kg body weight; PND 1 to 7) or deltamethrin (DLT—pyrethroid insecticide; 0.7 mg/kg body weight; PND 1 to 7). The rotarod, elevated plus maze, open field, light and dark box tests, and other neurobehavioral assessments were performed on F1 rats at one, three, and six months of age. When LP rats were compared to age-matched control rats, it was shown that the former were much more susceptible to single or multiple exposures. Additionally, they had quite severe behavioural abnormalities, including low anxiety, hyperactivity, and attention deficits—all of which are indicative of neuropsychiatric illnesses including ADHD and schizophrenia. This shows that exposure to several hits throughout early childhood may put individuals at risk for developmental abnormalities.

1 INTRODUCTION

As a result of widespread consumer rejection and their perceived role as catalysts for the carcinogenesis of artificial food additives, the use of chemically synthesised antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) has decreased in the current context (Stojkovic et al., 2013). Consequently, scientists throughout the world are becoming more interested in the creation and use of natural antioxidants as substitutes for synthetic ones. It is possible to extract new, safe antioxidants, such plant material derived from natural sources, to slow down oxidative damage, particularly in food systems.

In addition to one or more additional aromatic rings, antioxidant molecules also have extra hydroxyl groups. Studies have shown that substances with phenol groups are thought of as naturally occurring antioxidants (Lopez-Velez et al., 2003). Additionally, it has been noted that polyphenolic compounds are becoming increasingly common in the typical human diet and are drawing the attention of food producers and consumers alike to the need of preserving good health (Xiong et al., 2019). The researchers found that polyphenols may function as potent antioxidants by quenching singlet oxygen, lowering reactive oxygen species (ROS), and refilling other antioxidants by donating hydrogen.

During the electron transport cycle, ROS are produced (Apak et al., 2016). Important biomolecules suffer less oxidative damage when this free radical is scavenged by antioxidant molecules (Sassi et al., 2021). The compounds containing polyphenol groups can be further classified into the following categories: flavonoids (flavones, anthocyanins, and flavanols), phenolic acids (hydroxybenzoic acids and hydroxycinnamic acids), isoflavonoids (isoflavones, coumestans), phenolic polymers (pro-anthocyanidins—condensation of tannins and hydrolysable tannins), stilbenes, lignans, and flavonoids (Jia et al., 2021). Two prevalent structures for phenolic acids are hydroxybenzoic and hydroxylcinn amic acid.

Gallic acid, vanillic acid, syringic acid, and protocatechuic acids are the derivatives of hydroxybenzoic acid, whereas the derivatives of hydroxycinnamic acid are caffeic acid, ferulic acid, p-coumaric acid, and sinapic acids. Another significant family of phenolic chemicals found in organisms' cell walls are phenols. Cell wall phenolics are primarily divided into two groups: lignins and hydroxylcinn amic acids. Phenolic acid's main function is generally to shield the body from UV rays and microbial infections. In addition, it contributes to plant growth, reproduction, and coloration. The primary food sources of phenolic acid include fruits, vegetables, drinks, grains, and leguminous plants. According to a recent research, microalgae have strong antioxidant activity and are a rich source of secondary metabolites such phenolic compounds.

Similar to phytoplankton, microalgae are autotrophic cyanobacteria that thrive in open systems. Despite being members of the kingdom Plantae, they essentially have the look of unicellular bacteria. Because they contain pigments that allow for photosynthetic reactions, alkaline settings are suitable for the growth of microalgae. In addition, a variety of significant biomolecules, including xanthophylls, flavonoids, and carotenoids, may be synthesised by microalgae. Microalgae come in a range of hues, including blue-green, green, and red (like Chlorella sp. and Scenedesmus sp.), in addition to brown, purple, pink, yellow, and black microalgae.

The oldest of these are blue-green microalgae, which lack chloroplasts and a nucleus. All natural waterways include cyanobacteria, or blue-green microalgae. They are able to take part in the organic cycle of nutrients in the environment and food chain. Due to its ability to synthesise a broad range of bioactive chemicals, microalgae research is getting greater interest and offers several opportunities in the field of food biotechnology. Spirulina is a prokaryotic cyanobacterium with arthosporium filaments that is readily cultivated and collected in an open environment. Spirulina thrives in a variety of environments, including freshwater and brackish water, but it prefers an alkaline body of water. Generally speaking, Spirulina platensis and Spirulina maxima are utilised as food in many parts of the world, including Asia, Europe, and the US. In nutritional supplements, spirulina is available as a powder and a tablet. Natural compounds developed from Spirulina platensis have drawn the interest of recent studies due to their potential health benefits in

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relation to diabetes, blood cholesterol, and the prevention of ageing.

2 LITERATURE SURVEY

Reduced oxygen species have harmful interactions that lead to oxidative stress. It is a significant phenomena seen in all aerobic living forms. In air, oxygen is present as the diatomic molecule O2. Geological evidence indicates that its appearance in the earth's atmosphere in considerable proportions over 2.5 x 10^9 years ago was caused by the cyanobacteria's development of photosynthesis. Extremely high levels of oxygen were discharged into the environment by these bacteria as they split water to generate the hydrogen required to fuel metabolic decreases. The stratosphere's ozone (O3) layer formed as a result of the increase in atmospheric oxygen, which was beneficial. Living things were able to leave the water and settle on land thanks to the capacity of ozone and oxygen to filter a large portion of the powerful solar ultraviolet radiation (UV-C). However, oxygen itself must have put a great deal of stress on the creatures that were already there. There must have been a mass extinction of primordial creatures when atmospheric oxygen level increased.

Most of the ones that made it have evolved defence mechanisms against too much oxygen. Some of the bacteria restricted themselves to conditions that did not allow oxygen to get through, following the evolutionary route that allowed them to adapt to growing atmospheric oxygen levels. With oxygen making about 21 percent of the atmosphere, oxygen is now the most common element in the earth's crust. At other times, oxygen levels could have been significantly greater. According to several theories, oxygen climbed from around 18% to 20% in the mid-to-late Devonian period and then significantly to 35% in the late Carboniferous period as plant life developed.

Additionally, organisms that could withstand oxygen could have evolved to use it for electron transport chains that use oxygen as the terminal electron acceptor for efficient energy production, as well as for metabolic transformations (e.g., oxidase, oxygenase, and hydroxylase, such as tyrosine hydroxylase or cytochrome P450) (Halliwell and Gutteridge, 1998). Reactive oxygen species (ROS), sometimes referred to as "free radicals," are byproducts of aerobic metabolic activities including respiration and photosynthesis that eventually accumulate in the mitochondria, chloroplast, and peroxisomes. One thing that all ROS have in common is that and

they may lead to oxidative damage.

Free radicals are produced by a number of metabolic processes, metabolic damage, and some exogenous sources. They also have a significant impact on metabolism and overall development. Any species that has one or more unpaired electrons and is capable of independent life is referred to as a free radical. A radical may be created when a non-radical loses an electron or when a non-radical gains an electron.

There are many ways in which free radicals may interact with other molecules. Chain reactions are often the result of free radicals reacting with nonradicals. Free radicals are often extremely reactive and mildly attracted to a magnetic field when one or more unpaired electrons are present. The structure and function of every organic molecule in biological systems may be altered by the combination of highly reactive oxygen species. These are oxygen derivatives, sometimes called oxidants, that are both radical and non-radical.

These radicals are mostly produced in mitochondria and chloroplasts. While photosynthesis in plants produces the majority of ROS, mitochondria are the main generator of ROS in animal cells. The mitochondria of plants are not the main generator of ROS. This might be because ubiquinone catalyses the tetravalent reduction of O2 by an alternate oxidase (AOX). By battling the cytochrome b ci complex for electrons, the AOX may be able to lessen the generation of ROS (Wagner, 1995).

The main source of singlet oxygen in plants is the chlorophyll pigments connected to the electron transport system. PSII continually produces singlet oxygen during photosynthesis. The production of singlet oxygen ('O2) is another possible consequence of lipoxygenase action. It reacts with the majority of biological molecules at speeds that are almost diffusion-controlled and is very damaging (Arora et al, 2002).

During photosynthesis and respiration, the cell produces superoxide and hydrogen peroxide. Reduction of ferredoxin during photosynthesis is

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thought to be the primary source of most superoxide radicals, since it converts molecular oxygen to the superoxide radical (reaction I). Reaction II: This radical's dismutation results in the spontaneous production of H2O2, which is accelerated by superoxide dismutase (SOD)

3 METHODOLOGY

Green copper protein SOD was initially identified in 1938 by Mann and Keilin from the blood of cows. Its biological role was thought to be copper storage. McCord and Fridovich identified the enzyme's catalytic activity in 1969. Aerobes, aerotolerant anaerobes, and some obligatory anaerobes are all often found to have this enzyme.

Uncertainty surrounds the evolutionary genesis of the different SODs. Four forms of SOD are suggested by the sequence data that are now available, each of which has one or more metal ions—Cu, Zn, Fe, Mn, and Nickel—at the active site. While Ni-containing SOD has been described from a number of Streptomyces species, Cu, Zn, Fe, and Mn SODs are the predominant types (Choudhury et al., 1999). Because of their strong structural similarities and shared amino acid sequence, the Fe and Mn SODs are thought to be closely related to Cu Zn SOD and Ni SOD. Regardless of their origin, all SODs are multimeric metalloproteins. Either dimers or tetramers describe them. According to Imlay (2003), the Cu Zn enzymes are often located in the periplasmic region of a variety of Gram-negative bacteria, chloroplasts, and the cytosol of eukaryotic cells. According to one research, the cyanobacterium Synechococcus sp. WH7803 may contain Cu Zn SOD (Chadd et al., 1996). Prokaryotes and mitochondria have a matrix that contains Mn SODs. Prokaryotes and some plants are often home to the Fe SODs (Duke and Salin, 1985).

The cytoplasm of bacteria often contains the Fe SOD and/or the Mn SOD, both of which have very similar protein structures. The catalytic portion of Fe SOD was shown to be identical to MnSOD based on sequence analysis. It comprises one aspartate and three conserved histidine residues, both of which act as ligands for the metal ion (Regelsberger et al., 2002). SOD derived from many bacteria, including Bacteriodes fragilis. Often referred to as cambialisitic SOD, Streptococcus mutans and Propionibacterium shermanii exhibit action with any of the

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metals (Gabbianelli et al., 1995; Martin et al., 1986). The activity of cambialisitic SOD from P. shermanii declines sharply at pH6.0, according to a study of the reaction kinetics. This suggests that as the pH of the culture medium becomes acidic, the enzyme may have some physiological uses.

It has been shown that some cyanobacterial species have cytoplasmic Fe and Mn SOD linked to the cell and thyiakoid membranes. Fe SOD is found in the heterocyst of the nitrogen-fixing cyanobacterium Anabaena cylindrica (Henry et al., 1978). Anabaena PCC7120, formerly known as Nostoc PCC7120, has two superoxide dismutase genes: one that contains iron and the other that contains manganese. Fe SOD is cytosolic, whereas Mn SOD is a membrane-bound homodimeric protein with a transmembrane helix, a spacer region, and a soluble catalytic domain, according to an examination of both sequences. Its location is in the thyiakoid membrane and the plasma membrane in equal amounts. This suggests that the location of this enzyme in plasma and thyiakoid membranes is not dependent on any distinct signals.

One of the protons needed to create H2O2 in the Cu Zn SOD is thought to come from the imidazolate bridge that forms between the Cu and Zn ions when the reduced active site oxidises. For the active site to maintain the same positive charge in both the reduced and oxidised forms of the enzyme, protonation and the bridge's loss during the reduction of the Cu site are crucial. It's unknown where the other proton came from. The protons in Mn SOD and Fe SOD come from a pool of protons that are part of a network of hydrogen bonds that include ligands such as water and aspartate as well as Tyr and Gin residues close to the metal site. Cysteine ligands act as proton donors in Ni SOD.

Cyanide selectively inhibits the activity of Cu Zn SOD, whereas Fe and Mn forms are not. Long-term exposure to H2O2 inactivates Cu, Zn, and Fe SOD, but does not inhibit Mn SOD. Fe SOD is more susceptible to azide's inhibition. Cu Zn, Mn, and Fe SOD are inhibited by 10 mM azide at pH 7.8, by about 10%, 30%, and 70%, respectively (Halliwell and Gutteridge 1998) Azide inhibits NiSOD less than cyanide does (Choudhury et al., 1999).

Various species, including certain cyanobacteria, have been found to

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contain CuZn, Fe, and Mn SOD DNA sequences (Laudenbach et al., 1989; Campbell and Laudenbach, 1995; Li, et al., 2000; Shirkey et al., 2000). According to these investigations and genomic research, cyanobacteria have one or more SOD genes. One Fe SOD gene is present in the unicellular cyanobacterium Synechocystis PCC6803, one Fe SOD gene and two Mn SOD genes are present in Nostoc punctiforme, and one Fe SOD gene and three Mn SOD genes are present in Plectonema boryanum.

Cyanobacterial SODs have vital physiological functions in defending the constituents of the cell from oxidative and environmental damage. Desiccated cells of Nostoc commune have been shown to have copious SOD mRNA and active FeSOD. When cellular SOD activity was reduced, the beginning of photoinhibition-induced mortality in A. nidularis and Plectonema boryanum became more noticeable. During photooxidation, the Plectonema boryanum photooxidation-resistant mutant exhibited continuous SOD activity. Herbicide application, as well as a number of environmental factors including freezing, dryness, anoxia, and pathogenic damage, have all been linked to increased SOD activity in plants (Scandalios, 1993). Treatment with paraquet results in an overexpression of SOD because the herbicide generates its cytotoxic effects via a free radical mechanism.

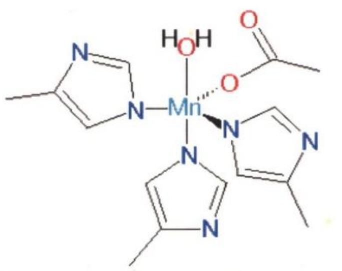
4 RESULTS

The molecular mass of Mn SOD, which was isolated from some organisms, is 40 k Da. It demonstrates that manganese is present at the place of activity. Ethanol combined with chloroform destroys the activity. Other superoxide dismutases have a similar chemical mechanism.

$$\mathrm{Mn(III)} + O_2^{\bullet -} \rightleftharpoons [\mathrm{Mn(III)}\text{-}O_2^{\bullet -}] \longrightarrow Mn^{2+} + O_2$$

$$Mn^{2+} + O_2^{\bullet -} \rightleftharpoons [\mathrm{Mn(III)}\text{-}O_2^{\bullet -}] \longrightarrow \mathrm{Mn(III)} + H_2O_2$$

The rate of O2' dismutation for Mn and Cu Zn SODs is equal at pH 7.0, however it lowers at the alkaline pH for Mn SODs. Compared to Cu Zn SOD, they are more susceptible to denaturation by heat or chemicals like detergents.



Sequence and structural homology between Mn SOD and Fe SOD are quite high, but not with Cu Zn SOD. The mechanism of action was discovered by chemically altering these enzymes. H2O2 inactivates Cu Zn SOD, Fe SOD, and Mn SOD, but not Mn SOD, despite their structural resemblance to Fe SOD. Cu Zn SOD is inactivated by H2O2 as the H02' anion by an affinity mechanism, which also results in the destruction of a metal-liganding histidine.

Iron is necessary for E. Coli Fe SOD to function at its active site, because H2O2 inactivates the enzyme by causing each subunit to lose four tryptophan residues. Fe SOD that was isolated from Pseudomonas ovalis also reports this (Yamakura, 1984). Based on biochemical data, certain amino acid residues such as 76, 77, 79, 84, 87, 154, and 155 are most likely to be in charge of giving the iron and manganese classes of SOD their distinctive characteristics.

Ni SODs differ from Cu Zn, Fe, and Mn SODs in terms of amino acid content, protein sequences, and immunological characteristics (Choudhury et al., 1999).

$$Ni(III) + O_2^{\cdot -} + H^+ \longrightarrow Ni^{2+} + O_2$$

$$Ni^{2+} + O_2^{\cdot -} + H^+ \longrightarrow Ni(III) + H_2O_2$$

The enzyme was observed to elute strongly in fractions 6–9 during the first DEAE-Cellulose Chromatography (DE-I), with fraction 8 having the greatest recovery and specific activity.

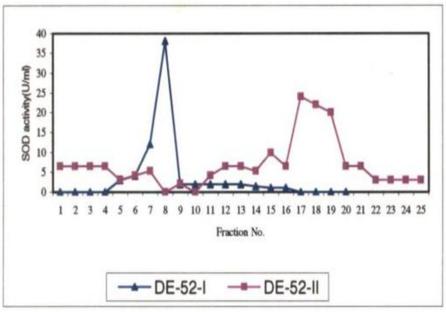


Fig 1 . Elution of active SOD fractions from DE-I (-A-) and DE-II (-■-) fractions.

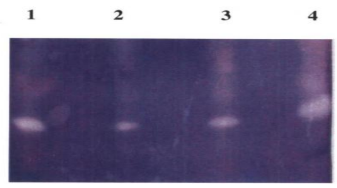


Fig 2 . Activity staining of sonicate and different am m onium sulphate fractions on 1 0% native PAGE lane 1: sonicate lane 2: 0.30% lane 3; 30-60'/- lane4: 60-90%.SOD staining was applied to aliquots of these active fractions that were run on native-PAGE gels. Every fraction exhibited the SOD-corresponding achromatic bands. Nevertheless, these fractions' silver staining revealed some small impurities.

5 CONCLUSION

Only after passing in vivo testing is a medicine considered effectively validated; some treatments that performed well in vitro were shown to

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be ineffective in in vivo research. Therefore, the effectiveness of SPME in the brine shrimp larvae model was confirmed in the present investigation. The results shown that treating Artemia with SPME boosted its survival rate, decreased mortality by up to 90%, and completely protected against Artemia. Additionally, the bacterial enumeration in brine prawns indicated a seven-fold reduction in vibrio load compared to the control group. This demonstrates unequivocally how effective SPME is as an anti-infective agent. The outcome is similar to that of Defoirdt et al.'s (2012) study on thiophenone chemicals as a means of preventing vibriosis in brine shrimp. It was discovered that the SPME was an effective treatment.

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