Lablab purpureus (L) bean flour ameliorates plasma proteins and accretion of docosahexaenoic acid (DHA, 22:6, ω-3) in the plasma, liver, and brain of malnourished rats

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Abstract
Lablab purpureus (L) bean is an undervalued or underutilized orphan crop in many tropical countries, where different forms of malnutrition are associated with stunting growth and cognitive deficiencies. We previously reported that L. purpureus contains ω-3 α-linolenic acid (ALA, C18:3, ω-3), which can act as the precursor of ω-3 docosahexaenoic acid (DHA, C22:6, ω-3). Inadequate level of DHA impairs growth, development, and cognitive performance. Therefore, we evaluated if supplementation of L. purpureus seed flour (LPS) affects the nutritional status, in terms of body weight gain, plasma proteins, and DHA levels of malnourished model rats. Three groups of rats, namely, controls, malnourished alone (MN), and 15% LPS-supplemented malnourished (MN + LPS) rats were fed with LPS for 12 weeks. Afterward, body weight, liver weight, brain weight, plasma proteins, micronutrients, lipid profile, and fatty acid profile of plasma, liver, and brains were determined by standard methods. The levels of liver lipid peroxide (LPO) and proinflammatory TNFα were also measured. The body weight, liver weight, serum total proteins and micronutrients (iron/potassium), and the levels of docosahexaenoic acid significantly increased in the plasma, liver, and brain of MN + LPS rats. Moreover, LPO and TNFα levels reduced significantly in MN + LPS rats. In vitro analysis revealed a significant free radical scavenging and antioxidative potential of L. purpureus seed extract. Thus, L. purpureus not only replenishes protein-energy malnutrition, but also increases the levels of DHA, an indispensable polyunsaturated fatty acid for brain cognition. Finally, our results suggest that L. purpureus might benefit human malnutrition and related cognitive deficits.

KEYWORDS
brain, docosahexaenoic acid (DHA), Lablab purpureus, malnutrition
Animal proteins are relatively scarce and expensive. People in low income countries do not get enough of them to meet their requirements of protein and other essential nutrients. Beans, on the other hand, are cheap and readily available; they are also important sources of nutrients like proteins, carbohydrates, fibers, and minerals (Duke, Reed, & Weder, 1983; Deka & Sarkar, 1990). If used judiciously in the diet, they can contribute to alleviate malnutrition and stunted growth among infants and young children in the least-developed or developing countries.

*Lablab purpureus* is an unpopular bean with a poor image as foodstuff. There has also been relatively little research and discussion about its nutritional attributes. Over four decades ago, Hamilton, Lambourne, Roe, and Minson (1970) described that feeding of *L. purpureus* increased the protein content and milk production in cows. Abeke et al. (2007) reported that *L. purpureus* supplementation increased the body weight of hens, their egg weight, and duration of egg production. Afterwards, several other studies reported that feeding of *L. purpureus* decreased blood glucose and cholesterol levels in hens (Ragab, Abdel Ati, Kijora, & Ibrahim, 2012) and rats (Hartoyo, Dahrulsyah, & N., & Nugroho P., 2008; Hartoyo, Muchtadi, Astawan, & Dahrulsyah dan., & Winarto, A., 2011). Besides, *L. purpureus* has previously been reported to have a broad spectrum of medicinal uses as anthelmintic, antispasmodic, and antistomachic agent (Chetty & Rao, 2003).

Recently, we have reported that *L. purpureus* seed flour (LPS) contains polyunsaturated fatty acids (PUFAs), such as ω-6 linoleic acid (LLA, C18:2, ω-6) and ω-3 α-linolenic acid (ALA, C18:3, ω-3; Hossain, Ahmed, Bhowmick, Mamun, & Hashimoto, 2017), LLA and ALA are well-known essential fatty acids (EFA); they act as the precursors for the physiologically most important long-chain (LC) PUFAs, such as ω-6 arachidonic acid (AA, C20:4 ω6) and ω-3 docosahexaenoic acid (DHA, C22:6 ω3), respectively (Nakamura & Nara, 2004). Deficiency of DHA impairs the growth and development of brain, concurrently with reductions of learning-related memory cognitions and mental health (Hashimoto, Hossain, Al, Matsuzaki, & Arai, 2017; Nguyen et al., 2014). Between the LCPUFAs, DHA is crucial for sperm and retinal outer-segment membrane lipid composition (Crawford et al., 1999). DHA may improve attention deficit hyperactivity disorder (ADHD)–a common childhood behavioral disorder (Königs & Kiliaan, 2016). An oral administration of DHA increases the memory of elderly (Gamoh et al., 1999) and Alzheimer’s disease model rats (Hashimoto et al., 2002). Though consumption of purified DHA from commercial sources and/or directly from sea-based fish/animal sources is the best way to have an adequate blood/brain levels DHA, it is, however, difficult to afford for the people in poor countries struggling continuously against malnutrition and economic burden. Also, the people living in the landlocked geographical regions without/with limited access to the seafood cannot get sufficient DHA. Given the clear role of DHA in brain functions, much interest has generated whether the levels of blood/brain DHA could be enhanced by dietary means. Despite of having many nutritional components and health-promoting factors, including precursor of DHA (i.e., α-linolenic acid, ALA) and high protein content, *L. purpureus* has remained a neglected crop. The feeding of low-protein diet is believed to cause protein malnutrition in the experimental rats. Proteins not only play roles for the structural purposes of the cells, they also perform important roles as enzymes and hormones, and participate in boosting immune systems, regulate cell development and transport functions. When proteins fail to play these roles, an imbalance sets in and protein-energy malnutrition (PEM) may develop in animals. Protein malnutrition may also result in an inadequate intake of micronutrients (Brito et al., 2016; Leite, Jordao, de Andrade, Masson, & Frade, 2011). Therefore, in this investigation, we studied whether the supplementation of LPS improves protein malnutrition and affects the plasma, liver, and brain DHA composition of the experimental malnourished rats. Alongside, we also determined the effects of *L. purpureus* on the body/liver weight gain, plasma micronutrients, including Na, K, Ca, and Fe levels, lipid profiles, and oxidative stress (OS) in these rats.

## 2 | MATERIALS AND METHODS

### 2.1 | Collection of *L. purpureus* seeds

Dried *L. purpureus* seeds were collected from a local market and authenticated by a botanist from the Department of Botany, Jahangirnagar University, Savar, Dhaka. Dried seeds were powdered into fine flour using a mechanical grinder. The diet containing LPS was autoclaved for 20 min at 121 °C under 15lb/in and stored in an airtight container.

### 2.2 | Animals

Protein malnutrition in animals can be induced by variations of composition and duration (weeks to months) of the feeding of restricted diets. The severity of malnutrition depends on the protein contents, namely, diets with low-protein contents may induce severe malnutrition, whereas those with moderate protein contents may induce moderate malnutrition (Lobe, Marica, Bernstein, & C., & German, R.Z., 2006; Miñana-Solis et al., 2008; Leite et al., 2011; Brito et al., 2016; Triawanti et al., 2018). In our experiments, the control rats received a normal quantity of protein, whereas the malnutrition model rats were prepared by providing them with low quantity of protein. Briefly, inbred albino Wistar rats (8-WKs, BW ~100 g) were divided into three groups: control group (control diet-fed group), malnourished (MN) diet-fed rat group, and 15% *L. purpureus*-supplemented malnourished rat group (MN + LPS; Figure 1). The MN diet—a low protein diet—contained a lower amount of pure casein (2% by weight, one sixth that of the control diet, Table 1). The experimental MN + LPS diet was supplemented with 15% protein-rich *L. purpureus*. Moreover, approximate composition analyses of the diets revealed that among all the diets, the
MN diet contained the lowest amount of total proteins (control, MN, and MN + LPS diets: 22.6%, 12.85%, and 19% protein, respectively).

Water was supplied ad libitum to all rats. The diet composition is shown in Table 1. Rats were housed in polypropylene cages under controlled laboratory conditions (12 hr light: 12 hr dark; temp. 25 ± 2°C; relative humidity 50 ± 10%). The experimental diet was provided for 12 weeks. All the experiments were conducted in accordance with the guideline for the care and use of laboratory animals and approved by the Institutional Animal Ethical Committee at Jahangirnagar University, Savar, Dhaka, Bangladesh (Resolution No. 10/(i)/a/CPCSEA/IACE/SVU/PSR-MRA).

2.3 | In vivo studies

2.3.1 | Brain tissue preparation

At the end of the experiment, the rats were killed after sodium pentobarbital (65 mg/kg of body weight) anesthesia (i.p., intraperitoneally).
After drawing blood, the livers and brains were perfused with ice-cold saline to remove blood. The brain cortex was separated from the whole brain on ice. Tissues were homogenized (10 mg/ml) in phosphate buffer (50 mM, pH 7.4, containing phenylmethylsulfonyl fluoride [PMSF] protease inhibitor), using Dounce glass homogenizer and centrifuged at 500 × g to remove unruptured tissues. The resulting supernatants were assigned as whole homogenates. Whole homogenates were again centrifuged at 10,000 × g for 1 hr to separate the supernatants (cytosolic fractions) for ELISA of tumor necrosis factor alpha (TNFα). All samples were immediately subjected to the assays and/or stored at −80 °C.

2.3.2 | Blood biochemistry

Plasma total cholesterol (TC) was determined enzymatically using the cholesterol oxidase method, whereas high-density lipoprotein (HDL)-cholesterol (HDL-C) were determined by the same procedure after precipitating low-density lipoprotein (LDL) and very low-density lipoprotein (VLDL), using assay kits for total cholesterol (TC) and HDL-C (total cholesterol E-test and HDL-C test; Wako Pure Chemical Industries, Osaka, Japan). The absorbance of the color complex was measured at 560 nm. Plasma triglyceride (TG) was also measured using a commercially available reagent kit (TG-test; Wako Pure Chemical Industries). In the TG assay protocol, TGs are converted to free fatty acids and glycerol. The absorbance of the color was measured at 570 nm.

LDL-cholesterol (LDL-C) was calculated as follows: LDL-C = (TC − (TG/5) + HDL-C) and VLDL-C was calculated as: VLDL-C = (TC − (HDL-C + LDL-C)).

Serum glucose was measured by glucose oxidase method using a reagent kit (Wako Pure Chemicals, Osaka, Japan). Briefly, glucose oxidase catalyzes the oxidation of glucose to gluconate, concurrently with the production of hydrogen peroxide (H₂O₂). The formed H₂O₂ is detected by a chromogenic oxygen acceptor (phenol)4-amino anti-pyrene (4-AAP) in the presence of peroxidase. Within 15–30 mins of color development, the color was detected at 510 nm against glucose standard.

Serum protein was assayed by protein assay BCA (bicinchoninic acid) reagents. The BCA method quantifies protein in a short time. The principle of this method is that proteins reduce Cu²⁺ to Cu⁺ in an alkaline solution (the biuret reaction) and result in a purple color formation by bicinchoninic acid. Absorbance was recorded at 560 nm against BSA standard (Wako Pure Chemicals).

Electrolytes were measured by potentiometry (Maas & Sprokholt, 1990). The method used here measured the voltage that develops between the inner and outer surfaces of an ion selective electrode. The electrode (membrane) is made of a material that is selectively permeable to the ion being measured. This potential is measured by comparing it with the potential of a reference electrode. Because the potential of the reference electrode is held constant, the difference in voltage between the two electrodes is attributed to the concentration of ions in the sample. Values for Na and K were expressed as mmol/L, whereas those for Ca and Fe as mg/dl and μg/dl, respectively.

2.3.3 | Lipid peroxide (LPO) assay

LPO levels, as indicator of OS, were assessed as previously described by Hossain et al., (2019). Briefly, 200 μl of 8.1% sodium dodecyl sulfate, 3.0 ml of 0.4% thiobarbituric acid in 20% acetic acid (pH 3.5), and 700 μl distilled water were added to 100 μl of homogenate; the mixture was then incubated at 95 °C for 1 hr. After cooling, 1.0 ml water and 4.0 ml n-butanol-pyridine (15:1, v/v) were added and the mixture was vigorously agitated for 20 min. After centrifugation at 1800 × g for 10 min, the absorbance of the upper organic layer was determined at 535 nm.

2.3.4 | TNFα assay

TNFα levels were measured as described previously (Hossain, Bhowmick, Sarkar, Basunia, & Mamun, 2019). Briefly, the multi-well plate coated with 20 μl of cytosolic fraction in 180 μl of 100 mM sodium bicarbonate buffer (pH 9.6) was incubated at 4 °C for 12 hr. The wells were then washed, blocked with BSA (1%) in tris-buffered saline (TBS) for 4 hr. After wash, anti-rabbit TNFα 1° antibody (at 1:1000 dilutions) was added and incubated at 4 °C for 12 hr. On the next day, the wells were washed, incubated with HRP-coupled 2° anti-rabbit antibody for 2 hr at room temperature. After wash, tetramethylbenzidine was used to develop color; the color reaction was stopped by adding 0.1 N HCl. The absorbance of the plate was determined at 450 nm. All absorbance values were normalized to protein concentration.

2.3.5 | Fatty acid composition

The fatty acid profiles of plasma, liver, and brain tissues were determined by direct transmethylation (Lepage et al., 1986), as described previously (Hashimoto et al., 1999). Briefly, to 100 μl of plasma or brain/liver tissue homogenates, 2.0 ml methanol–n-octane (4:1, v/v) containing 10 μg tricosanoic acid as an internal standard and 200 μl acetyl chloride were added. The mixture was incubated at 100 °C for 60 min and cooled, then neutralized with 0.5 N aqueous NaOH solution containing 10% sodium chloride. The neutralized mixture was shaken for 10 min at room temperature and centrifuged at 1800 × g for 5 min. The octane phase with the fatty acid methyl esters was directly subjected to gas chromatography. The gas chromatographic separation was carried out on a Model 5890II (Hewlett-Packard, Avondale, PA, USA) equipped with a flame ionization detector and an automatic sampler Model 7673. A 30-m × 0.25-mm capillary column (DB-WAX P/N 122-7032, J & W Scientific, CA, USA) was initially maintained at 100 °C for 1 min, raised to 180 °C at 20 °C/min, then raised to 240 °C at 2 °C/min, further raised
to 260 °C at 4 °C/min and maintained for 5 min. The identities of the peaks were established by comparing with those of reference compounds and, in part, by gas chromatography–mass spectrometry.

### 2.4 | In vitro studies

#### 2.4.1 | Preparation of methanolic extract of LPS

A part of LPS was soaked in methanol for 24 hr with continuous shaking (SI-300/300R/600/600R, Jeio Tech, South Korea). Afterwards, the filtrate was concentrated under vacuum rotary evaporator (J.P. Selecta, s.a, Spain) and stored for the in vitro tests of antioxidative potentials (Figure 2). The extract was used to determine total polyphenol contents, total flavonoid contents, DPPH-free radical scavenging, and anti-LPO activity.

#### 2.4.2 | Antioxidative potential assay

Total polyphenol content (TPC) of *L. purpureus* flour extract was determined spectrophotometry by using the Folin-Ciocalteu method (Hossain et al., 2012). Briefly, 0.50 ml of the diluted methanic extract was transferred in triplicate to separate tubes and allowed to dry. Then, 5.0 ml of a 1/10 dilution of Folin-Ciocalteu’s reagent in water was added to each tube and vortexed. Polyphenols present in the extract reduce phosphotungustomolybdic acid of Folin-Ciocalteu reagent in alkaline solution to produce a highly blue colored solution. Afterwards, 4.0 ml of a sodium carbonate solution (7.5% w/v) was added. The tubes were then allowed to stand at room temperature for 60 min. The color complex was measured at 765 nm. The concentration of total phenol compounds in the extract was determined against gallic acid as standard (μg of gallic acid equivalent [GAE]/gm of dry flour). The total flavonoid content of the extract was measured by the aluminum chloride colorimetric assay against catechin standard (Akter, Haque, Islam, Rahaman, & Bhowmick, 2015) and its concentration in the extract was expressed as catechin equivalent (mg of catechin/mg of extract).

In vitro antioxidant effect of *L. purpureus* flour extract was performed by determining: (a) the DPPH-free radical scavenging ability, and (b) anti-LPO ability, as described previously from this laboratory (Haque et al., 2014; Islam, Haque, Rahaman, & Hossain, 2014).

(a) The DPPH (2, 2 diphenyl-1-picrylhydrazyl) free radical scavenging activity of *L. purpureus* extract was evaluated as described previously (Hossain et al., 2012). The free radical scavenging ability was evaluated through recording the change of absorbance produced by the reduction of 0.2 mM of DPPH. DPPH scavenging activity was calculated as (%):

\[
\frac{A_{DPPH} - A_{DPPH + Scavenger}}{A_{DPPH}} \times 100,
\]

where, \(A_{DPPH}\) = absorbance of the DPPH (0.2 mM)-alone; \(A_{DPPH + scavenger}\) = absorbance of the DPPH in the presence of scavenger (here, the *L. purpureus* extract).

(b) In vitro anti-LPO activity of the *L. purpureus* extract indicates whether it can repress the upsurges of the levels of LPO produced by the OS of Fenton’s reagent (FR)—a free radical (●OH, hydroxyl radical) producer. For the determination of in vitro anti-LPO ability of *L. purpureus* extract, liver whole homogenates (WH) were prepared from some new rats (Figure 2). After killing, the livers were collected, perfused with cold saline to remove RBCs, and then homogenized in 50 mM phosphate buffer (pH 7.4). An OS was induced by incubating the whole homogenate samples with the FR (FeSO₄ + H₂O₂). We then examined whether the in vitro OS was inhibited in the presence of the methanolic extract of *L. purpureus* flour, as shown in

![Figure 2](image-url)
Figure 2B. The level of LPO was determined as an indicator of OS. The higher the LPO level, the greater is the degree of OS in the homogenates. The levels of LPO were measured as described for the in vivo samples (Section 2.3.3).

Protein concentrations were measured by BCA method.

2.5 | Statistical analysis

Results are reported as mean ± SEM values. Data were subjected to Student's t test and/or one-way ANOVA followed by Fisher's PLSD test for post hoc comparisons. A level of $p < .05$ was considered statistically significant.

2.6 | Data availability statement

All data generated or analyzed during this study are included in this published article.

3 | RESULTS

3.1 | Effects of *L. purpureus* supplementation on body, brain and liver weights

The body weight (BW) of MN rats decreased by 16%, when compared with that of the control. The BW of MN + LPS rats rose to that of the control rats (Figure 3a). The brain weight of the MN rats decreased slightly (by ~5%) when compared with that of the control rats; however, the values did not reach significance.

The brain weight of MN + LPS rats, though increased by ~8% as compared with that of the MN rats (g), the difference was not statistically significant (Figure 3b). However, the changes in the body weight and brain weight gave rise to a significant increase in the brain:body weight ratio in MN rats when compared with that of the control rats. *L. purpureus*-supplementation decreased the brain:body weight ratio to that of the control rats in the MN + LPS rats (Figure 3c).

Liver weight of the MN rats decreased significantly when compared with that of the control rats. However, the supplementation with *L. purpureus* increased significantly the liver weight when compared with that of the MN rats (Figure 3d). Food intake was not statistically different among the rat groups (data not shown).

3.2 | Effect of *L. purpureus* supplementation on plasma total protein and micronutrients

Malnutrition decreased significantly the level of plasma total protein in the MN rats, when compared with that of the control rats. Total protein level, however, increased significantly in MN + LPS rats, as compared with those of the control and MN rats (Table 2). The levels of Na, K, and Ca decreased in the MN rats, but not significantly. Fe level decreased significantly in the MN rats. *L. purpureus* supplementation, however, increased significantly the levels of K and Fe in the plasma of these rats. Blood glucose levels were not altered in either of the rat groups (Table 2).
3.3 | Effects of *Lablab purpureus* supplementation on plasma fatty acid profile

The effect of *Lablab purpureus* supplementation on plasma fatty acid profile of the MN is shown in Table 3. The levels of almost all fatty acids decreased in the MN rats. Compared with the control rats, the plasma levels of DHA decreased significantly in the MN rats; however, it increased significantly in MN + LPS rats. Eicosapentaenoic acid (EPA) levels also decreased significantly in the MN rats. The levels of essential PUFA such as LLA and ALA also decreased in the plasma of the MN rats. The levels of AA decreased in the MN rats; however, it did not reach significance, as compared with those of the control rats. The levels of the saturated fatty acid palmitic acid (PLA) decreased, whereas those of stearic acid (STA) increased in the MN rats. Levels of the monounsaturated fatty acids palmitoleic acid (POA) and oleic acid (OLA) decreased significantly in MN + LPS rats. Finally, the changes in the fatty acid profile gave rise to a significant increase in the unsaturation index (USI) in the MN + LPS rats.

3.4 | Effect of *Lablab purpureus* supplementation on liver fatty acid profile

The effects of *Lablab purpureus* supplementation on liver fatty acid profile of the MN rats are shown in Table 4. Levels of PLA and STA, ω-6 AA, and ω-3 docosapentaenoic acid (DPA) increased significantly in the liver tissues of the MN rats. Compared with the control rats, levels of the saturated PLA and STA were not altered in MN + LPS rats. However, *Lablab purpureus* supplementation decreased significantly the levels of AA and increased the levels of DHA in MN + LPS rats. All these changes in the fatty acid profile led to a significant increase in the degree of USI in the liver tissue of MN + LPS rats.

3.5 | Effect of *Lablab purpureus* supplementation on brain fatty acid profile

The levels of the saturated PLA and STA and monounsaturated POA and OLA increased significantly in brain tissues of the MN rats (Table 5). Levels of PUFA such as LLA, AA, DPA were also high in the MN rats, when compared with those in brains of the control rats. However, feeding of MN rats with *Lablab purpureus* increased AA and DHA levels, concurrently with increases in the molar ratios of DHA/AA in their brains. The degree of USI was also significantly higher in the (MN + LPS) rats.

3.6 | Effect of *Lablab purpureus* supplementation on OS and TNFα in liver tissues of malnourished rats

Malnutrition augmented the degree of OS, as indicated by significant increases in the levels of LPO in the liver tissues of MN rats, as compared with those in the control rats. *Lablab purpureus* supplementation, however, decreased significantly LPO levels in the hepatic tissues of these rats (Figure 4a). Levels of proinflammatory TNFα also rose significantly in the liver tissues of MN rats. However, supplementation of *Lablab purpureus* to MN rats decreased significantly the TNFα levels in these rats when compared with those of the malnourished (MN) rats (Figure 4b).

3.7 | In vitro results

Total polyphenol and total flavonoid contents of *Lablab purpureus* were 17.15 mg gallic acid equivalent (GAE)/gm dry flour and 9.55 mg catechin equivalent (CTE)/gm dry flour, respectively. The IC50 (concentration required to scavenge 50% of 0.2 mM DPPH) for *Lablab purpureus* extract was 21.2 mg/ml extract. in vitro
### TABLE 3  Effect of *Lablab purpureus* supplementation on the plasma (μg/ml) fatty acid profile of the rats

| Rat | PLA | POA | STA | OLA | LLA | ALA | AA | EPA | DPA | TCA | DHA | NVA | DHA/AA | USI |
|-----|-----|-----|-----|-----|-----|-----|----|-----|-----|-----|-----|-----|--------|-----|
| Cont | 231±3 33 | 20.3±4.7 | 98.0±12 | 234±22 | 272±12 | 3.10±0.15 | 113±3.0 | 1.92±0.05 | 1.90±0.03 | 4.30±0.20 | 7.16±0.20 | 0.86±0.04 | 0.05±0.003 | 1.274±0.003 |
| MN | 201±10.5 | 25.2±1.5 | 79.9±3.5 | 171±7.5 | 192±9.5 | 2.75±0.05 | 105±2.5 | 1.43±0.05 | 2.28±0.15 | 3.61±0.15 | 5.24±0.25 | 0.97±0.45 | 0.04±0.011 | 1.28±0.004 |
| MN+ LPS | 198±10.5 | 11.7±1.5 | 121±5.5 | 177±9.5 | 225±10.5 | 3.35±0.15 | 168±6.5 | 1.45±10.5 | 1.76±0.75 | 5.0±0.25 | 9.80±0.35 | 1.21±0.05 | 0.05±0.003 | 1.439±0.005 |

Note. Results are mean ± SEM (n = 6) for duplicate determinations. PLA = palmitic acid (C16:0); POA, palmitoleic acid (C16:1, ω-6); STA = stearic acid (C18:0); OLA, oleic acid (C18:1, ω-9); LLA = linoleic acid (C18:2, ω-6); ALA, α-linolenic acid (C18:3, ω-3); AA = arachidonic acid (C20:4, ω-6); EPA, eicosapentaenoic acid (C20:5, ω-3); DPA = docosapentaenoic acid (C22:5, ω-3); TCA = tetracosanoic acid (C24:0); DHA = docosahexaenoic acid (C22:6, ω-3) and NVA = nervonic acid (C24:1, ω-9). DHA/AA = molar ratio of DHA and AA. USI (unsaturation index) = ∑(mol% of each (poly)unsaturated fatty acid × number of double bond(s)) per (poly)unsaturated fatty acid)/100. Data were analyzed by one-way ANOVA, followed by Fisher’s PLSD test for post-hoc comparisons. Cont: Control diet-fed rats. MN: Malnourished group. MN + LPS: Malnourished+*Lablab purpureus* seed flour. Values in the same column that differ in superscript are significantly different at † p < .05. [Correction added on 27 March 2020, after first online publication: In Table 3 note, changes have been made to the following: (1) the word 'linolenic' in essential fatty acid 'LLA' was corrected to 'linoleic' so it reads 'LLA = linoleic acid (C18:2, ω-6)'; and (2) the word 'linoleic' in essential fatty acid 'ALA' was corrected to 'α-linolenic acid (C18:3, ω-3)'].

### TABLE 4  Effect of *Lablab purpureus* supplementation on the liver (μg/mg protein) fatty acid profile of the rats

| Rat | PLA | POA | STA | OLA | LLA | ALA | AA | EPA | DPA | TCA | DHA | NVA | DHA/AA | USI |
|-----|-----|-----|-----|-----|-----|-----|----|-----|-----|-----|-----|-----|--------|-----|
| Cont | 10.2±1 76 | 0.28±0.19 | 12.6±1.96 | 5.25±1.6 | 4.60±0.99 | 0.08±0.03 | 2.50±0.36 | 0.00±0.00 | 0.11±0.03 | 0.54±0.11 | 0.56±0.09 | 0.05±0.01 | 0.20±0.01 | 0.739±0.03 |
| MN | 14.9±2.3 | 0.27±0.04 | 18.1±1.2 | 4.04±0.50 | 4.44±0.50 | 0.00±0.00 | 4.2±0.03 | 0.00±0.00 | 0.16±0.01 | 0.52±0.02 | 0.50±0.03 | 0.06±0.00 | 0.127±0.01 | 0.656±0.02 |
| MN+ LPS | 12.3±1.75 | 0.12±0.18 | 18.3±1.9 | 4.30±1.70 | 4.43±0.90 | 0.01±0.00 | 3.4±0.03 | 0.00±0.00 | 0.11±0.01 | 0.59±0.03 | 0.96±0.09 | 0.03±0.80 | 0.256±0.01 | 0.694±0.03 |

Note. Results are mean ± SEM (n = 6) for duplicate determinations. PLA = palmitic acid (C16:0); POA, palmitoleic acid (C16:1, ω-6); STA = stearic acid (C18:0); OLA, oleic acid (C18:1, ω-9); LLA = linoleic acid (C18:2, ω-6); ALA, α-linolenic acid (C18:3, ω-3); AA = arachidonic acid (C20:4, ω-6); EPA, eicosapentaenoic acid (C20:5, ω-3); DPA = docosapentaenoic acid (C22:5, ω-3); TCA = tetracosanoic acid (C24:0); DHA = docosahexaenoic acid (C22:6, ω-3) and NVA = nervonic acid (C24:1, ω-9). DHA/AA = molar ratio of DHA and AA. USI (unsaturation index) = ∑(mol% of each (poly)unsaturated fatty acid × number of double bond(s)) per (poly)unsaturated fatty acid)/100. Data were analyzed by one-way ANOVA, followed by Fisher’s PLSD test for post-hoc comparisons. Cont: Control diet-fed rats. MN: Malnourished group. MN + LPS: Malnourished+*Lablab purpureus* seed flour. Values in the same column that differ in superscript are significantly different at † p < .05. [Correction added on 27 March 2020, after first online publication: In Table 4 note, changes have been made to the following: (1) the word 'linolenic' in essential fatty acid 'LLA' was corrected to 'linoleic' so it reads 'LLA = linoleic acid (C18:2, ω-6)'; and (2) the word 'linoleic' in essential fatty acid 'ALA' was corrected to 'α-linolenic acid (C18:3, ω-3)'].
### Table 5

| Rat               | PLA (μg/mg protein) | POA (μg/mg protein) | OLA (μg/mg protein) | STA (μg/mg protein) | LLA (μg/mg protein) | ALA (μg/mg protein) | AA (μg/mg protein) | EPA (μg/mg protein) | DPA (μg/mg protein) | TCA (μg/mg protein) | DHA (μg/mg protein) | NVA (μg/mg protein) | DHA/AA | USI   |
|-------------------|---------------------|---------------------|---------------------|---------------------|---------------------|---------------------|---------------------|---------------------|---------------------|---------------------|---------------------|---------------------|---------|-------|
| Cont              | 28.4 ± 2.30         | 0.40 ± 0.04         | 38.0 ± 2.4          | 24.0 ± 2.0          | 5.1 ± 2.27          | 51.3 ± 2.27         | 0.72 ± 0.07         | 56.7 ± 4.3          | 40.2 ± 3.7          | 1.34 ± 0.5         | 14.6 ± 1.0          | 0.92 ± 0.01         | 1.23 ± 0.01 |
| MN                | 51.3 ± 2.27         | 0.72 ± 0.07         | 56.7 ± 4.3          | 40.2 ± 3.7          | 1.34 ± 0.5          | 14.6 ± 1.0          | 0.92 ± 0.01         | 1.23 ± 0.01         |                    |                    |                    |                     |         |       |
| MN + LPS          | 55.1 ± 3.18         | 0.92 ± 0.01         | 56.7 ± 4.3          | 40.2 ± 3.7          | 1.34 ± 0.5          | 14.6 ± 1.0          | 0.92 ± 0.01         | 1.23 ± 0.01         |                    |                    |                    |                     |         |       |

**Note.** Results are mean ± SEM (n = 6) for duplicate determinations. PLA = palmitic acid (C16:0); POA, palmitoleic acid (C16:1, n-8); STA = stearic acid (C18:0); OLA, oleic acid (C18:1, ω-9); LLA = linoleic acid (C18:2, ω-6); ALA, α-linolenic acid (C18:3, ω-3); DPA = docosapentaenoic acid (C22:5, ω-3); TCA = tetracosanoic acid (C24:0); DHA = docosahexaenoic acid (C22:6, ω-3); NVA = nervonic acid (C24:1, ω-9). DHA/AA = molar ratio of DHA and AA. USI (unsaturation index) = number of double bond(s) per (poly)unsaturated fatty acid)/100. Data were analyzed by one-way ANOVA, followed by Fisher’s PLSD test for post hoc comparisons.

**Cont:** control-diet fed rats. **MN:** malnourished group. **MN + LPS:** malnourished+Lablab purpureus L. purpureus extract-

The results of this study suggest that *L. purpureus* (L) flour supplementation improves the body weight and increases the levels of protein, potassium, and iron in plasma. Moreover, levels of DHA increases significantly in plasma, liver, and brain tissues, with concurrent ameliorations of hepatic oxidative and proinflammatory stress, and plasma lipid profile in MN rats. Usually, the loss of body weight is used as a marker for the first sign of malnutrition. In this regard, Fletcher and Carey (2011) reported that a loss of 10% of body weight reflects a profound effect of malnutrition. Thus, the decrease in the body weight by 16% authenticates that protein-malnutrition was generated in our experimental MN model rats. Malnutrition can evidently be replenished by the supplementation of adequate diet. Consequently, the increase (p < .05) in the body weight of MN + LPS rats suggests that *L. purpureus*, at least partially, was able to replenish the malnutrition of the MN rats in our experimental paradigm. Malnutrition, however, caused only a 5% decrease in the brain weight (Figure 3b). This indicates that malnutrition could not sufficiently affect the brain weight of our MN model rats. Here, we speculate that the brain was somehow resistant to malnutrition at the adult age of the rats. *L. purpureus* supplementation increased the brain weight by only 8% of the MN + LPS rats; however, the effect on the brain weight was not statistically significant. In contrast, the liver weight of the MN rats decreased significantly (by ~17%), as compared with that of the control rats (Figure 3d). The mechanism as to why malnutrition decreased the weight of liver remains to be clarified. We speculate that protein-energy malnutrition is accompanied by metabolic changes, whereby the animals attempt to guarantee the energy supply to organs of utmost importance at the expense of structural fat and protein. Our assumption was consistent with the previous reports (Cahill, 1970). Opleta et al. (1998) also reported that malnutrition decreases both cell number and cell size in the hepatic tissues and hence decreases the liver weight. Thus, the increased turnover of the metabolites from the liver and/or worsening of liver cells might be the reasons for which the liver weight of the low protein-supplemented MN model rats decreased. *L. purpureus* supplementation significantly increased the plasma proteins of the MN + LPS rats, indicating that proteins contained in *L. purpureus* flour might have contributed to increase the plasma proteins as well as liver weight in these rats.

**4 | DISCUSSION**

The antioxidants effect of the *L. purpureus* extract is shown in Figure 5. FR induced a significant increase in the degree of OS in the whole homogenates of hepatic tissue (Figure 5), as indicated by an increase in LPO levels. *L. purpureus* extract, however, significantly withstood the FR-induced in vitro OS. This was demonstrated by significant reductions in LPO levels in the extract-pretreated whole homogenates (i.e., OS+Ext samples). All these outcomes thus suggest that *L. purpureus* extract could be used as a decent source of antioxidants to withstand the OS.
ω-6 PUFAs in the diet is also important, because of their competitive nature and their different biological roles at the cellular levels. Compared with those of the control rats, the plasma levels of ω-6 LLA and ω-3 ALA as well as those of the AA and DHA decreased significantly in the MN rats. The relationship between protein-energy malnutrition and essential fatty acid deficiency remains to be clarified. Several previous studies have shown that protein-energy malnutrition is associated with reduced levels of PUFAs in the plasma and RBC membranes (Holman et al., 1981; Wolff et al., 1948; Koletzki, Abiodun, Laryea, & Bremer, 1986). The results of decreased levels of LLA and ALA in the MN rats of our investigation are also consistent with the reports of Smita, Muskiet, and Boersma (2004). The LLA and ALA, respectively, represent the major ω-6 and ω-3 PUFAs in LPS [9]. Therefore, the increase (p < .05) in LLA and ALA levels in the plasma of MN + LPS rats are consistent with the presence of LLA and ALA in L. purpureus seed flour. Furthermore, the change in AA and DHA levels in the plasma of the MN + LPS rats might be due to in vivo conversion (elongation) of the dietary LLA and ALA (derived from L. purpureus flour) to AA and DHA, respectively. Moreover, changes in the fatty acid profile resulted in an overall increase of the DHA/AA molar ratios and the degree of USI in the plasma of the MN + LPS rats. Changes in plasma LLA and ALA levels were also reflected in the liver fatty acid profile of the MN rats. Levels of LLA decreased (though not significantly), whereas ALA levels decreased significantly. Levels of atherogenic AA increased (p < .05), whereas DHA levels decreased (p < .05) in the liver tissues of MN rats. All these changes (Table 4) brought about a significant decrease (p < .05) in the DHA/AA molar ratio and degree of USI. However, the supplementation of L. purpureus to MN rats significantly decreased the levels of AA and increased DHA levels, DHA/AA molar ratios and the USI of the liver tissues.

Levels of ω-6 LLA and AA increased, whereas those of DHA decreased in the brain tissues of MN rats. Interestingly, the supplementation of L. purpureus increased DHA levels (by approximate ly twofolds), concurrently with an increase in the DHA/AA molar ratio. The DHA/AA ratio is considered an antioxidative indicator in the brain tissues (Gamoh et al., 1999; Hashimoto et al., 2002). Recently, Serrini and Calviello (2015) also reported that ω-3, in particular DHA, reduces OS in neurodegenerative diseases. Reductions in the levels of DHA and/or DHA/AA molar ratios are associated with an impairment in learning and memory (Hashimoto et al., 2002, 2017). Neurodegenerative diseases such as Alzheimer’s disease is also accompanied with a decrease in brain DHA levels (Hashimoto et al., 2002). The results of increased levels of DHA or DHA/AA ratio are, therefore, suggestive of the beneficial effects of L. purpureus flour in malnutrition-induced impairments of learning and memory. Furthermore, the USI increased significantly in the plasma, liver, and brain tissues of MN + LPS rats.

FIGURE 4 In vivo effects of supplementation of Lablab purpureus on the levels of lipid peroxide (LPO) (a) and (b) tumor necrosis factor alpha (TNFα) in the hepatic tissues of the in vivo experimental rats. Results are means ± SEM for six rats per group. Data were subjected to one-way ANOVA, followed by Fisher’s PLSD for post hoc comparisons. Bars with different symbols are significantly different at †, ‡, § p < .05. Control: Standard pellet-diet fed rats. MN = malnourished-diet fed rats. MN + LPS = L. purpureus-supplemented malnourished rats

FIGURE 5 Effect of Lablab purpureus flour extract on the in vitro Fenton reagents (FR)-induced oxidative stress (OS) in the whole homogenate samples of liver tissues. Data (n = 5) were subjected to one-way ANOVA followed by Fisher’s PLSD post hoc test for multiple comparisons. Bars with different symbols are significantly different at †, ‡, § p < .05. LPO: lipid peroxide. Con = whole homogenate (WHs) only. Ext: extract-only treated WHs. OS: oxidative stress was induced in the WHs with FR. OS+Ext: extract-pretreated WHs + OS
The larger USI indicates a greater membrane fluidity, which plays a crucial role in many cellular and physiological functions. For example, an increase in membrane fluidity ameliorates hypertension (Hashimoto et al., 1998), platelet aggregation (Hossain et al., 1998; Hashimoto et al., 2006), hepatic secretory functions (Hashimoto et al., 2001), exo/endocytosis or neurotransmitter release from the synaptic plasma membranes/brain cognitions (Hashimoto et al., 2017), and cellular glucose transport (Weijers, 2012). These results thus demonstrate that LPS could be used not only in replenishment of protein-energy malnutrition, it may also replenish essential DHA levels in brain and, consequently, may contribute positively to brain cognition impairments. In contrast, malnutrition increased significantly the levels of saturated fatty acids such as PLA, STA, and TCA, and monounsaturated fatty acids such as POA, OLA, and NVA in brain tissues. It, therefore, remains to be clarified as to whether the increase in the levels of saturated fatty acids was an adaptive response to the EFA-deficiency during malnutrition in our MN rats (Table 5).

LPS contains a considerable amount of polyphenols and flavonoids: it exhibited in vitro DPPH-free radical scavenging knock as well as anti-LPO effects (Figure 5). We, therefore, infer that the antioxidant contents and/or antioxidative ability of *L. purpureus* flour reduced the OS in the malnourished (MN + LPS) rats, as indicated by decreased levels of LPO in the liver tissues of *L. purpureus*-fed rats (Figure 4). The hepatic OS was accompanied with an elevated level of proinflammatory stress, as characterized by increased levels of TNFα in the MN rats. Hepatic LPO levels were 90% more in the MN rats than those in *L. purpureus*-supplemented well-nourished rats. The OS is severely compromised in malnutrition (Aly, 2014). It is, therefore, conceivable that the hepatic rise in LPO levels, induced by dietary protein depletion, may be one of the important contributing factors in the activation of proinflammatory response. The speculation is well consistent with the increase (p < .05) in hepatic TNFα levels in the MN rats than those in *L. purpureus*-supplemented rats.

The beneficial effects of *L. purpureus* supplementation should, nevertheless, be claimed with precautions. Some previous reports claim that the antinutrients such as phytate reduces the absorption of essential trace elements and minerals during gastrointestinal passage, and hence leads to calcium, iron, and zinc deficiencies (Zhou & Erdman, 1995; Jenab & Thompson, 2002; Gemede, 2014). In contrast, the beneficial properties of phytate, for example its antioxidant (Graf, 1987; Minihane & Rimbach, 2002) and anticancer activities (Shamsuddin, 1995, 2002), have also been reported by some other investigators. In the present investigation, the levels of Na, K, and Ca decreased, though not significantly; Fe levels, however, decreased significantly in the MN rats when compared with those of the control rats. We have previously reported that *L. purpureus* contains 1% phytate (Hossain et al., 2016). If the phytate present in *L. purpureus* could reduce the absorption of microelements/minerals, the levels of these elements would decrease further in MN + LPS rats. Instead, the levels of plasma Fe and K increased in MN + LPS rats. As malnutrition is usually accompanied with electrolyte imbalance, it is essential that the imbalance be corrected. *L. purpureus* contains considerable amounts of micronutrients and minerals (Shahhu et al., 2014). Therefore, the levels of Fe and K increased and contributed to the electrolyte and mineral pools in the malnourished rats (MN + LPS).

Plasma levels of TG, TC, HDL-C, LDL-C, and VLDL-C were not noticeably altered in the MN rats when compared with those of the control rats. In contrast, Oyagbemi and Odetola (2013) reported an increased level of both plasma TC and TG in the malnourished rats. In our investigation, *L. purpureus* supplementation to the malnourished rats rather decreased (p < .05) the levels of all these parameters of the lipid profile. The mechanism(s) of action as to why the feeding of *L. purpureus* flour decreased plasma TC and TG in the MN + LPS rats is not clear. We infer that the fiber content of the seed powder might have played an important role in decreasing the intestinal absorption of fats including cholesterol and, consequently, increased the fecal excretion of cholesterol and other fats. Though LDL-C levels did not decrease significantly (it decreased by 8% only), levels of plasma VLDL-C decreased (by ~35%) significantly in MN + LPS rats. Here, we speculate that, before being secreted from the hepatic cells to the plasma pool as VLDL-C, a portion of hepatic-pool cholesterol might have been bypassed to biliary-secretory pathway and excreted via feces, decreasing thereby the levels of plasma VLDL-C. If any other mechanisms exist, it has remained without further predications. Plasma glucose levels were not significantly altered either in the MN or in the MN + LPS rats when compared with those of the controls.

5 | CONCLUSION

The results of the present study clearly suggest that the supplementation of *L. purpureus* bean flour increases the body weight as well as levels of plasma protein, potassium, iron, and essential fatty acids (LLA and ALA) in the plasma, liver, and brain. All these results suggest that *L. purpureus* may replenish not only protein-energy malnutrition, but may also augment DHA levels in brain. However, further research in malnourished human subjects is important.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this paper.

DATA AVAILABILITY STATEMENT

All data generated or analysed during this study are included in this published article (and its supplementary information files).

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