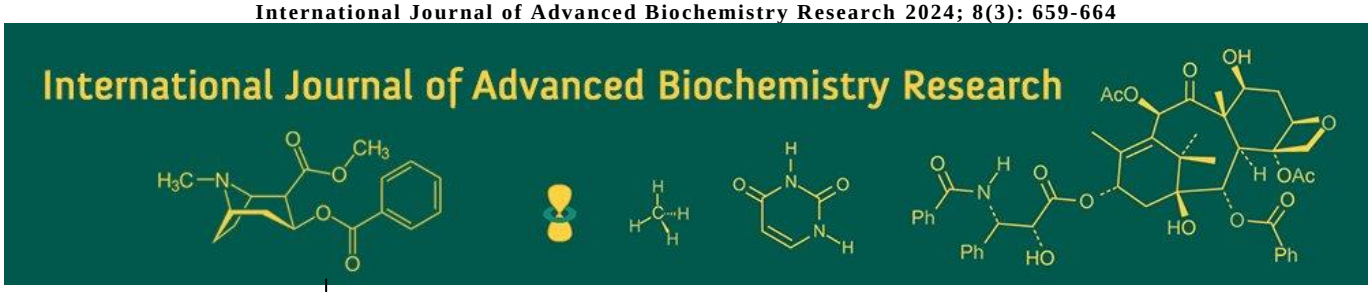

ISSN Print: 2617-4693
ISSN Online: 2617-4707
IJABR 2024; 8(3): 659-664
www.biochemjournal.com
Received: 21-12-2023
Accepted: 26-01-2024

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Mungbean seed protein: A potent functional food with health promoting biological potential

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DOI: https://doi.org/10.33545/26174693.2024.v8.i3h.813

Abstract
Mung bean protein (MBP), an important component of vital amino acids, minerals, and vitamins, as well as an adequate source of protein, carbs, and fibre, has drawn a lot of interest in the growing trend of plant-based proteins. When compared to other pulses, the protein quality is higher in MBP. Mungbean consumption is associated with favourable health benefits. Increasing interest is being paid to dietary peptides derived from mungbean protein. MBP has a variety of bioactivities, such as improvements in growth deficiencies and cognitive dysfunction, type 2 diabetes, anticancer, anti-obesity, antihypertensive, antioxidant, anti-inflammatory, and antimicrobial activity. Balanced diet plays an essential role in prevailing against many illnesses. Numerous bioactive substances found in mungbean, such as phytochemicals, bioactive peptides and antioxidants have protective and preventive impacts on health. This review outlines the most recent research on proteins obtained from mungbeans, their effects on human health and their use as a valuable functional food.

Keywords: Mungbean seed protein, potent functional food, health promoting biological potential

Introduction
Proteins serve as essential nutrients needed by cells, tissues and organs to perform vital functions. Consuming protein in the diet has a number of health benefits, and because animal and plant proteins have superior bio-acitivity so, they are frequently utilised as food components (Gupta *et al*., 2022) [14]. Consumers are now paying greater attention to plant proteins than animal proteins due to their benefits, which include reduced costs, benefits for cardiovascular disease, natural abundance, favourable environmental effect, and sustainability (Gupta *et al*., 2021; Gupta *et al*., 2018a) [9, 12]. Plant-based diets have been encouraged by several healthcare facilities in an effort to reduce malnutrition and improve human health. Thus, a variety of nutrient-dense plant-based diets have been adopted by health care programmes (Gupta *et al*., 2016; Gupta *et al*., 2017; Gupta *et al*., 2018b, c, d; Gupta etc., 2019a,b,c; Bhagyawant *et al*., 2019) [12, 13, 15-17, 10, 11, 2]. The mung bean (*Vigna radiata* L.) one of these foods, is well-known for its health advantages. It is well recognised for its detoxifying bioactivities and is a substantial source of plant-based nutrition (Gupta *et al*., 2018a) [12].
India's native mungbean crop is utilised extensively both in Asia and the Western countries for fresh salad vegetables and ordinary cuisine of seeds and sprouts (Ezekekwu *et al*., 2021) [5]. Mung beans typically possess a high protein content (25 to 28%) and are rich in carbohydrates, vitamins, minerals and active substances. Due to its high lysine content and balanced amino acid ratio, mung bean protein (MBP) is considered a high-quality protein (Hou *et al*., 2023) [17]. The biological potential of MBP for health and wellness has been further emphasised by a previous studies. These biological potential include antidiabetic, anti-obesity, lipid-lowering, antihypertensive, antioxidant, and anti-inflammatory activities, as well as the capacity to modulate mental impairment, growth deficiencies and gut microbiota (Gupta *et al*., 2018a) [12].
As a source of commercial protein, MBP has garnered significant interest in the food and pharma industry due to its superior yield, accessibility, elevated nutritional value and biological potential. The review will provide readers with a comprehensive grasp of MBPs and assist them in extending the range of possible uses for MBPs as food additives with biological potential.

Biological potential

Plant-based proteins have several biological potentials in addition to their nutritional value, which dictates how MBP could be used in the field of functional foods. The principal biological potential both *in vivo* and *in vitro*, which includes effect on antidiabetic, anticancer, anti-obesity, antihypertensive, antioxidant, cognitive dysfunction, gut microbiota modulation, anti-inflammatory and antimicrobial (Figure 1). These explanations will aid in directing MBP consumption for distinct physiological demands.

Antidiabetic activity

Chronic metabolic disease known as type 2 diabetes mellitus (T2DM) is an important global health issue. Elevated blood glucose levels are the most common symptom in people with type 2 diabetes (T2DM), which is linked to changes in insulin action, secretion, and endogenous glucose production. The most favoured and successful method for regulating patients blood glucose levels is through dietary changes. Food ingredients that have the ability to block α-amylase and α-glucosidase can prevent the breakdown of starch and the absorption of glucose (Miller & Joubert, 2021; Gupta *et al*., 2022) [14]. The α-amylase inhibitor found in MBP has a molecular weight of 27 kDa and a specific activity of 14.5 U/(mg of protein), which is lower than that of common beans (495.2–1925.5 U/(mg of protein)) (Wisessing *et al*., 2010) [30]. Furthermore, MBP peptides derived from Alcalase hydrolysis demonstrated greater α-glucosidase inhibitory activity (42.17%) (Li *et al*., 2022) [22]. The mice on a high-fat diet experienced a 24.93% decrease in their fasting blood glucose level after 5 weeks of MBPs intake (245 mg/kg/day) (Li *et al*., 2022) [22]. In addition, investigations on both humans and animals have verified MBP's hypoglycemic action. According to a research, glucagon-like peptide-1 (GLP-1) production was elevated by around 3.6% in a high-fat diet when supplemented with 26% MBP (Nakatani *et al*., 2018) [39]. GLP-1 can reduce blood sugar and prevent diabetes by suppressing the brain's appetite, skeletal muscle's absorption of blood glucose, pancreatic glucagon secretion, inhibition of endogenous hepatic glucose production, and blood sugar normalisation (Whang *et al*., 2019) [29]. In addition, an 8-week clinical trial revealed that individuals with low insulin sensitivity could improve insulin resistance and lower their serum insulin levels without experiencing fluctuations in their blood glucose levels if they consumed at least 3 g of MBPs before breakfast and dinner (Kohno *et al*., 2018) [20]. The possible use of MBP in the production of hypoglycaemic foods is supported by these findings.

Anticancer activity

Studies have demonstrated that dietary choices made throughout life can impact the likelihood of carcinogenesis at every stage of the disease process, typically lowering the risk in a positive way. However, there are other factors that contribute to cancer as well, including radiation, genetic predisposition, smoking, obesity, chronic inflammation, and immunosuppression (Gupta *et al*., 2021) [9]. A diet high in beans may be associated with a lower risk of developing several forms of cancer, according to substantial evidence. Peptides and proteins from mung beans were shown to have dose-dependent anti-proliferative effects on a variety of cancer cell lines, including leukemia (HL-60), digestive system cancer cells (CAL27, AGS, HepG2, and Caco-2), and human breast cancer cells (MCF-7 and MDA-MB-231) (Gupta *et al*., 2018a) [12]. Though the precise regulatory mechanism preventing cancer cells has not yet been fully established, it is already evident that the chemicals in mung beans have an anti-proliferative impact on cancer cells. An essential component of the host's defense against infection and malignancy is the cell-mediated immune response.

Anti-obesity and lipid-lowering activity

According to McIntyre *et al*. (2006) [40], obesity promotes the development of chronic metabolic diseases, cognitive decline, and even mental disorders. It also results in weight increase and fat buildup. Over 600 million people globally suffer from obesity, a frightening proportion that seems to be rising, especially among kids and teenagers. The primary cause of obesity is an imbalanced diet high in excess calories. Recently, there has been a growing interest in the use of plant proteins to treat obesity due to their safety and lack of negative side effects. The administration of MBP (26% in diet) to mice for 4 weeks resulted in a 16.7% reduction in body weight when compared to the casein control group (Nakatani *et al*. 2018) [39].

According to a clinical investigation, when healthy individuals took 6.0 g of commercially available MBP daily for four weeks, their body mass indices decreased dramatically from 29.1 to 28.9. (Kohno *et al*., 2018) [20]. Typically, obesity and hyperlipidemia coexist due to aberrant lipid metabolism (Ul Islam *et al*., 2021) [25]. Consuming MBP lowers cholesterol levels. In earlier research, we demonstrated that 2% MBP-supplemented diets improved the elimination of cholesterol, increased the production of mRNA3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase and cholesterol-7α-hydroxylase (CYP7A1), and enhanced sterol excretion in mice (Yao *et al*., 2014) [33]. Moreover, mice given a diet containing 23.4% MBP for four weeks had blood adiponectin levels that were around three times greater than those of the casein control group (Tachibana *et al*., 2010) [41].

Adiponectin stimulates fatty acid oxidation and improves insulin sensitivity in the liver, thereby regulating lipid metabolism. It also has a positive correlation with serum levels of high-density lipoprotein cholesterol and a negative correlation with serum levels of low-density lipoprotein cholesterol and total cholesterol (TC) (Matsuzawa *et al*., 2011) [24]. By blocking sterol regulatory element binding factor 1, which controls fatty acid production at the gene level, MBP expedites lipid breakdown in the liver through an adiponectin signal cascade (Tachibana *et al*., 2010) [41]. According to Yao *et al*. (2014) [33], MBP also had the ability to lower TC levels more successfully (17.1%). Additionally, studies have indicated that MBP lowers triglycerides in a manner akin to that of β-conglycinin (Tachibana *et al*., 2010) [41]. According to Kohno *et al*. (2018) [20], there might be a correlation between the physiological effects of MBPs and β-conglycinin because of their comparable amino acid makeup and sequencing. As previously mentioned, MBP is preferred for individuals who are obese or hyperlipidemic after it is included as a functional element.

Antihypertensive activity

According to Gupta *et al*. (2022) [14] hypertension is a highly prevalent factor in cardiovascular mortality and morbidity. Plant-based bioactive substances often exert their antihypertensive effects by blocking the activity of the

enzyme angiotensin-converting enzyme (ACE), which facilitates the conversion of angiotensin I to angiotensin II in the mammalian renin-angiotensin system. By directly affecting blood vessels, sympathetic nerves, and adrenal glands, angiotensin II raises blood pressure. With bioactive peptides that inhibit angiotensin II–converting enzyme activity in vitro, 8S globulin, which makes up more than 80% of MBP, has been found (Li *et al*., 2006) [21]. According to research, alcalase hydrolyze to produce MBP hydrolysate (MBPH), which has ACE dose-dependent inhibitory action (Gupta, *et al*., 2018a) [12].

The alkaline hydrolysate of MBP was found to have higher ACE inhibitory activity (IC50 value: 0.32 mg/mL; Gupta *et al*., 2018) [8]. Furthermore, research conducted on animals have demonstrated that a single intragastric injection of high-dose aqueous extracts of enzyme-digested mung bean sprouts (600 mg/kg body weight) dramatically lowers blood pressure in spontaneously hypertensive rats. Both the raw and dried sprout extracts dramatically lowered the systolic blood pressure in rats after two weeks of therapy in a long-term treatment for four weeks in the same study. According to these findings, MBPH may be utilised to both prevent and treat hypertension (Hou *et al*., 2019) [18]. Mung bean antihypertensive peptides may generally be obtained by the enzymatic hydrolysis of MBP. As a result, using an enzymatic mung bean polypeptide intervention diet has been shown to be a successful method of helping hypertension individuals regain their health and may even be an adjunct for drugs that treat hypertension.

Antioxidant activity

The primary causes of illnesses including ageing, inflammation, and cancer are oxidative stress and an inadequate concentration of free radicals in the body. The majority of food ingredients that are good for one's health also have chelating and antioxidant properties (Zeb, 2020) [35]. The antioxidant properties of mung bean peptides have been the subject of several investigations. The antioxidant activities were found to be negatively correlated with the molecular weight of MBPH, according to Xie *et al*.'s (2019) [31] evaluation of the scavenging capacity of 2,2-diphenyl-1-picrylhydrazyl (DPPH), hydroxyl radicals, superoxide radicals, and Fe^{2+} chelating activities in three MBPH fractions hydrolyzed by Alcalase. Furthermore, MBPHs derived from ficin and bromelain demonstrated remarkable DPPH free radical-scavenging activity, with IC_{50} values of 9.45 and 8.67 µg/ml (Zheng *et al*., 2020) [38]. In contrast, the Fe^{2+} chelating activity of MBPH with IC_{50} of 14.84 µg/ml (Zheng *et al*., 2022) [37]. Because hydrophobic and aromatic amino acids are highly present in injured NCTC-1469 cells, MBPH has been shown to scavenge reactive oxygen species (ROS). At the same time, oxidative stress and lipid peroxidation were suppressed (Xie *et al*., 2020) [32]. In this study d-galactose-induced ageing mice, MBP was given at a dosage of 250 mg/day, or 84 g/day for people, for 42 days in order to assess the impact of the medication on oxidative stress indicators.

The consumption of MBP led to a significant increase in the serum total antioxidant capacity as well as the activities of superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSH-Px) in both the serum and liver tissue. However, there was a significant decrease in the content of malondialdehyde (MDA) in liver homogenates (Abeyrathne *et al*., 2022) [1].

Improvement in growth deficits and cognitive dysfunction

In malnourished rats generated by a low-protein diet, dietary treatment with MBP (20% MBP in the meal) for 6 weeks markedly reversed growth deficits and cognitive impairment. Rats given MBP fared well in the Morris water maze test, and their body weight/tail length and insulin-like growth factor $(IGF)^{-1}$ levels, which are linked to early hippocampus development, were recovered. Prior research indicated that adding MBP to the diet raised the (IGF)-1 content (19.88%). However, the data also indicated that MBP is crucial for addressing memory impairment (Wei, Wang *et al*., 2021) [28].

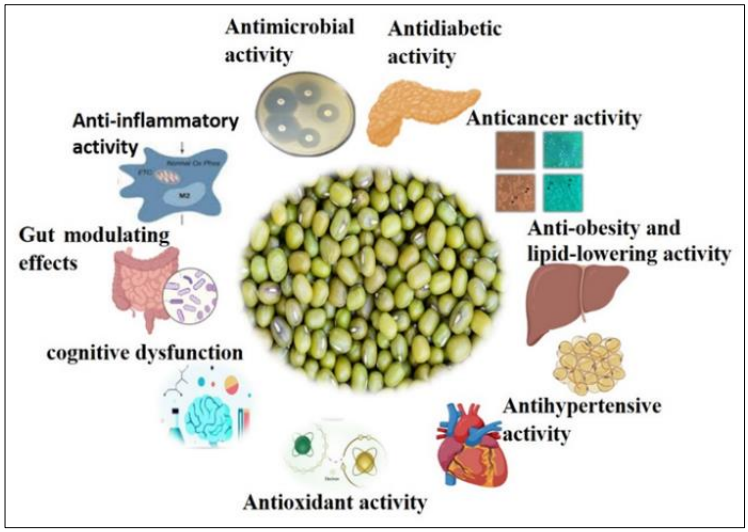

Fig 1: Biological potential of mungbean protein

Gut microbiota–modulating effects

Research has demonstrated that consumption of MBP can result in notable alterations in gut microbiota, including an upsurge in *Bifidobacterium longum* subsp., *Alloprevotella*, and *Lactobacillus* and a reduction in pathogenic bacteria [Ruminococcus] torques group and Enterococcus (Wei, Wang *et al*., 2021) [28]. Additionally, the majority of MBP's biological potential as reported in in vivo investigations is associated with its ability to modify the makeup of the gut microbiota. Changes in dominating bacterial species, alpha

diversity indices (Shannon index), and operational taxonomic unit reconfiguration were shown to be related to the effects of MBP on growth deficits and cognitive impairment. MBPs increase the number of *Lactobacillus*, *Alloprevotella*, and *B. longum* subsp. (Wei, Wang *et al*., 2021) [28]. When comparing the 20% MBP diet to the 7% casein diet, the beneficial bacteria *Lachnospiraceae* NK4A136 group and *Ruminococcaceae* UCG-014 were elevated by 96.06% and 92.20%, respectively.

The modulatory effects of MBP on the gut microbiota composition are also related to its potential anti-obesity effects. An expansion of taxa belonging to the phylum *Bacteroidetes* along with a reduction in *Firmicutes* (Nakatani *et al*., 2018) [39]. In addition, an increase in the members of the genus *Parasutterella*, the family *Ruminococcaceae*, and the *Clostridium* cluster XIVa was also observed, among which the *Clostridium* cluster XIVa was associated with an increase in the production of secondary bile acid deoxycholic acid by 7*α*-dehydroxylation.

Enhancing the variety and richness of gut bacteria in mice is another benefit of MBPs' antioxidant action. After examining the connection between the intestinal flora and the antioxidant index, scientists discovered that the major bacterial species involved in controlling oxidative stress include *Ruminococcus*, *Muribaculaceae*, *Lachnospiraceae* NK4A136 group, and *Roseburia*. A negative correlation was seen between the Lachnospiraceae NK4A136 group and CAT in both liver tissue and serum, as well as between SOD and plasma GSH-Px. Both the MDA and GSH-Px levels showed a favourable correlation with Ruminococcus. Zhang, Ma, *et al*. (2022) [36] found a negative correlation between MDA and *Roseburia* and *Muribaculaceae*. Through the relationship of metabolites, changes in the makeup of the gut microbiota are linked to a number of disorders, including cancer, diabetes, and cardiovascular disease.

Anti-inflammatory activity

MBP has been shown to have anti-inflammatory effect in vivo in addition to its resistance against a number of disorders. Cognitive impairment results from altered synaptic plasticity caused by neuroinflammation. In lipopolysaccharide (LPS)-stimulated macrophages, MBPH has been shown to have anti-inflammatory properties in vitro. Interleukin (IL)-6, IL-1β, tumour necrosis factor-α (TNF-α), and other pro-inflammatory cytokines have been observed to be secreted less often by LPS-induced macrophages when MBPH is present, all without compromising cell survival. The secretion levels of IL-6 were considerably decreased by MBPH (200 μg/mL) (Diao *et al*., 2019) [3].

MBPH effectively decreased the secretion levels of IL-1β by 49.7%. The fundamental process has been ascribed by researchers to the suppression of IκBα phosphorylation, the nuclear translocation of p65 protein, and the control of the over-activation of the nuclear factor-κB NF-κB) signalling pathway (Diao *et al*., 2019, 2022) [3-4]. Furthermore, the brains of rats fed MBP showed reduced levels of pro-inflammatory cytokines (TNF-α, IL-1β, and monocyte chemoattractant protein-1) which also tended to attenuate the toll-like receptor 4 (TLR4)/NF-κB pathway. Li and colleagues (2022) [22] observed that following the successful modelling of mice fed a high-fat diet for five weeks, MBPs (245 mg/kg/day) for five weeks significantly reduced the serum levels of TNF-α and IL-6 in diabetic mice by 28.56% and 21.18%, respectively, compared with the high-fat diet group (Li *et al*., 2022) [22]. Hence, in order to further understand the underlying mechanism of MBP's health advantages, future research should concentrate on examining how MBP ingestion alters metabolites.

Antimicrobial activity

Biocides are the natural antibacterial agents derived from bioactive proteins found in dietary legumes (Gupta *et al*., 2022; Keawpeng *et al*., 2022) [14]. Mungin (18 kDa) and chitinase (30.8 kDa), proteins that were extracted from mung bean seeds, have antifungal properties against *Fusarium oxysporum*, *Botrytis cinerea*, *Mycosphaerella arachidicola*, *Rhizoctonia solani*, and *Coprinus comatus* (Ye & Ng *et al*., 2000) [34]. Mungin and chitinase block α- and β-glucosidases, preventing mouse splenocytes from producing [3H] thymidine. Notably, mungin demonstrated anti-mitogenic action in addition to its anti-fungal properties, akin to cyclophilin (Wang *et al*., 2005) [20]. The protein nsLTP (9.03 kda) found in mung bean seeds also has anti-pathogenic properties. In vitro, a wide range of varied lipids may be bound and transferred across membranes by the nsLTP protein (Wang *et al*., 2004) [27]. It has antibacterial activity against *Staphylococcus aureus* and antifungal effect against *Fusarium solani*, *Fusarium oxysporum*, *Pythium aphanidermatum* and *Sclerotium rolfsii*, but not *Salmonella typhimurium*.

Conclusion

Studies on MBP have already increased in recent years due to the global trend of replacing animal protein with plant protein. MBP has a low sulfur-containing amino acid content but high amounts of leucine and other important amino acids, and it has excellent in vitro digestibility. In this situation, eco-friendly items have been created to meet consumer needs. The practical development of products and the health advantages of MBP must still exist together. In general, MBP, being essential plant protein, with biological potential that needs further investigation to fully grasp its immense possibilities in the functional food and pharmaceutical sectors.

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