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Original Article

Comparative effect of horse gram and black gram on inflammatory mediators and antioxidant status

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ABSTRACT

A balanced diet is important for the overall wellbeing of an individual. Pulses are an important part of a nutritive diet. Pulses have been consumed for at least 10,000 years and are among the most extensively used foods in the world. They are a rich source of protein and fiber, as well as a significant source of vitamins and minerals, such as iron, zinc, and magnesium. The purpose of this study was to compare the effect of two pulses, horse gram and black gram, on inflammatory mediators and the antioxidant enzymes. Two sets of experiments were conducted in rats which were fed with boiled and unboiled horse gram and black gram, at a dose of 100 mg/100 g body weight, for 21 days and 60 days. The results showed that horse gram supplementation for 21 days and 60 days significantly increased the activities of antioxidant enzymes such as superoxide dismutase, catalase, glutathione peroxidase and showed no significant changes in the activities of the inflammatory mediators such as cyclooxygenase, lipoxygenase, myeloperoxidase, nitric oxide synthase, monocyte chemoattractant protein-1 (MCP-1), tumor necrosis factor-alpha (TNF-α), interleukin-1-beta (IL-1β), etc. However, the black gram (with skin and without skin) supplementation significantly increased activities of the inflammatory mediators and showed a significant decrease in the antioxidant enzymes in both the 21-day and 60-day experiments. Thus, these preliminary results demonstrate the anti-inflammatory and antioxidant potential of horse gram and the proinflammatory effects of black gram in rats. This is in accordance with the dietary regime advised by Ayurveda practitioners, where horse gram is to be included and black gram is to be excluded from the diet for conditions such as rheumatoid arthritis. Further studies are to be conducted to validate the same.

1. Introduction

Consuming a well-balanced diet is essential for maintaining good health of the body. A nutritious diet helps to keep the immune system functioning properly and contributes to the overall wellbeing of the organism. Without good nutrition, the body becomes susceptible to disease, infection, fatigue, etc. Eating many different foods helps maintain a healthy and interesting diet which provides a range of different nutrients to the body. However, eating too much or too little of a particular food variety can cause issues. Some foods have

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antioxidant or anti-inflammatory potential and can be highly beneficial to the body, whereas some others may be detrimental by eliciting immune reactions in the body. Pulses are a very important food source particularly in Middle and South America, the Middle East, China, Africa, and Asia. India is one of the world's largest producers and consumers of pulses. Pulses have significant nutritional and health advantages for consumers. They are rich in proteins, carbohydrates, fiber, the minerals phosphate, calcium, and iron, and the vitamins of the B-complex. They are low in sodium and saturated fats. Pulses are an important source of protein for vegetarians and should be included in their diet. Soaking dried beans for several hours brings them back to life, activating enzymes, proteins, minerals, and vitamins [1,2]. Horse gram (*Macrotyloma uniflorum*) is one of the lesser known beans. Horse gram is a legume of the tropics and subtropics, grown mostly under dry-land agriculture. Studies have shown that unprocessed raw horse gram seeds not only possess antihyperglycemic properties, but also have qualities which reduce insulin resistance. Raw horse gram seed is rich in polyphenols, flavonoids and proteins, the major antioxidants present in fruits and other food materials [3,4]. Also, a solid state fermentation product, Kaulath, obtained from horse gram, showed an increased free radical scavenging property and can be included in the diet and in formulated foods [5]. Dehulling in combination with germinating the seeds has also been shown to improve the nutritive value of horse gram [6]. These studies in horse gram show the importance of including it in the diet. Black gram (*Vigna mungo*), also known as black lentil, white lentil and black matpe bean, is a bean grown in the Indian subcontinent. Ground into flour or paste, it is also extensively used in South Indian culinary preparations. *Vigna mungo* is used in traditional Indian (Ayurveda) medicine. Pharmacologically, extracts have demonstrated immunostimulatory activity in rats [7].

Inflammation is a localized protective reaction of cells/tissues of the body to any kind of noxious stimulus such as allergic or chemical irritation, injury, and/or infections. This initial response may further initiate a series of biochemical, immunological, and cellular events, ending with physical repair and restoration of function of the injured tissue. Inflammation is a protective attempt by the organism to remove the injurious stimuli and to initiate the healing process. Without inflammation, wounds and infections would never heal [8]. The symptoms of inflammation are characterized by pain, heat, redness, swelling, and loss of function that result from dilation of the blood vessels leading to an increased blood supply and from increased intercellular spaces resulting in the movement of leukocytes, protein and fluids into the inflamed regions [9]. Prostaglandins, thromboxanes and leukotrienes are inflammatory mediators called eicosanoids and are synthesized by cyclooxygenases (COX) and lipoxygenases (LOX) in cell types that are associated with inflammatory disorders. COX-2 catalyzed synthesis of prostaglandin E2 plays a key role in inflammation and its associated diseases [10].

Free radicals are produced by chemical or biological aggression to the body caused by stress, physical damage, infection and cytotoxic or carcinogenic compounds. This may cause peroxidation of cell components such as proteins, lipids and can also break strands of DNA [11]. This oxidative damage caused by the free radicals is considered to play a causative role in ageing and several diseases including cataract, cognitive dysfunction, cancer, myocardial infarction, diabetes, arthritis and several heart diseases [12]. Reactive oxygen species are playing a dual role as deleterious and beneficial species, since they can be either harmful or beneficial to living systems [13]. Our bodies try to protect us from free radical damage by producing antioxidant enzymes that neutralize them. However, they are not capable of handling this function without nutrients and antioxidants provided by our diets. Antioxidants are protective molecules also referred to as free radical scavengers and hence prevent and repair damage done by these free radicals [14]. Antioxidants may also enhance immune defense and therefore lower the risk of cancer and infection. Fruits, pulses and vegetables, the main source of antioxidants in the diet, are associated with lower risk of degenerative disease [15].

The antioxidant enzymes help to maintain the oxidant/antioxidant status by tackling excess of free radicals in the system. The antioxidant enzymes should be studied in order to evaluate the extent of lipid peroxidation during inflammation. The enzymes involved in inflammatory processes are seen to be increased in many of the disease conditions. Hence, such inflammatory mediators should be studied in order to understand the progress of inflammation and also to figure out good therapeutic targets for inflammation. The present study is designed to evaluate the effects of two pulses, horse gram and black gram, on oxidative stress and inflammatory mediators in normal rats.

2. Materials and methods

2.1. Chemicals and solvents

Histopaque, linoleic acid, and arachidonic acid were purchased from Sigma-Aldrich Chemicals (St Louis, MO, USA) and Spectrochem Pvt. Ltd. (Mumbai, Maharashtra, India). All other chemicals and biochemicals used were of the highest grade available.

2.2. Collection of the pulses

Pulses, horse gram and black gram were purchased from the local market of Thiruvananthapuram district, India. Two types of black gram were purchased: with skin and without skin varieties.

2.3. Animal experiment

Female albino rats (Sprague–Dawley strain) of body weight 150–200 g which were breed and reared in the department animal house were used for this study. They were provided laboratory chow (Hindustan Lever Lab diet) and water *ad libitum* throughout the experimental period. The rats were housed in polypropylene cages in a room with temperature maintained at $26 \pm 1^{\circ}C$ and a 12 hour light and dark cycle. The animals received human care, in compliance with the