Chapter 29

Use of *Moringa* and Barley for the Control of Hyperlipidemia

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ABSTRACT

Cardiovascular diseases affect the heart and blood vessels. Increased level or deposition of circulating lipids that is hyperlipidemia is among the most significant hazards for coronary heart disease. Coronary heart diseases caused 17.5 million deaths worldwide. Due to chronic heart diseases, 20.28% deaths occur in Pakistan. In recent years, the popularity of natural products has grown. Phytosterol is effective in treating hyperlipidemia. Beta sitosterol, that is a phytosterol, is present in *Moringa Oleifera* leaves which lowers the cholesterol by inhibiting the reabsorption of cholesterol. Soluble fiber Beta glucan present in Barley binds bile acid and prevents the body's ability to breakdown and eliminate cholesterol. There were significant increases in HDL. The data shows a significant reduction in low-density lipoproteins, serum triglyceride level, total cholesterol and increase in high-density lipoproteins. It is concluded from the present study that *moringa* and barley have great ability to manage the above-described hyperlipidemia biomarkers.

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INTRODUCTION

Hyperlipidemia, a chronic ailment connected to cardiovascular disease, has a significant impact on community health as the world's leading cause of death (WHO, 2015).

The risks associated with people's bad lifestyles, such as consuming alcohol, smoking, eating poorly, and not exercising, are linked to hyperlipidemia. According to WHO statistics, 15 million people between the ages of 39 and 69 die each year from noncommunicable diseases (NCDs), with more than 80% of these deaths occurring in developing countries. Because of the rise in their prevalence and the relationship between them and hyperlipidemias that have reached epidemic statistical proportions, it is more vital than ever to keep an eye on how NCDs effect premature mortality, particularly in the population of young people. The total prevalence of hyperlipidemia was 33.8%, with risk values for triglycerides, hypercholesterolemia, cholesterol on high density lipoproteins and cholesterol on low density lipoproteins of 12.8, 15.0, 16.1, and 6.1%, respectively (Ramírez et al., 2020).

Moringa oleifera is a multipurpose herb that is used in human diets around the world. Many detractors argue that a plant with numerous medical benefits includes healing and therapeutic harmonizing circumstances. The *Moringa* plant possesses a high concentration of carotenoids in its leaves and other sections that have nutraceutical qualities. The leaves and stems have grown to an unusually healthy appearance. One of the most important aspects is the therapeutic application of *Moringa oleifera*. It contains all the necessary antioxidants, anti-infection agents, nutrients, and minerals (Abdull Razis et al., 2014).

Hyperlipidemia is the medical term for high lipid levels in the blood or lipid accumulation. The biggest issue with the cardiovascular system is atherosclerosis, which is caused by elevated cholesterol levels. It is characterized by hypercholesterolemia and inflammation. It can also lead to many other health problems, such as failure of heart and stroke, which raise mortality and morbidity (Verma, 2017).

Various studies show that grains rich in soluble fibre, like barley and oats, are useful in decreasing cholesterol as compared to cereals with mostly insoluble fibre, such as wheat or rice. β -glucan is a non-starch polysaccharide soluble fibre found in barley and oats that has the ability to lower LDL by increasing intestinal viscosity, which may reduce cholesterol absorption (EI Rabey et al., 2013). An active component of barley β -glucan has the ability to reduce cholesterol. β -glucan binding bile acid and preventing the body's ability to breakdown and eliminate cholesterol (Andersson et al., 2010). Table 1 shows the lipid profile reference range.

Hyperlipidemia Reference Ranges:

The lipid profile parameters along with their ranges are as follow:

Classification of Lipids

A naturally occurring diverse set of biomolecules is referred to as lipids, which dissolve in organic solvents that are non-polar. Small compounds that are either hydrophobic or amphiphilic can be used to characterize them. These include fatty substances, oils, waxes, and fats. Fat-soluble vitamins, phospholipids, monoglycerides, diglycerides, and triglycerides, as well as sterols and steroids. Lipids are necessary for many bodily biological activities because they make up a large part of the cell membrane, which acts as a mechanical barrier between the cell and its external environment.

They give 9Kcal/g of fat intake and are also a dense source of energy. They are crucial to signaling as well. Mammals who live in cold climates use their subcutaneous fat to slow down the loss of body heat. Lipoproteins are made up of proteins and lipids. They play a crucial role in the transit of lipids and are a component of the cell membrane. In addition to being lipid-based, fat-soluble vitamins provide a source of vital fatty acids (Kumar et al., 2019).

Composition-based Classification

The lipids are divided into three categories based on the makeup of their molecules: simple, complex, and derived lipids.

Simple Lipids

Simple lipids are fatty acid esters with different alcohol contents. They are also categorized as waxes and fats. Triglycerides make up the majority of fats and are present in adipose tissue. These different fat sources include fish, lard, butter, and oils derived from plants like corn or olives. Three fatty acids and a glycerol molecule are joined in triglycerides. The fatty acids could differ or be the same. Esters of fatty acids, waxes are composed of monohydric alcohol with a molecular weight greater than glycerol. Wax has been discovered in a variety of sources, including lanolin, carnauba oil, sperm whale oil, and bee wax.

Lipid Compounds Lipids

Esters of fatty acids that have an extra group added to them in addition to an alcohol and a fatty acid are known as compound lipids. The many kinds of lipid compounds are as follows:

Phospholipids

Phospholipids, sometimes referred to as phosphatides, are mostly present in the tissues of animals. They are made up of two fatty acids, one alcohol-modified 9-phosphate group, and one glycerol molecule. Both hydrophilic (phosphate group) and hydrophobic (uncharged fatty acid) characteristics are present in phospholipids.

Glycolipids

Glycolipids, sometimes known as glycosphingolipids, are molecules made up of carbohydrates, sphingosine, and fatty acids.

Lecithin

A phosphatide connected to choline is present in lecithin. Another name for it is phosphatidyl either serine or choline. It is mostly present in the brain, egg yolk, and organ meats. It facilitates the movement and metabolism of fats. In the food business, it is also employed as an emulsifier.

Cephalin

It is attached to serine or ethanolamine via a phosphatide bond. This primarily affects nerve tissue and aids in blood clotting.

Plasmalogen

Plasmalogen is a key component of structural membranes, particularly when it comes to the stability of cholesterolrich membranes used in cellular signaling. They are classified as glycerophospholipids and have ester and vinyl-ether bonds at positions sn-1 and sn-2. It is present in the heart, brain, and muscles.

Lipositol

When phosphatide is joined to inositol, it is also referred to as phosphatidyl inositol. In the brain, it is generated and broken down quickly. Lipositol is mostly found in the kidneys, heart, brain, and tissues of plants. It is involved in the mechanisms of cell transport.

Sphingomyelin

It is a phosphatide that contains sphingosine that produces choline, fatty acids, and phosphoric acid, and upon hydrolysis, sphingosine. But no glycerol is generated on the hydrolysis. The brain, nerve tissue, and red blood cells are the main suppliers. It serves as a source as well of the body's phosphoric acid.

Cerebrosides

Ceramide, which is a sphingosine and fatty acid, along with a mono sugar attached to the C1 of esfingol via a βglycosidic link, makes up cerebrosides. In these, galactose is the most frequently occurring carbohydrate, and the fatty acids are either 24 carbon lignoceric, cerebonic, or hydroxylignoceric acid. Cerebrosides containing lignoceric acid are named kerasin, whilst those containing cerebronic acid are referred to as phrenosin. Cerebrosides originate from the white matter of the brain and the myelin sheaths surrounding nerves. Other tissues' cell membranes also contain trace amounts of it.

Ganglioside

A glycosphingolipid makes up ganglioside. It has an oligosaccharide or ceramide that is connected to fatty acids, sphingosine, n-acetylneuraminic acid, and hexose sugar. The spleen, brain, and nerve tissue all contain this.

Sulfolipid

This glycolipid has sulfur in it, and the sulfur is linked to galactose through an ester bond. This can be found in the chloroplasts of plants, testicles, liver, and brain. lipids in proteins.

Proteolipids

These consist of proteins that are covalently attached to lipid molecules such as cholesterol, fatty acids, isoprenoids, and glycosylphosphatidylinositols. They exist in the nerve and brain.

Precursors and derivatives Lipids

By hydrolyzing simple or conjugated lipids, these lipids are produced. Fatty acids, glycerol, fatty aldehydes, ketone bodies, steroids, hydrocarbons, lipid-soluble vitamins, and hormones are all members of this class.

Steroids, Terpenes, and Terpenoids

Terpenes are basic hydrocarbons made up of five carbon isoprene units connected in various ways to one another. These make up the greatest class of secondary metabolites and are present in rubber, resin acids, essential oils, and colors derived from plants, such as lycopene and carotenoids. Terpenoids are modified terpenes that have distinct functional groups. Terpenoids can be categorized as monoterpenes, diterpenes, triterpenes, sesterpenes, and sesquiterpenes based on the units of carbon.

Classification on the basis of Chain Length and Unsaturation

Lipids are made up of tiny units called fatty acids. These are even carbon atoms in a linear hydrocarbon chain. The carboxyl group (-COOH) is at one end of this chain and the hydrogen atom (H) is at the other. FAs often assemble in a group and are not seen in nature in their free form. trio used with alcohol to create triglycerides. The length of the chain, the degree of unsaturation, and the quantity of essential acids present can all be used to categorize fatty acids. Cerebrosides are found in the brain and nerve myelin sheaths of fatty acids with less than eight carbon atoms in their chain, eight to twelve carbon atoms in their chain for medium chain fatty acids, and fourteen carbon atoms or more for long-chain fatty acids. Other tissues' cell membranes also contain trace amounts of it.

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Classification on the basis of Chain Length and Unsaturation

Lipids are made up of microscopic units called fatty acids (FAs). These are even carbon atoms in a linear hydrocarbon chain. The carboxyl group (-COOH) is at one end of this chain and the hydrogen atom (H) is at the other. In nature, FAs are not found in their free form. Instead, they usually combine with alcohol in groups of three to produce triglycerides. The length of the chain, the degree of unsaturation, and the quantity of essential acids present can all be used to categorize fatty acids. A fatty acid is classified as a short chain if it has less than eight carbon atoms, medium chain if it has eight to twelve carbon atoms, and long chain if it has fourteen or more carbon atoms.

Mechanism of Action of *moringa* **in our Body**

The *Moringaceae* family, which contains the plant species *Moringa*, can be found across the tropics and subtropics (Boonchum et al., 2011). Leaves of these trees have been shown to be useful in treating a variety of chronic conditions, including hypercholesterolemia (Vergara-Jimenez et al., 2017). *Moringa* leaves, for example, are high in vitamin C, vitamin A, calcium, iron, and potassium (Aborhyem et al., 2016). *Moringa* has been around since 150 B.C. Old kings and queens drank *Moringa* leaves and fruit to maintain mental alertness and healthy skin, according to historical records (Zaku et al., 2015). *Moringa's* bioactive component, Beta-sitosterol lowers cholesterol. The impact of lowering cholesterol is caused by a decrease in endogenous cholesterol reabsorption (Mehta et al., 2003).

It is considered one of the old and useful trees; it is famous for curing a lot of diseases like asthma, bronchitis, liver fibrosis, cholera, renal disorders, chest congestion, as well as several other kinds of illnesses (Khawaja et al., 2010; Hamza, 2010). Different parts of the *Moringa* plant have cholesterol lowering properties. Because many phenolic compounds exist like zeatin, quercetin and sterol like beta sterol (Anwar et al., 2007).

Leaves of *moringa* are riches are enriched with beta sitosterol, a plant sterol that is same as cholesterol but varies in structure due to the presence of ethyl group in the side chain at C24 which exist in cholesterol. It lowers the cholesterol of serum cholesterol in the body by stopping the absorption of cholesterol in the intestine. Moreover, it also promotes its excretion and acts as natural steroids. This mechanism directly contributes to the hypolipidemic activity of MO (Jain et al., 2010).

Classification of Beta-Sitosterol

Synthesis of Beta-Sitosterol

Even though β-sitosterol hasn't been entirely synthesized yet, there are two methods that can be used to create it from pure stigmasterol. The first route involves selectively hydrogenating the side chain Δ22–23 alkene to yield βsitosterol, as well as varying amounts of stigmasterol and fully saturated stigmastanol. The second approach aims to achieve the same selective hydrogenation while shielding the Δ5–6 alkene from cyclopropylcarbinyl ether. After this procedure, the Δ22–23 double bond should be hydrogenated, and the cyclopropane should be solvolyzed to create the C3–alcohol and Δ5–6 alkene once more. The latter approach appears to be highly beneficial, since it produces high-purity β-sitosterol. In actuality, semi-synthesis of β-sitosterol remains a challenge due to the production of methyl ether byproducts, which are difficult to remove.

β-Sitosterol Biosynthesis

Membrane biogenesis involves the regulation of phytosterol biosynthesis. Research has indicated that the synthesis of β-sitosterol occurs naturally through the routes of both deoxyxylulose and mevalonate. The mechanism of β-sitosterol production has been examined using the 13C-labeling technique. Cycloarteol has been identified as an initial substrate, however, the exact identification depends on the organism utilized. Farnesyldiphosphate (FPP) is actually created when two molecules of dimethylallyldiphosphate (DMAPP) combine with one molecule of isopentenyl-diphosphate (IPP). After that, two of the latter molecules (FPP) are joined tail to tail to generate squalene, a triterpene, and ultimately cycloartenol (Saeidnia et al., 2014).

Distribution of β-Sitosterol in Plants and Algae

β-sitosterol is an ancient plant-based molecule. From single-celled organisms to vascular plants, simple sterols have developed into more complex forms. β-sitosterol has been extracted and purified from a variety of plant families using

various chromatographic techniques, according to a literature study. The plants included here are only a few of the wellknown sources of the compound's distribution and its derivative components, which span many different plant families.

Mechanism of Action of Barley in Our Body

Barley with reduced glycemic index and rich soluble fiber such as β -glucan and high level of magnesium and chromium are beneficent grains to prevent and treat diabetes and changes in blood fat composition. The usage of barley, as well as the consumption of β -glucan supplements made from barley, resulted in a reduction of lipids in healthy persons (Babitha and Priyamvada, 2016). β -glucan binding bile acid and preventing the body's ability to breakdown and eliminate cholesterol (Andersson et al., 2010).

Recommended Dosage of *Moringa* **and Barley**

The recommended dosage of *moringa* leaves powder is 10-15g per day per person to cure hyperlipidemia. And 30g barley daily is effective to overcome hyperlipidemia. This dosage is proved effective after so many clinical research trials.

Product Develpoment with *Moringa* **Leaf powder and Barley**

Procurement of Raw Material

Moringa leaves and barley were purchased from the local market. All standards or chemicals were obtained from the local market.

Preparation of Procured Material

Sorting of *Moringa* **leaves**

Green, fresh and undamaged *moringa* leaves were selected whereas damaged, willed, bruised and discolored leaves were discarded. The reason is that damaged and decayed leaves give bad flavor as well as it also leads to loss of essential nutrients.

Washing of *Moringa* **leaves**

Selected *moringa* leaves were washed under the running water and the leaves were soaked in the solution of 1% saline for five minutes in order to remove all microbes. Leaves were washed with distilled water. This step is considered crucial in order to get rid of all types of microbes present on the surface of *moringa* leaves.

Preparation of Barley Flour

Barley was purchased from the local market then sun dried and milled into fine flour.

Preparation of Product

- Weighed the barley flour and *moringa* powder
- Mixing of all the ingredients
- Bake at 180°C for 10-15 minutes

Total Phenolic component

Total phenolic component of *moringa* and barley-based cake was determined by Folin- ciocalteu method. This method is described by Kulkarni et al., (2006). The method states, the sample solution was prepared by 5g *moringa* and barley-based cake powder and extracted it with 20ml of n-hexane. The solution was kept for 1 hour. After that, the resultant solution was centrifuged. The centrifugation process took only two minutes at exactly 6000 rpm then filters the solution. Supernatant was saved and was also extracted with 25ml 80% methanol solution for 3 hours. Then filter the solution again the filtrate then evaporates in rotatory evaporator until 3-5ml solution remained left. However, the total phenolic content of *moringa* and barley-based cake was evaluated by using gallic acid as the standard.

Take 125µl sample in the test tube and add 500µl distilled water and 125µl of Folin-Ciocalteu reagent in it. The whole mixture was mixed well after that, the mixture was incubated for about 8 minutes at room temperature. In the mixture 1.25ml of 7% sodium carbonate solution was also added to make the final volume 3ml with distilled water and give stay time of 90 minutes. The absorbance was taken 760nm with the help of UV light spectrophotometer.

In the whole process, Gallic acid was used as a calibration standard. All the readings were recorded with gallic acid equivalents. TPC of extract in gallic acid was calculated with the help of the following formula:

 $C = c \times V/m$

- C= Total phenolic content (mg/g plant extract, in GAE)
- $c =$ Concentration of gallic acid (mg/mL)
- V= volume of extract (mL)
- m= Weight of *moringa*-based cake extract (g)

Total Flavonoids Content

In a test tube, combine 0.25ml phytochemical extracts as needed for TPC with 1.25ml distilled water. After mixing, pour 75μl of sodium nitrite (5% solution) into the test tubes and leave for 6 minutes at 25°C. After 6 minutes, add 150μl aluminum chloride (10%) and distil water to make a volume of 2.5ml and thoroughly mix. The absorbance of the sample was immediately measured at 510nm against a blank using a UV-Vi spectrophotometer. Catechin was used as the standard, with methand concentrations of 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, and 0.9mg/ml.

TFC is calculated against a standard curve of catechin [µg/g catechin equivalent (CE)] as below.

 $TFC=C \times (V/m)$

Where,

C= concentration of catechin in mg/ml

 $V =$ volume of plant extract in ml

m= weight of *moringa* based cake extract in g

Free Phytosterol Determination by UV-Vis Spectrometry using method of Extraction

The samples were macerated for three days in between 100 and 150mL of ethanol for the ethanolic extraction. The obtained ethanolic extract was filtered to remove solid residues, and the filtrate was concentrated at 50°C on a rotary evaporator under reduced pressure. The residue was resuspended in 30mL of ethanol and centrifuged at 8000g for 15 minutes to obtain a clear supernatant. This was transferred to a 50 mL volumetric flask and the volume was adjusted with ethanol. The absorbance was recorded in UV-Vis spectrophotometer equipment at 650nm, in a time range no longer than 10 min after the end of the incubation. Comparative graph of the phytosterols concentrations of each sample by extraction methods (Badilla et al., 2020).

Conclusion

This evaluation has explored the potential curative effects of *Moringa oleifera* and barley cake on hyperlipidemia. *Moringa* and Balrey plant renowned for its health benefits, whole grain known for its potential impact on heart health, are both theoretically capable of beneficial impacts on lipid profiles. This evaluation has explored the potential curative effects of *Moringa oleifera* and barley cake on hyperlipidemia. *Moringa* and Balrey plant renowned for its health benefits, whole grain known for its potential impact on heart health, are both theoretically capable of beneficial impacts on lipid profiles.

It is also the substituted way against the higher prices of pharmacological and traditional food products day by day. So, high prevalence of hyperlipidemia is the major health sickness throughout the world. The important factors in the initiation of hyperlipidemia is the lifestyle modification, high amount of energy intake, unhealthy eating patterns and decreased physical activity. Unhealthy dietary behavior usually causes uncontrolled fat intake and also responsible for the elevation of adiposity.

It is high nutritional value, especially the leaves of *moringa* contains beta sitosterol in highlighted amount, which acts as binding agent and allow the body to flush out all the unwanted substances from the body. It also protects from blood coagulation and increases energy production. Barley contains an active component beta-glucan binding bile acids and preventing the body's ability to breakdown and eliminate cholesterol.

Antioxidant potential of *moringa* based cake was checked by TPC and TFC present in the product.

Cake containing *moringa* powder had shown to possess the capability of lowering the Cholesterol of hyperlipidemic subjects. *Moringa* and Barley based cake are highly nutritious food and used any time during the day, so patients enjoy the food and overcome their higher lipid profile. This product also contains sufficient calories, minerals and antioxidants.

Recommendations

- Nutritional and health awareness programs must be developed, as well as educating people about the health benefits of *moringa* and barley-based cakes.
- Hyperlipidemic patients can use this as a snack at any time during the day to overcome CVD. This is also beneficial to treat obesity.

Therapeutic potential of *moringa* and barley utilized for reducing cholesterol.

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