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Review on "Donkey Milk and its Potential Therapeutic Uses"

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ABSTRACT

Milk is nutrient-rich. Due to its high content of saturated fatty acids, some animal's milk fat is thought to have a health risk. Donkey's milk has gained more and more interest. In the event that a baby has a milk protein allergy from cows, donkeys' milk is a suitable substitute for formula and a promising new dietetic food. Donkey milk shares similarities with human milk in terms of its high lactose concentration, low fat content, and low casein/whey protein ratio. Its nutritional composition is comparable to that of human milk, and it also has characteristics with it such as immunological homeostasis, antibacterial activity, and hypoallergenicity. The scientific data supporting donkey milk's positive health effects on humans is examined in this article. The present review also discusses a number of other donkey milk-related topics, with a focus on the composition, shelf life, antibacterial, therapeutic, and cosmetic properties of the milk.

KEYWORDS: *Donkey milk, Milk chemical composition, Immunomodulation, Antimicrobial activity, cosmetic*

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INTRODUCTION

Milk has been an essential component of human diets since the agricultural revolution because milk provides the body with vital nutrients, which are essential for both nutrition and overall health. Equus africanus asinus, the donkey, belongs to the Equidae family and is a domesticated animal. [(1)]

In terms of lactose concentration, protein content, and amino acid profile, donkey milk is said to be the naturally occurring milk that most closely resembles human milk in composition. Over the past several years, there have been an increasing number of scientific research attempting to clarify the composition of donkey milk and the existence of beneficial substances. Up till now, studies have been conducted to draw attention to compounds that may be bioactive, including functional proteins, vitamins, polar lipids, phytosterols, polyunsaturated and omega 3 fatty acids, and the diversity in milk composition. Studies examining the possible effects of donkey milk on people in vitro and in vivo have been developed as a result of new understanding. This study looks at the data from scientific research on the benefits of donkey milk for human health and its potential uses as a supplement to address ageing, malnutrition, and cardiometabolic disorders. [(2)]

The market price range for donkey milk in India is between Rs 2000 and Rs 7000 per litre. In Hisar, Haryana, the National Research Centre on Equines intends to begin selling donkey milk from the Halari breed. Owing to the demand for donkey milk,

pasteurisation of the milk is required to ensure the safety and quality of the product while also protecting human health. Giribaldi and associate researchers utilised donkey milk to evaluate the effectiveness of a recently developed small-scale high-temperature short-time (HTST) pasteurizer that operates at 72°C for 15 seconds. The Enterobacteriaceae was entirely eliminated and the overall number of bacteria was reduced thanks to the HTST device. When Bacillus cereus was present, it was reduced with little effectiveness. They said that pasteurising donkey milk may be done safely using the HTST apparatus. This correspondence aims to provide a succinct overview of the nutritional composition and possible health advantages of donkey milk and related products. [3]

Table: Basic chemical composition of human, donkey, cow, buffalo, sheep, goat, camel and horse milk[4-9]

Composition (%)	Human	Donkey	Cow	Buffalo	Sheep	Goat	Camel	Horse
Water Content (%, wet basis)	86.80-90.50	90.63	87.80	82.40	81.60	87.80	88.44	89.86
Fat	2.10-4.00	0.76	3.60	7.10	7.30	3.60	3.60	1.21
Lactose	6.30-7.00	6.30	4.70	4.60	4.60	4.70	4.30	6.37
Ash	0.20-0.30	0.40	0.70	0.90	0.80	0.70	0.71	0.42
Protein	0.90-1.90	1.91	3.20	5.00	5.70	3.20	2.95	2.14
Energy (kcal)	47.70-71.60	39.68	64.00	102.30	89.80	64.00	61.40	48.00

Functional characteristics of donkey milk that relate to health:

In order to promote health and avoid disease, a balanced diet is essential. Although donkey's milk has long been used for medicinal purposes, current study focuses on the nutrients it contains and any possible health benefits.[10]

Due to the significant presence of milk constituents like immunoglobulins, lysozyme, lactoferrin, Ω3-fatty acids, bioactive peptides, and a favourable casein:whey protein ratio, donkey milk's functional qualities are primarily attributed to (a) antimicrobial activities, (b) immunomodulating activities, and (c) hypoallergenicity.[11]

1. Antimicrobial activity-

A number of antibacterial proteins found in donkey milk, including lactoferrin and lysozyme, can stop the growth of a wide range of bacteria. They also have the capacity to prevent mammary gland infections or lower the frequency of gastrointestinal illnesses in the digestive system.Donkey milk contains the enzyme lysozyme, which comes in two varieties, A and B. These variations differ in three amino acid substitutions that occur at separate locations. In other species (cow, goat, sheep) that produce milk, it is essentially nonexistent.

Donkey milk's high lysozyme content may be the cause of the low bacterial count that has been virtually entirely documented in the literature. This milk is also ideally suited to prevent or lessen infants' gastrointestinal ailments.

Furthermore, lysozyme boosts the immune system in early childhood and has other vital roles, such as high microbial inhibitory action, inactivation of some viruses, anti-inflammatory, and anti-tumor properties. [12,13]

As an antibacterial agent, lactoferrin, an iron-binding protein, breaks down the glycosidic linkages in mucous polysaccharides found in bacterial cell walls. There are reports that the content of lactoferrin in donkey's milk is greater than in cow's milk.

It was found that a strain of L. paracasei that produced bacteriocin could be isolated from donkey milk. It was discovered that the bacteriocin had antibacterial effects against a number of harmful bacteria, including Salmonella typhi, Pseudomonas aeruginosa, and Escherichia coli. Similar to this, Murua et al. observed that a bacteriocin (LP08AD) generated by an isolation of Lactobacillus plantarum from donkey milk inhibited the development of food spoilage bacteria and pathogens. [14]

2. Bioactive peptides in donkey milk-

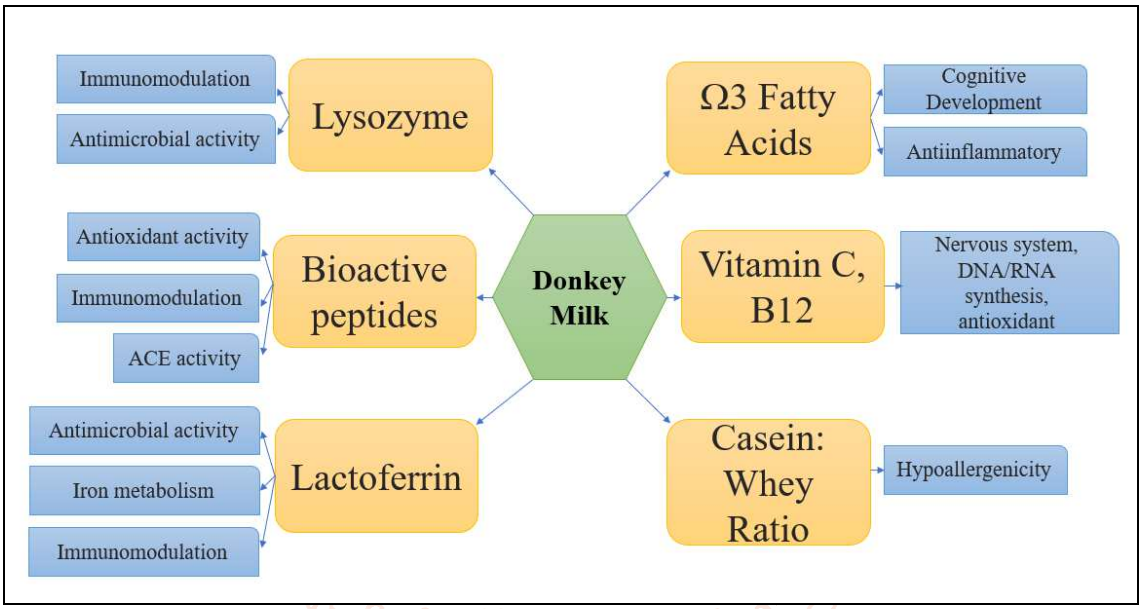
The amino acid sequence of the milk proteins contains a variety of bioactive peptides in addition to the bioactive milk proteins (casein and whey proteins).

Pepsin was used to hydrolyse and acidify donkey milk in order to extract antibacterial components other than lysozyme. The inhibition halo test was used in this investigation to evaluate the inhibitory action of donkey milk following hydrolysis against a variety of pathogenic bacteria, including B. cereus, S. aureus, Enterococcus faecalis, and E. coli. In a dose-dependent way, hydrolyzed milk shown activity against S. aureus and E. faecalis. Hydrolyzed milk exhibits dose-dependent efficiency in relation to the B. cereus strains; however, the inhibitory activity varied among the strains, indicating strain-dependent action within the same species.

The E. Coli strain was the most resilient. The results that have been published demonstrate the existence of biomolecules that are produced when milk proteins are hydrolyzed, which may be a factor in milk's antibacterial action.

A different study found that antimicrobial peptides generated during in vitro digestion may be found in donkey milk. The study's findings demonstrated that both raw and digested donkey milk stop the infections under investigation from growing. There was no effect shown against B. cereus, however there was a notable decrease against E. coli and Listeria monocytogenes. The digested samples had a notably greater inhibitory impact as compared to the raw milk samples. This suggests that the antibacterial activity might be attributed to the combined action of intact proteins, such as lysozyme, and peptides produced by gastrointestinal enzymes.

Moreover, it was shown that hormones and growth factors such ghrelin, insulin-like growth factor 1, human-like leptin, and triiodothyronine T3 are present in donkey milk. These chemicals directly affect food intake regulation, body composition, and metabolism. (16)



3. Immunomodulation activity-

Human microbiome naturally contains Lactobacillus rhamnosus, which is crucial to human health because it regulates the gut microbiota and offers both systemic and local immunomodulation. L. rhamnosus ZDY114 and donkey milk on mice's immune systems, concentrating on humoral, cellular, and nonspecific immunity. This works in concert to improve the immune system's performance in mice by stimulating the transformation of splenic lymphocytes, quickening the elimination of carbon particles, and enhancing the activity of natural killer cells.

To demonstrate if this collaboration between L. rhamnosus and donkey milk also occurs on the human immune system, more study is required. Donkey milk has the capacity to trigger the release of interleukins such as IL-2, IFN-γ, IL-6, TNF-α, and IL-1β from lymphocytes and macrophages. It also causes the expression of activation cell surface molecules on peripheral blood mononuclear cells (PBMCs), such as CD69, a signal transmitting receptor in lymphocytes, and CD25, which is used to track the progression of disease. Donkey milk enhances immunity in part because of these cytokines. Donkey milk also causes PBMCs to produce nitric oxide. Since donkey milk is a potent vasodilator and effectively blocks the growth of infections or their byproducts, all these immunological actions may help prevent atherosclerosis. (17,18)

4. Hypoallergenicity-

A food allergy is an aberrant immune system response that arises every time food is consumed, regardless of the amount. Food allergies are frequent in young children and newborns. It's interesting to note that food allergies in newborns appear in the same sequence as new foods are added to their diet. More than 85% of food allergies are caused by major allergens that are proteins found in shellfish, wheat, sesame seeds, peanuts, eggs, milk, and soy. As the name suggests, in certain people, this is an aberrant immune reaction to cow milk proteins.

Cow's milk proteins are often the first foreign proteins given to newborns in affluent nations. It affects 2–7% of infants under the age of six months and thereafter drops to 0.1–0.5% in adults. Cow's milk allergies are caused by a variety of

immunological mechanisms, including (a) delayed non-IgE mediated reaction, which manifests symptoms hours to days after food ingestion, or (b) immediate IgE-mediated hypersensitivity reaction, which manifests symptoms within 30 minutes of food ingestion. Caseins (αs1- and β-caseins) and β-lactoglobulin are the primary allergens found in cow's milk, with α-lactalbumin occurring to a lesser degree.

It is crucial to use milk from diverse species since, up to the age of two, milk is a significant source of nutrients and shouldn't be cut out of a diet. The chosen milk must be fairly priced, tasty, and provide adequate nutrients with few allergens. [19,20]

5. Potential Antioxidant and Antihypertensive Effects-

Animal models have been used in double-blind, randomised experiments to assess the antioxidant properties of donkey milk. Rats given donkey milk have demonstrated improvements in their detoxifying enzymes and antioxidant defence systems.

When comparing the plasma of diabetic rats to rats that were not treated, the consumption of donkey milk tended to elevate the activity of superoxide dismutase (SOD). The superoxide radical is progressively dismutated (or partitioned) into regular molecular oxygen and hydrogen peroxide by the SOD enzyme. Additionally, compared to the untreated group, the diabetic rats treated with DM showed an improvement in total anti-oxidation ability, approaching levels observed in the healthy (control) group of rats.

Donkey milk contains a large number of peptides that are known to be angiotensin-converting enzyme (ACE)-inhibitory peptides, which may reduce the function of ACE. In vitro bioassays verified the inhibitory effects of angiotensin converting enzyme on donkey milk fractions with varying peptide contents. The production of angiotensin II may be inhibited and bradykinin levels may be raised by milk-derived bioactive peptides, which have vasoconstrictor and vasodilator effects, respectively. The combined effects of the acts reduce blood pressure. Donkey milk that has undergone fermentation exhibited ACE-inhibitory action in vitro. Furthermore, it was shown in vitro that peripheral blood mononuclear cells treated with donkey milk released nitric oxide. There has been speculation that donkey milk may help prevent atherosclerosis since nitric oxide is a potent vasodilator. [21,22]

6. Effects on Glucose Metabolism and Potential Coadjutant Action in the Diabetes Treatment-

Lactose is the main carbohydrate in donkey milk, as in horse milk and cow milk (about 70, 60 and 49 g/L of milk respectively) and it is in charge of maintaining the osmotic balance between blood and milk, respectively and the breast gland's alveolar lumen.

Due mostly to the loss of intestinal lactase, lactose intolerance is frequent in adults and varies greatly in geography. A large number of lactose intolerant people can handle little amounts of lactose (5–10 g spread throughout the day), and using lactase supplements and fermented foods may usually solve the issue. As far as we are aware, no particular research has been done on the application of donkey milk in individuals who are lactose intolerant. There isn't any lactose-free donkey milk on the market at the moment. [23]

The advantageous benefits of donkey milk on glucose metabolism can be attributed, at least partially, to: [24]

1. decrease in leptin/adiponectin ratio and inflammatory state. The leptin/adiponectin ratio and blood inflammatory mediators were both lower in the donkey milk-treated animals. These two adipocyte-derived hormones have roles in inflammation, energy balance, and lipid metabolism. Insulin resistance is associated with a high leptin to adiponectin ratio, and in animal models, the start of type 2 diabetes was found to be correlated with a reduction in adiponectin.
2. enhancement of antioxidant defence systems, which fights against the development of insulin resistance.
3. alteration of mitochondrial dynamics that affects metabolism within the mitochondria.

   Variations in the kinetics, function, and efficiency of mitochondrial activity can affect several clinical situations, including metabolic illnesses like type 2 diabetes and obesity. Rats fed donkey milk had skeletal muscle mitochondria that were bigger, more numerous, more electron-dense when examined under an electron microscope. These traits have been linked to enhanced glucose metabolism, better respiratory capacity, and more active mitochondria.
4. Phosphoenolpyruvate carboxykinase 1 (Pck1) and glucose-6-phosphatase (G6PC), two essential enzymes for gluconeogenesis, are downregulated.

7. Effects on Lipid Metabolism-

The use of DM in low-calorie diets and the treatment of dyslipidemia is supported by certain nutritional characteristics of the grain. When DM is compared to other milks used in human nutrition, it actually has a lower percentage of fat and calories (fat in DM ranges from 0.20% to 1.7%). Furthermore, while the

UFA:SFA ratio is larger (0.75 vs. 0.41, respectively), the quantity of saturated fatty acids (SFA) in DM is much lower than that of CM (3.02 g/L vs. 26.27 g/L, respectively). Moreover, with 7.25 g/100 g of fat, DM has the highest concentration of alpha-linolenic acid (C18:3 n-3;ALA) of any farm animal milk.

C18:3 n-3 ALA has positive health benefits and is a precursor to long n-3 fatty acids. [25]

8. Antiproliferative and Antitumor Effect-

There are contradicting claims in the literature about the link between eating dairy products and milk and the risk of developing cancer. Few research has looked at DM's potential antiproliferative and anticancer properties, with inconsistent findings.

In rats with Ehrlich ascites cancer (EAC), DM kefir treatment for ten days decreased the amount of the tumour and raised the number of apoptotic cells in comparison to the control group, according to a randomised trial. The down-regulation of the NO synthase enzyme (isoforms iNOS and eNOS) was identified as the cause of the effects of DM kefir on tumour volume and apoptosis. Specifically, as compared to the DM kefir group, the iNOS levels in the control and DM groups were significantly greater. [26]

9. Protection of the Intestinal Barrier and Modulatory Effect of the Intestinal Flora-

A complicated family of bioactive carbohydrates with no discernible nutritional value are milk oligosaccharides.

Because of their prebiotic function, oligosaccharides in milk are important for nutrition (reported for breast milk). In horse milk, oligosaccharides function as prebiotics and act as a substrate for the good gut microbiota, preventing pathogenic germs from adhering to the intestinal wall and delaying the onset of enteric illnesses. DM oligosaccharides have not received much research attention, despite the potential protective effect of oligosaccharides on the gut and the interest in nutritional applications for particular consumer groups.

Indomethacin-induced ileitis in mice was the subject of a randomised investigation that revealed the anti-inflammatory effects of DM on the gut. The study found that oral DM therapy reduced both macroscopic and microscopic damage, as well as the severity of symptoms (body weight loss, shortening of the small intestine, and increased faecal lipocalin-2). The authors attributed these activities to the restoration of intestinal immunity, namely to the Paneth cells' production of antimicrobial peptides that directly lower dysbiosis. [27]

Cosmetic properties of donkey milk-

It's even more amazing when donkey milk is used as a moisturiser in cosmetics. Donkey's milk is utilised in cosmetics because of its ability to cleanse, moisturise, and function as an antioxidant to slow down the ageing process. In actuality, the fat in donkey's milk softens and nourishes the skin. These days, several investigations appear to support the purported medicinal and cosmetic benefits of donkey milk. Donkey milk has anti-aging, anti-oxidant, and regeneration substances that are naturally active in hydrating and preventing skin ageing. It is also rich in vitamins and polyunsaturated fatty acids.

High concentrations of essential fatty acids and low amounts of saturated fatty acids define the lipid fraction. Donkey milk is far more beneficial in preventing inflammatory, autoimmune, and cardiovascular disorders than ruminant milk due to its higher concentration of unsaturated fatty acids. [28]

Shelf life of donkey milk:

The shelf life of milk is a crucial factor that affects its processing, storage, packaging, and supply. The longer the shelf life, the more acceptable the milk will be. Aseptically collected mare and donkey milk samples were incubated at 37°C for 24 hours, and the periodical changes in acidity and pH in equine milk were regularly studied at 2-hour intervals. The results of the shelf-life analysis showed that mare and donkey milk were stable at 37°C for up to 8 hours and 10 hours, respectively. Lysozyme acts as a natural preservative, extending the shelf life of raw donkey milk. [29]

Conclusion:

It is becoming increasingly evident that donkey milk contains special nutritional qualities that are similar to those of human milk. In several scientific fields, further study is needed. For instance, animal nutrition and its impact on (a) milk quality, (b) production, and (c) nutritional content of

investigation of additional potential bioactivities, such as antibacterial activity against Bordetella pertussis (whooping cough), and donkey milk. More in vitro and in vivo research is required to confirm the potential use of donkey milk as a bioactive compound in the formulation of new functional food products (such as fermented milk) and the impact of processing on bioactivity (i.e., raw versus minimally treated versus heat treated versus lyophilized).Thus, it is imperative to use an interdisciplinary strategy that includes food technologists, clinical nutritionists, veterinarians, and animal nutritionists in order to demonstrate through well planned clinical investigations in various vulnerable populations. the

potential benefits to health for particular groups (children and the elderly, for example).

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