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From Feed to Fork: Mycotoxins in Aquaculture as a Threat to the World's Food Security and Human Nutrition

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One of the most crucial pillars of World food security is aquatic production because it offers a source of high-quality protein to billions of inhabitants and livelihood to millions of communities around the world. Nevertheless, in my capacity, this crucial industry has been endangered by a looming menace that stands at all times, and that is mycotoxin contamination [1]. Mycotoxins are heat-resistant and highly toxic secondary metabolites that are synthesized by filamentous fungi such as Aspergillus, Fusarium, and Penicillium, and may enter aquatic environments and production systems through a variety of different pathways. They have greater impacts than reducing the well-being of aquatic life, including fish, shrimp, and benthic invertebrates, as well as imposing dire health risks to the health of well-being of humans in the food chain, hence posing a long-term threat to the sustainability of aquaculture [2].

The large presence of mycotoxins in water bodies can be explained by a variety of interrelated reasons, of which the use of polluted feed contributes significantly to intensive aquaculture. Cereals (e.g., corn and soybean meal) are the most common feed staples vulnerable to fungi during production, harvesting, storage, and processing in warm and damp climates. The sources of protein (e.g., fish meal) are also vulnerable to fungi. Due to this, feeds can have an array of mycotoxins, i.e., aflatoxins, zearalenone, deoxynivalenol, and ochratoxins. When the water creatures ingest these toxins, they might have a direct impact on their health and are thus released back into the water, leading to secondary environmental pollution [3]. Other sources, such as agricultural runoffs, industrial effluents, and improper handling or disposal of mold-infested substances, are some of the other sources that further augment the mycotoxin loads on the environment. Moreover, the impact of toxin-producing fungi is enlarged within the ecological niche due to climate change (high temperatures and more precipitation), which aggravates the risk of contamination. The problems are compounded by the processes of bioaccumulation and biomagnification in the water food webs, whereby the mycotoxins are concentrated in the apex predators and further enhance the potential human health risks [3,4].

Mycotoxins have diverse toxicological impacts on aquatic organisms, which include effects on the digestive systems, immune systems, reproductive systems, and neurological systems. Consumption of polluted food may cause intestinal integrity breach, diminished digestive enzyme activity, and deterioration in growth performance. More importantly, mycotoxins suppress their immune functions, increasing their vulnerability to bacterial and viral diseases, and this increases mortality and causes heavy financial losses to aquaculture. Some of these mycotoxins are also endocrine disruptors, disrupting the balance of reproductive hormones, reducing the quality of gametes, and decreasing reproductive productivity, and as a result jeopardizing the long-term sustainability of wild and farmed populations [5]. Also, the toxins cause oxidative stress and genotoxicity, which causes DNA damage, lipid peroxidation, and tumorigenesis. Regardless of awareness of these negative effects, there are notable gaps in knowledge regarding the molecular pathogenesis of mycotoxin toxicity in aquatic organisms, the interactive effect of many concomitant co-occurring mycotoxins, and the

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ecological implications of chronic low-dose exposure [6].

Mycotoxin contamination of aquaculture needs to be mitigated using a holistic, systems-based, interdisciplinary approach. The quality assurance and quality control should be implemented throughout the feed supply chain, including the optimization of storage and the processing environment, the creation and implementation of efficient detection, prevention, and detoxification technology [7].

The seemingly underestimated risk of mycotoxins in aquatic production systems is the one that should be handled by the scientists and regulators in a concerted effort. It will require concerted interdisciplinary measures, technological advancement, and the systematization of evidence-based policies and management practices to minimize the ecological and economic impacts of them, preserve the aquatic life forms, and guarantee the further provision of this essential source of animal protein to human food.

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Review Article

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Hop Shoots (*Humulus lupulus*): Emerging Bioactive Components, Functional Properties, and Opportunities for Food and Bio-Industrial Applications

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ABSTRACT

The common hop (*Humulus lupulus L.*) is a dioecious perennial climbing plant and is referred to as a member of the Cannabaceae family, and is located in the northern temperate regions. Female plants are grown solely due to the large quantities of constituents they possess, which are primarily bitter principles and essential oils that are not only used in the industry but are also used as medicinal products. It is endowed with bioactive properties, including prenylated flavonoids, bitter and phenolic acids, terpenoids, and vitamins, antimicrobial, anti-inflammatory, antioxidant, estrogenic, neuroprotective, and hormonal modulation properties. The hop shoots contain the optimal composition of any micronutrient that is not caloric and thus can be added to health-promoting meals. They have a niche potential to produce and market because of their high market value, thus the high economic importance they have in the European market; the agro-climatic conditions of the regions in northern Pakistan create the possibility of the renewal of rural development. This paper intends to provide an overview of its novel bioactive compounds, properties, and food and biotechnological uses. A multidisciplinary approach to the application of the idea of harnessing the full potential of hop shoots will entail the use of both agricultural science, food technology, and pharmacology, as well as implementing it in the market preparation.

INTRODUCTION

The hop plant (*Humulus lupulus L.*) is a dioecious perennial climbing species of the Cannabaceae family, having historically been grown to be used as female inflorescences of the plant commonly referred to as cones or strobiles, which have achieved considerable success in the brewing industry because of its aromatic, bittering, and preservative qualities (Figure 1) [1]. However, among the cones, it possesses the beams of tenderness, young shoots every year sprouting out of the perennial rhizomes. They have even been called the vegetable so much desired by the Europeans in their cuisine, the most expensive vegetable in the world, and called simply the hop shoot in virtue of the subtle asparagus flavor, short growing season, scarcity [2]. They appeared in the pharmacopoeias of European pharmaceutical collections and folk medicine of the Middle Ages and were said to act on digestion, skin, and

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blood purification [3]. In recent decades, hop shoots have been receiving renewed attention due to renewed interest in functional foods and niche crops with high value. The scientific advances have enhanced the degree of knowledge with regard to the phytochemical composition of hop shoots. The previously described cone-related compounds (alpha -acids (humulones), 2 -acids (lupulones), xanthohumol, desmethylxanthohumol, and 8 -prenylnaringenin) also occur in shoots with varying concentrations [4]. They are metabolites, and they have diverse biological activities, including antimicrobial, antioxidant, anti-inflammatory, estrogenic, and anticancer activities [5-7]. The hop shoot is a functioning food with high amounts of vitamin C (as much as 40mg/100g), dietary fiber, essential minerals, and very small amounts of oxalic acid (less than 67mg/100g), making the Hop Shoot a food item as well as a culinary delight [8]. With the propagation of hop growers to every corner of the world, the hop shoots have drawn a lot of agricultural research. The interested areas, such as the Mediterranean Europe, which are interested in commercial production of hops due to the limitation of the photo period and the climate, are now wondering about the dual use of the cones and shoots production [9]. Pakistan has virtually no experience of hop cultivation and hop shoot production. However, the variation in agro-ecological conditions (diverse agro-ecological conditions), i.e., the temperate valleys of Gilgit-Baltistan, the elevated regions of Khyber Pakhtunkhwa, and the irrigated plains of the northern part of the Punjab, would support the growth of hop in case of the introduction of hop-photoperiod-adapted varieties. The main aim of the present review was to conduct a representative study regarding the new bioactive components, functions, and opportunities of food and bio-industrial exploitation of hop shoots. This review highlights the potential and the unfulfilled potential of hop shoot production in Pakistan and in the Pakistani agro-climatic regions, the opportunities, challenges, and research opportunities in the future.

Despite growing interest in *Humulus lupulus* for its brewing and medicinal value, the scientific literature has predominantly focused on hop cones, with comparatively limited attention given to the nutritional, pharmacological, and agro-economic potential of hop shoots. Existing studies are fragmented, often addressing isolated phytochemicals or specific biological activities without providing an integrated perspective on their functional food and bio-industrial applications. Moreover, region-specific evaluations, particularly regarding the feasibility of hop shoot cultivation in Pakistan's agro-climatic zones, remain largely unexplored. This gap underscores the need for a comprehensive review synthesizing bioactive composition, health-promoting properties, and commercialization prospects of hop shoots.

Figure 1: *Humulus lupulus L.* Cones

Biologically Active Compounds

Hop shoots (*Humulus lupulus L.*) are biochemically rich vegetation since secondary metabolites are complex, and a great number of them are concentrated in the hop cones, but also in the young aerial shoots. These compounds belong to various chemical families, which are bitter acids, pre-phenylated flavonoids, volatile essential oils, phenolic acids, and other polyphenols [10]. The best concentrations of the compounds in the hop extracts were catechin and xanthohumol, with the level of the compounds being 1.22mg per gram to 2.70mg per gram and 1.09mg per gram to 2.67mg per gram, respectively. The concentrations of Rutin and chlorogenic acid were found to be 0.612-0.877 mg g -1, and the p-hydroxybenzoic acid, protocatechuic acid, syringic acid, and ellagic acid were 0.262-0.654 mg g -1. Epigallocatechin, p-coumaric, caffeic, and ferulic acids were also found in small amounts and at varying levels depending on the extraction mode, which is taken [11]. Future research opportunities, challenges, and directions.

Bitter Acids

Bitter acids are a hallmark of the *Humulus* genus and represent one of its most pharmacologically relevant phytochemical groups. They are classified into two structurally related subclasses: α-acids, also known as humulones, and β-acids, known as lupulones (Figure 2).

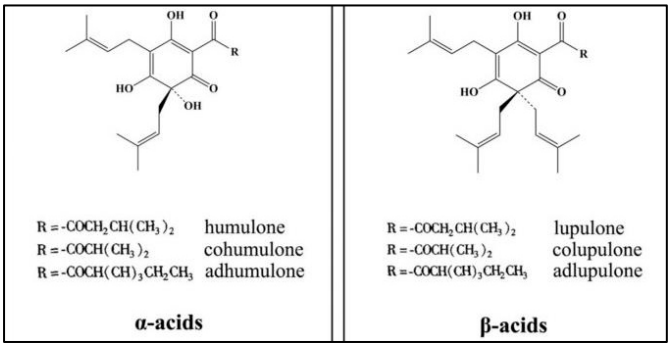

Figure 2: α-acids and β-acids of Humulus Genus

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Both have a comparable acylphloroglucinol backbone and differ in prenylation patterns, which give different physicochemical properties and biological activities. The 1,5-acids, such as humulone, cohumulone, and adhumulone, are biosynthesized in hop tissues in the glandular trichomes of the branched-chain amino acid precursors by the action of the enzyme valerophenone synthase, and then by successive prenylations by prenyltransferases [12]. The 2 -acids such as lupulone, colupulone, and adlupulone are more hydrophobic than their 2 -acid analogues and exhibit strong antifungal effects due to their ability to disrupt ergosterol production in fungal membranes. In addition to antimicrobial activities, β-acids have also exhibited selective cytotoxicity in some cancer cell lines, which indicates that they can be used in cancer chemoprevention [13].

Prenylated Flavonoids

Prenylated flavonoids are also another important bioactive in the hops shoots and contribute to the hop shoot pharmacology and possible health applications as functional foods. Among them, the prenylated chalcone xanthohumol is the best-researched. The amounts of hop shoots, which, though lower than those of cones, are calculable, are also in their biological characteristics. Having diverse bioactivity, xanthohumol has been identified to exhibit antiproliferative, antioxidant, and anti-inflammatory properties on a diverse array of cancer cell lines, as well as inhibiting lipid peroxidation and inducible nitric oxide synthase (iNOS) and COX-2 expression [14]. The other prenylated chalcone identified in hop shoots is desmethylxanthohumol, which serves as a biosynthetic precursor to xanthohumol and the phytoestrogen 8-prenylnaringenin. The conversion can either occur enzymatically in the plant or in the metabolic conversion of the human gut microbiota. Of particular interest is one of the prenylated flavanones, 8-prenylnaringenin, which, in addition, is one of the strongest plant estrogens, being 100-fold weaker than genistein, the well-known soy isoflavone. They are prescribed to alleviate menopausal symptoms, osteoporosis prevention, and treatment of physiological functions that are dependent on hormones [15].

Essential Oils (*Volatile Terpenoids*)

Although the essential oil fraction of hop shoots is less compared to mature cones, it retains a typical blend of monoterpenes and sesquiterpenes, which can be found in aroma and bioactivity, and 2-myrcene is prevalent, which has anti-inflammatory, analgesic, and muscle-relaxant effects. The other monoterpenes are alpha pinene and limonene that were reported to possess anti-microbial and anti-inflammatory properties. Short-chain sesquiterpenes, which are selective agonists of the cannabinoid receptor CB2 (alpha-humulene, 2-caryophyllene, 2-farnesene), are also non-psychoactive and exhibit anti-inflammatory properties in addition to analgesic properties [16].

Phenolic Acids and Other Polyphenols

The phenolic acids are also very significant in the antioxidant properties of the hop shoots. Other typical ones are p-Coumaric, ferulic, and caffeic acids, which are low-density lipoproteins (LDL) oxidation inhibitors and free radical scavengers [17]. The other effects of the compounds include the protection of the vascular endothelium that could be translated into cardiovascular effects. The other polyphenols, such as flavonols and flavan-3-ols, are also biologically favourable to the human organism since they enhance the anti-inflammatory and vascular protective properties of hop shoots [18].

Minor Bioactive Constituents

The minor constituents of hop shoots along with the major bioactive groups, can make contributions to the functional properties of this group. These include amino acids and small peptides that have the potential of antioxidants or antimicrobial activity and traces of saponins. They have not been fully studied as to their pharmacological usefulness in the hop shoots, although are a hopeful research potential in the future [19].

Biological Activities of Hop Shoots (*Humulus lupulus*)

The principal classes of bioactive compounds present in hop shoots (*Humulus lupulus*), their model molecules, and the biological effects that they have are outlined (Table 1).

Table 1: Major Biologically Active Compounds in Hop Shoots and Their Biological Activities

Compound Class	Representative Compounds	Chemical Nature	Major Biological Activities	References
Bitter Acids (α-acids)	Humulone, Cohumulone, Adhumulone	Acylphloroglucinol derivatives	Antioxidant, Antibacterial against Gram-positive bacteria, anti-inflammatory	[20]
Bitter Acids (β-acids)	Lupulone, Colupulone, Adlupulone	Acylphloroglucinol derivatives	Antifungal, antimicrobial synergy, and selective cytotoxicity in cancer cells	[21]
Prenylated Flavonoids	Xanthohumol	Prenylated chalcone	Anticancer, antioxidant, anti-inflammatory, chemopreventive	[22]
	Desmethylxanthohumol	Prenylated chalcone	COVID-19, Precursor to phytoestrogen 8-prenylnaringenin	[23]
	8-Prenylnaringenin	Prenylated flavanone	Potent estrogenic activity, relief of menopausal symptoms, bone health	[24]
Essential Oils	β-Myrcene, α-Humulene, β-Caryophyllene, β-Farnesene	Mono-/sesquiterpene hydrocarbons	Anti-inflammatory, analgesic, antimicrobial, sedative	[25]

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Phenolic Acids	p-Coumaric, Ferulic, Caffeic acids	Hydroxycinnamic acids	Antioxidant, cardiovascular protection	[26]
Other Polyphenols	Quercetin Derivatives, Catechins	Flavonols, flavan-3-ols	Anti-inflammatory, antioxidant, vascular protection	[27]

In recent years, the biological functions of hops have been the subject of multiple investigations. The biological activities of hops include anti-inflammatory, anticancer, anti-Alzheimer, antiviral, antidiabetic, antimicrobial, and antifungal activities. The possible therapeutic uses of hop bioactive components in the management of human illnesses are given (Figure 3) [25].

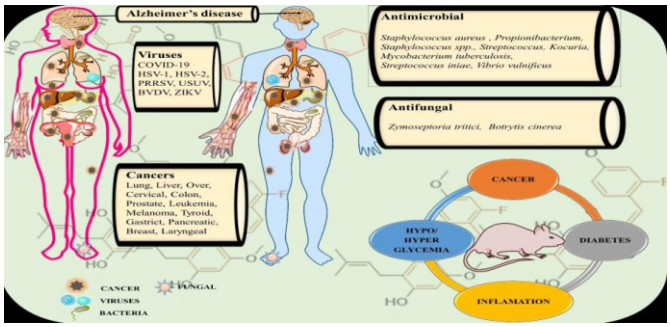

Figure 3: Overview of the components of hop shoots in related human diseases

Antimicrobial Activity

Hop-derived bitter acids, particularly α- and β-acids, have well-documented antibacterial and antifungal properties. In shoots, these compounds have been shown to inhibit gram-positive bacteria such as *Staphylococcus aureus* and *Listeria monocytogenes* by disrupting cytoplasmic membranes and inhibiting key metabolic enzymes. β-acids also display potent antifungal effects, interfering with ergosterol biosynthesis and fungal membrane stability. Some studies suggest that combining hop extracts with antibiotics can reduce bacterial resistance, offering potential in antimicrobial stewardship [26].

Antifungal Activity

Numerous investigations have demonstrated the antifungal action of prenylflavonoids. For illustration, cohumulone and desmethylxanthohumol (DXN) demonstrated antifungal activity against Zymoseptoria tritici with half-maximum inhibitory doses of 0.11 and 0.2 g/L, respectively. 88 With an EC50 value of 4.32 µg/mL, it was shown that iso-xanthohumol (IXN) drastically reduced antifungal activity against B. cinerea. This study demonstrates that phytopathogenic fungi can be treated with IXN [27].

Anti-inflammatory Effects

Some bioactive compounds in hop shoots, such as xanthohumol and alpha-humulene, have anti-inflammatory effects through the modulation of NF-κB signaling pathways and the downregulation of pro-inflammatory cytokines, TNF-alpha, and IL-1 beta. The sesquiterpene in hop oils, β-caryophyllene, acts on cannabinoid CB2 receptors and decreases inflammation without having a psychoactive effect. The properties have potential consequences in treating inflammatory diseases, including arthritis and metabolic inflammation [28].

Antioxidant Potential

Hop shoots are rich in phenolic acids (caffeic and ferulic) and flavonoids (quercetin derivatives) that exert high antioxidant capacity through reactive oxygen species (ROS) scavenging, lipid peroxidation inhibition, and up-regulation of endogenous antioxidant enzyme activities. This antioxidative activity is important in the prevention of oxidative stress-based pathology like cardiovascular disease, neurodegeneration, or some cancers [29].

Actions: Estrogenic, Hormone-Modulating

Hop shoots include 8-prenylnaringenin, which is one of the strongest phytoestrogens in the natural world. This compound acts on estrogen receptors, alpha and beta, and has the potential to provide a therapeutic benefit to menopausal symptom relief, prevention of osteoporosis, and hormone-related metabolic health [30]. It should be applied in a controlled manner because it is highly potent.

Neuroprotective Properties

Preclinical evidence indicates that prenylated flavonoids in hops could be neuroprotective owing to their antioxidant activity, neurotransmitter systems regulation, and anti-inflammatory effects. This has a possibility of future usage in the treatment of neurodegenerative diseases like Alzheimer's and Parkinson's diseases, but studies on shoots are few compared to cones [31, 32].

Antiviral Covid-19

Since 2019, millions of people worldwide have contracted the coronavirus, often known as SARS-CoV-2 or COVID-19. Nevertheless, hop illnesses were induced by the detected hop stunt viroid (HSVd), apple fruit crinkle viroid (AFCVd), citrus bark cracking viroid (CBCVd), and hop latent viroid (HLVd). Lin et al. showed that xanthohumol was a pan-inhibitor of the primary protease of COVID-19, with an IC50 value of 1.53 µM [33].

Anticancer activity

Numerous studies reported that xanthohumol (XN) from hop shoots induced cancer cell death and inhibited tumor growth *in vitro* and *in vivo*. The effect of xanthohumol was shown in various human cancers, such as estrogen receptor-positive breast cancer cells (MCF-7), ovarian (A2780), colon (HT-29), cervical cancer, melanoma, hepatocellular, and prostate (DU145, PC-3), and lung cancer [34].

Cardiovascular Impacts

Hops are typically extracted using any organic solvent or

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supercritical CO_2. The extractable hop resins and essential oils make up most of the hop extract, whereas cellulose and residual polar compounds make up the spent hop. A wide range of polyphenols can be obtained by extracting the wasted hop extract using either water or ethanol/water. The inhibition of ADP-induced platelet aggregation by the wasted hop extract may be advantageous for the control of platelet and endothelial function [35].

Metabolic Health Benefits

Some of the hop polyphenols affect glucose and lipid metabolism. It has also been demonstrated that xanthohumol (XN) enhances insulin sensitivity and decreases adipogenesis and plasma triglycerides in animal models. These effects suggest that hop shoot extracts may have a role in the prevention of metabolic syndrome and weight management programs. Humulone and lupulone are bitter acids that have antibacterial and antifungal effects, alter microbial membranes, and interfere with the synthesis of ergosterol. Prenylated flavonoids and terpenes, such as xanthohumol, alpha-humulene, and beta-caryophyllene, have shown anti-inflammatory action [24]. Other positive effects are neuroprotective and metabolic, which means that hop shoots are broadly therapeutic (Table 2).

Table 2: Biological Activities of Hop Shoot Compounds and Their Mechanisms of Action

Biological Activity	Key Compounds	Mechanism of Action	References
Antibacterial	Humulone, Lupulone	Disrupts bacterial membranes, inhibits enzymatic activity	[26]
Antifungal	Lupulone, Colupulone	Interferes with ergosterol synthesis, damages fungal cell membranes	[27]
Anti-inflammatory	Xanthohumol (XN) α-Humulene, β-Caryophyllene	Inhibits NF-κB signaling, reduces TNF-α, and IL-1β activates CB2 receptors	[28]
Antioxidant	Caffeic acid, Ferulic acid, Quercetin	Scavenges ROS, inhibits lipid peroxidation, and enhances antioxidant enzymes	[29]
Estrogenic Activity	8-Prenylnaringenin	Binds estrogen receptors α/β, modulates hormonal activity	[30]
Neuroprotective	Xanthohumol, Quercetin	Reduces oxidative damage, modulates neurotransmission, and inhibits neuroinflammation	[31, 32]
Metabolic Regulation	Xanthohumol	Improves insulin sensitivity, reduces adipogenesis, and lowers plasma triglycerides	[33]

Nutritional Composition and Functional Potential of Hop Shoots

Hop shoots (*Humulus lupulus*) are not only a source of pharmacologically active phytochemicals but also contain essential nutrients that contribute to their functional food value. Although much of the research has focused on the phytochemical profile of hop cones, recent analyses indicate that young shoots also possess a balanced composition of macronutrients, micronutrients, and bioactive non-nutrient compounds. This composition underpins their potential as a seasonal delicacy in gourmet cuisine and as a candidate for inclusion in functional foods.

Macronutrient Profile

The fresh hop shoots consist mostly of water (about 85-90%), and the levels of calories are low; thereby, they can be used in weight-conscious diets. Carbs are moderate, primarily consisting of dietary fiber, which helps gut health and contributes to the health of microbiota in the gut. The range of proteins is 2-3/100g fresh weight, which supplies essential amino acids in moderate amounts. There is also a small amount of lipids and a healthy percentage of polyunsaturated to saturated fatty acids that are beneficial to cardiovascular health [36].

Micronutrients

Hop shoots contain many vitamins, especially vitamin C, vitamin E, and some vitamins of the B-complex (folate, niacin). These play a role in antioxidant defense and metabolic roles. Analysis of the minerals has portrayed significant concentrations of potassium, calcium, magnesium, and traces of iron and zinc, which are vital in enzyme processes as well as bone conditions [37].

Fiber and Prebiotic Potential

The cellulose, hemicellulose, and pectin dietary fiber in hop shoots maintain intestinal motility and can have prebiotic activity by increasing the growth of helpful gut bacteria. This, together with their polyphenol content, could synergistically stimulate gut health, though little clinical evidence exists [36].

Nutritional and Culinary Relevance in Pakistan

In select regions of Europe, the hop shoots are regarded as a luxurious seasonal crop; there is little practice and consumption of these in Pakistan. The diverse agro-climate conditions in the country can, however, accommodate the cultivation of hops, especially in the cooler areas. The nutritional profile of hop shoots shows low-calorie, high-moisture food having moderate protein and dietary fiber content (Table 3). They contain the necessary vitamins, which include vitamin C and vitamin E, and the B-complex vitamin including folate. The minerals present are potassium, calcium, magnesium, iron, and zinc, which serve cardiovascular, bone, enzymatic systems, and immunity [37].

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Table 3: Nutritional Composition of Hop Shoots (per 100 g Fresh Weight)

Component	Content Range	Nutritional Relevance	References
Water (%)	85–90	Hydration, low energy density	[36]
Energy (kcal)	30–45	Low-calorie seasonal vegetable	[36]
Protein (g)	2–3	Moderate amino acid contribution	[36]
Total fat (g)	0.3–0.6	Minimal fat, favorable PUFA:SFA ratio	[36]
Carbohydrates (g)	5–7	Includes dietary fiber	[36]
Fiber (g)	2–3	Gastrointestinal health, prebiotic potential	[36]
Vitamin C (mg)	25–35	Antioxidant, immune support	[36]
Vitamin E (mg)	0.8–1.2	Lipid antioxidant, skin health	[36]
Folate (µg)	50–70	DNA synthesis, pregnancy health	[36]
Potassium (mg)	350–450	Blood pressure regulation	[36]
Calcium (mg)	25–40	Bone and dental health	[36]
Magnesium (mg)	15–25	Enzyme function, muscle relaxation	[36]
Iron (mg)	0.8–1.2	Hemoglobin synthesis	[36]
Zinc (mg)	0.2–0.4	Immune support, wound healing	[36]

Agronomic and Economic Potential of Hop Shoots

Hop shoots are the costliest vegetables in the world and retail from 1,000 to 1,200 euros per kilogram in seasonal European markets [38]. This is because of their labor-intensive harvesting methods, limited seasonal supply, and their distinctive culinary qualities, which made them highly valued. Although the hop plants are traditionally planted with cones that are used in brewing, they can be controlled in a manner that generates edible cones that give the growers another source of revenue.

Cultivation Requirements

The temperature and cool climate would be of great importance in the cultivation of hops; the temperature should be between 16 and 24C of the soil and well-drained soils that are rich in nutrients. The harvesting of shoots is usually done in early spring as the shoots are 10-15 cm long before lignification's make them less tender. The plants are perennial, such that production of annual shoots is possible without replacing the plants. Organic matter fertilization, as well as sufficient irrigation, improves the quality of phytochemicals and yield [39].

Harvesting and Postharvest Handling

Hop shoot harvesting is also a laborious activity because it requires manual picking of young apical shoots, as well as lateral buds. The critical stages of preservation of sensory quality and nutritional value are quick postharvest cooling and gentle handling. Because of their fragility, the shoots are best eaten freshly, but when stored under controlled atmospheres, the shelf life of the produce can take up to one week [39].

Economic Viability and Potential Market

Hop shoots can be sold as high-end gourmet vegetables in Europe, which are frequently utilized in luxury restaurants. Their low quantity, coupled with their distinct taste and texture, enables them to be highly valued. To growers in countries where hop growing has already occurred, new income can be obtained by diversifying the production of shoots, but without a significant impact on the resulting cone production. The economic model is based on the balance between the labor cost and market demand [37].

Potential in Pakistan

The north and northwestern parts of Pakistan, including Gilgit-Baltistan, Swat, and parts of Khyber Pakhtunkhwa, have agro-climatic features such as temperate hop cultures. The introduction of hop shoots would open niche exports to the Gulf and European markets, and provide completely new, nutritious food to the local markets. Small-scale high-value horticultural businesses (hop shoots) would be helpful to rural populations and, therefore, women-organized agriculture. Nonetheless, issues like poor awareness, upfront cost of investments, and the necessity to employ skilled labor would need to be solved via agricultural extension modules and pilot farm projects. The agronomic and economic factors are several on which the production of the hop shoot is dependent since the crop thrives well in temperate to cool climates (16 24 o C) and in soils that are well drained and consist of high organic matter levels [40]. In Pakistan, northwestern areas like Gilgit-Baltistan and Swat have a good environment, which has potential opportunities for niche, high-value exports and income diversification of rural farmers (Table 4).

Table 4: Agronomic and economic considerations for hop shoot production

Parameter	Details	References
Climate requirement	Temperate to cool (16–24°C)	[37, 39]
Soil requirement	Well-drained, fertile, organic matter-rich	[37, 39]
Harvest time	Early spring, shoots 10–15 cm long	[37, 39]
Harvest method	Manual cutting of tender apical shoots and lateral buds	[37]

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Postharvest handling	Rapid cooling, minimal handling, possible controlled-atmosphere storage	[37, 39]
Market price (Europe)	€1,000–€1,200/kg during season	[37, 39]
Potential regions in Pakistan	Gilgit-Baltistan, Swat, northern Khyber Pakhtunkhwa	[37]
Economic benefits	Diversification of farm income, high-value crop potential, niche export opportunities	[37, 39]

Limitations and Future prospects

This review is limited by the scarcity of clinical studies specifically investigating hop shoots, as most available evidence is extrapolated from research on hop cones or in vitro and preclinical models. Variability in phytochemical composition due to cultivar, climate, and extraction methods also restricts direct comparison across studies. Future research should prioritize controlled clinical trials, standardized extraction protocols, and detailed agronomic evaluations under Pakistani agro-ecological conditions. Multidisciplinary collaboration integrating agronomy, food science, pharmacology, and market analysis will be essential to validate health claims and enable sustainable commercialization of hop shoots.

CONCLUSIONS

Hop shoots are a bioactive and highly nutritional vegetable, which has potential uses in gastronomy, medicine, and agribusiness. These various phytochemical compositions provide antibacterial, anti-inflammatory, antioxidant, estrogenic, metabolic, and neuroprotective properties, which underscore their use as a functional food ingredient. In addition, the fact that hop shoots have high market value will enable agricultural diversification of climatically favorable areas such as the north of Pakistan, because well-structured clinical experiments are needed to demonstrate the bioactivities of hop shoots, and improvements in crop production and after-harvesting. There is a strong need, therefore, to integrate work on agronomy, food science, and biomedical research, facilitating their productive and sustainable production, value-added use, and commercialization.

Authors' Contribution

Conceptualization: MKS
Methodology: ZA, AA,
Formal analysis: STS, SS, NZ, AK, KS, IA
Writing and Drafting: AA
Review and Editing: MKS, STS, ZA, AA, SS, NZ, AK, KS, IA

All authors approved the final manuscript and take responsibility for the integrity of the work.

Conflicts of Interest

All the authors declare no conflict of interest.

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Review Article

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Exploring the Impact of Meal Timing on Metabolic Health: A Narrative Review of Recent Findings

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ABSTRACT

Lifestyle modification is the frontline therapy for preventing and treating dysmetabolic diseases. Meal timing is a favorable dietary regimen for managing metabolic dysregulation. To evaluate the impact of meal timing on metabolic output through the latest scientific findings. For this narrative review, various information retrieval databases were searched, including Google Scholar, PubMed, and Mendeley. Multiple combinations of keywords were used to identify relevant literature, such as "mealtime and metabolism," "circadian cycle," "intermittent fasting," "late eaters and metabolic health," and "appetite hormones and meal timing." Recent articles published between 2020 and 2025 in scientific journals were prioritized to provide the most up-to-date evidence. Eating habits such as speed of eating, timing of eating meals, and meal frequency have now gained more attention because of their potential effect on metabolic health. Mismatched circadian rhythms are common in shift workers, social gatherings, eating, jet lag, and those who sleep late at night and wake late in the morning. Various observational research studies report that a greater portion of meals are taken in the latter half of the day, which increases the risk of metabolic diseases. The Randomized Controlled Clinical Trial proved that intermittent fasting, time-restricted feeding, and eating small and frequent meals improve insulin sensitivity and reduce weight. Skipping breakfast or eating late at night has adversely affected hormone regulation. Hence, lifestyle modification according to a circadian cycle result in preventing metabolic disorders.

INTRODUCTION

The term Metabolism is used for all chemical reactions that take place in cells and are crucial for the existence of life. Chemical reactions comprise both anabolic and catabolic processes. Anabolism generates complex molecules, and Catabolism generates simpler molecules. Maintaining cellular and body functions requires a continuous supply of energy through metabolism [1]. A circadian word derived from the Latin "Circa diem" means "around the day" [2]. Circadian rhythms are biological, visceral 24-hour cycles that regulate the body's physiological, metabolic, and behavioral processes. This system initiates wake and sleep episodes and also gives feeding and fasting signals to the body [3, 4]. Eating/Feeding is the daily rhythm that is commanded by a circadian system, which is the Central master clock present in the hypothalamic suprachiasmatic nucleus (SCN). It is modified by different environmental stimuli [5]. When a person's eating and sleeping behavior does not coincide with the circadian signal, it will cause a misalignment of the circadian cycle. Over the past few decades, researchers have focused more on gaining insight into altering meal timing and its influence on metabolic output. Research studies indicate that eating food late in the day or near bedtime at night is correlated with metabolic issues. Research findings show that shift

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workers or Night duty staff also delay their morning meals and eat late at night, which disrupts the body's biological cycle and energy balance [3]. Humans are diurnal, and most of the physiological activity occurs in the daytime [6]. The body's circadian cycle regulates nutrient transport, utilization, and storage [7]. Various observational research studies reporting a greater portion of meals are taken in the latter half of the day, which increases the risk of metabolic diseases. According to the National Health and Nutrition Examination survey 2011-2015, most people take about 45 % of their daily energy intake in evening snacks and dinner, and it has a positive association with weight gain [6]. Consumption of food is transforming into energy that is used daily. Daily energy is classified into 3 ways: 1) Basal metabolic rate (60%), 2) Postprandial heat generation (10-15 %), 3) Physical activity (25-30 %). In a healthy population, there is a balance between food intake and physical activity [8]. Daily meal pattern is not only affected by biological factors and habits but also influenced by occupation and lifestyle, which leads to metabolic disruption and results in body weight change [9]. Many observational studies claim that over recent decades, eating patterns have changed and shifted towards skipping breakfast, eating throughout the day, prolonging the eating window for night shift working staff, and shorter sleep duration, which leads to metabolic disturbance in the body. Hence, proper mealtime is crucial for the metabolism of the body [10]. Availability of food, feelings of hunger and satiety, social gatherings, and convenience in making food all create hindrances in proper meal timing and eating patterns. If the consumption of meals is at the proper time (inherent time mechanism), then the circadian clock initiates a sensing pathway to maintain nutrient homeostasis, but if the consumption of meals occurs at a random time, the circadian clock is anticipated for new feeding time. Such disruption increases the risk of metabolic diseases (risk of obesity, type 2 Diabetes, and Chronic heart disease)[7].

Although growing evidence suggests that meal timing plays a crucial role in regulating metabolic health, the available literature remains fragmented across different dietary patterns, including intermittent fasting, breakfast skipping, and late-night eating. Many studies focus on isolated metabolic outcomes without integrating circadian biology, appetite-regulating hormones, and clinical implications into a comprehensive framework. Furthermore, inconsistencies in study designs and population characteristics have led to conflicting findings regarding optimal meal timing strategies. Therefore, a consolidated narrative review synthesizing recent evidence is necessary to clarify the relationship between meal timing and metabolic health outcomes.

Various information retrieval databases were used, including Google Scholar, PubMed, and Mendeley. To gather relevant literature for this narrative review, multiple keyword combinations were employed, including "Appetite-regulating hormone," "Circadian cycle," "Meal Timing," "Metabolic health," and "Time-restricted feeding." Articles included in this review were published in scientific journals between 2020 and 2025, written in English, and covered study designs such as cross-sectional studies, surveys, observational studies, randomized controlled trials, clinical trials, randomized crossover trials, and cohort studies. Articles published prior to 2020, non-English articles, and gray literature were excluded. Eating habits, such as meal timing, frequency, and speed, have recently attracted considerable attention due to their influence on metabolic health. Lifestyle modifications remain the frontline approach for preventing and managing metabolic disorders, and meal timing has emerged as a promising dietary strategy to regulate metabolic dysregulation. Evidence suggests that delayed evening feeding may adversely affect health by causing desynchronization between central and peripheral circadian clocks, particularly affecting gastrointestinal function and overall metabolism. All aspects of energy metabolism and appetite hormone regulation follow the body's circadian rhythm. Disruptions in eating patterns common among shift workers, individuals with late sleep-wake cycles, those traveling across time zones, or people engaged in social gatherings can lead to positive energy balance and weight gain. Conversely, consuming meals in alignment with endogenous circadian rhythms, such as early in the day, may reduce the risk of metabolic disorders, including obesity, hypertension, type 2 diabetes, dyslipidemia, and cardiovascular issues. Circadian rhythms involve physical, mental, and behavioral cycles over 24 hours. While light and dark are primary drivers, other factors such as dietary intake, food composition, psychological stress, physical activity, exposure to electronic devices, environmental and climatic conditions, and pharmacological or therapeutic interventions also modulate these rhythms. Proper alignment of food intake with circadian cues may therefore mitigate the risk of chronic metabolic diseases. Intermittent fasting, characterized by temporary abstinence from food through patterns such as alternate-day fasting or time-restricted feeding (e.g., 6 hours feeding and 18 hours fasting), has gained attention for its metabolic benefits. Evidence indicates that intermittent fasting promotes fatty acid metabolism to ketones, enhances thermogenesis, increases energy expenditure, and supports fat loss.

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Consequently, this dietary approach may benefit individuals with obesity, cardiovascular disorders, hypertension, and type 2 diabetes. Several studies have examined intermittent fasting as an intervention for weight management. A summary of key studies is provided in Table 1, which illustrates the positive impact of intermittent fasting on weight reduction and metabolic outcomes.

Table 1: Studies Related to Intermittent Fasting Impact on Metabolism

Study Design	Target Population	Results	References
Randomized Controlled Trial (Turkey) Sample Size=70 Study period 12 weeks	Patient with metabolic syndrome, age 18 to 65 years, and BMI (Body Mass Index) 27 or more.	The IER Group (Intermittent Energy Restriction) significantly reduced weight (reduction of 5.5 kg), the CER group (Continuous Energy Restriction) significantly reduced weight by 4 kg, and insulin reduction.	[11]
Randomized Controlled Trial (China) Sample Size=101 Study period 3 weeks	Age 18-65 years. Pre-diabetes, and BMI (Body Mass Index) 23 or more.	The ADF group (Alternate Day Feeding) observed a more significant reduction in body weight. The TRF group (Time Restricted Feeding) least significant difference as compared to the ADF group. No significant difference was observed in blood glucose level, waist circumference, or LDL (Low-Density Lipoprotein) in both groups.	[12]
Quasi-experimental clinical trial (Aga Khan University Hospital) Sample Size=40 Study period 6 weeks	Age 20-70 years, Serum High Density Lipoprotein < 40 mg/dl (men) and Serum High Density Lipoprotein 50 mg/dl (women)	The IF group (Intermittent fasting) showed a significant reduction in Body Mass Index, Waist circumference, Low Density Lipoprotein, and a significant improvement in High Density Lipoprotein.	[13]
Randomized Controlled Trial (Malaysia) Study period 12 weeks Sunnah Fasting 2 days per week (Consume a small meal before sunrise and a full meal after sunset)	Older males (Take a 300-500 kcal/ day diet with consumption of more healthy food)	Observed Weight loss approximates 3 %. Fat mass loss 6 -8 %	[14]

Breakfast is a kick-start to daily metabolism. It is considered the most crucial meal of the day that regulates energy metabolism. Despite this, skipping breakfast is common nowadays [15, 16]. According to various research studies, skipping breakfast is common among female, students in senior classes, and people who live in other cities [17]. In the last 4 decades, consumption of breakfast and lunch has been declining continuously. Many scientific research studies claimed that total calorie consumption in breakfast is inversely correlated with overweight/obesity and cardiovascular disease risk factors [18, 19] (Table 1).

Table 2: Influence of Skipping Breakfast on Metabolic Health

Study Design	Target Population	Results	References
Survey Study (Korea) Sample size=21193	Age 20-59 years Both gender	Regular Breakfast Eaters (56.4 %). The young adult population was significantly lower in this group. Irregular Breakfast Eaters have a higher abnormal metabolic outcome.	[16]
Cohort Study (United States of America) Sample size=9926 Study period 27 years	Age 20 years or older	Skipping breakfast shows higher cardiovascular and cerebrovascular disease mortality in MAFLD (Metabolic dysfunction associated with fatty liver disease), but not observed in the non-MAFLD group.	[20]
Survey-based (Brazil) Sample Size=776	Aged 18-65 years, Brazilian adults, both genders, and excluding night shift workers	Breakfast skippers had higher obesity than Breakfast eaters. Body Mass Index increased 0.74 kg/m2 for a delay of breakfast by every 1 hour from standard time.	[21]
Cross-Sectional Study (China) Sample size=70092	Chinese adult (having no cardiovascular disease and Cancer and with CRP (C-reactive Protein) concentration < 10 mg/l.	Serum CRP (C-reactive protein) was considerably higher in those individuals who skipped breakfast with poor diet quality.	[22]
Cross-sectional Study Sample Size= 112	3-12 years prepubertal children diagnosed with overweight/ obesity	There was no significant difference between the breakfast eater vs. breakfast skipper group in Growth Hormone, Cortisol, and Insulin-like growth factor-1, but total cholesterol, LDL-C (low density lipoprotein cholesterol), TGs (Triglycerides) were considerably higher in the breakfast skipper, and low HDL (High Density Lipoprotein) was observed in the breakfast skipper.	[23]

Glucose metabolism follows a circadian cycle, which peaks in the morning and declines in later days. Those who regularly skip breakfast and take more calories in the afternoon meal have peripheral tissues that do not manage glucose efficiently, which leads to a high post-prandial glucose level [24]. Skipping breakfast leads to more production of the ghrelin hormone, which encourages an individual's body to take more energy-dense food and leads toward overeating, which causes more fat storage

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and weight gain [25].

The modern lifestyle has a bad impact on people's health; most people consume high-energy food late at night or near bedtime. Late-night eating is classified in the literature as eating a meal after 10:00 pm or eating a meal 2 hours before bedtime, and if this activity is a minimum of 3 times per week, then it is considered late-night eating. This type of dietary change contradicts circadian rhythm, hence, increased risk of metabolic diseases (glucose intolerance, obesity, inflammation, and cardiac issues) and sleep disturbance [26, 27]. Epidemiological studies have highlighted that those who consume late-evening meals have a potentially negative impact on Cardio-metabolic health [28].

Table 3: Effect of Late-Night Eating on the Body's Metabolism

Study Design	Target Population	Results	References
Randomized Crossover Trial Sample Size=20	Age 18 -30 years, Healthy male/female	LD group (Late Dinner) had higher postprandial (4h) glucose and triglycerides levels than the RD group (Regular Dinner). There was no significant difference in morning fasting glucose and triglyceride levels in both groups. (Both groups' levels returned to normal in the fasting morning state.)	[29]
Randomized Crossover trial, Sample size=12	Age >20 years, non-smoker, and with no major disease.	The early dinner group (6:00 pm) had a significant decrease in postprandial respiratory quotient after breakfast, decreased blood glucose levels, and had a more positive effect on substrate oxidation than the late dinner group (9:00 pm)	[30]
Cross-sectional Study (Pakistan) Sample Size= 150	Age 18-30 years, University student, and late-night eaters.	95 % of participants usually consumed fried meals and confectionery items late at night. 75% headache, 66% sleep disorder, 63 % depression, 59 % stomach acidity, 29 % diarrhea, 25 % vomiting, and 21% piles.	[31]
Crossover Study, Sample size=8, and Study Period 3 days	Age 60 -70 years with body mass index < 30 kg/m2, Type 2 Diabetes HbA1c (Glycated Hemoglobin) 6.6 to 8.5 %, and not working at night.	By giving a test meal to both groups. Results showed that Postprandial glucose and insulin levels were high in the late -dinner group (21:00) as compared to the early-dinner group (18:00)	[32]
Prospective Observational Study	Patient age > 18 years with STEMI (ST-segment elevation myocardial infarction)	Skipping Breakfast (no food before lunch) with Late-night dinner (eating within 2 hours before bedtime) increases the risk of death four to five times more and re-attack of angina post 30 days of hospital discharge.	[33]

Small and frequent meal dietary habits are defined as the consumption of snacks or energy-giving beverages between main meals. It is still unclear whether consuming small and frequent meals or consuming a large meal has a greater potential benefit for metabolism or metabolic disorders [34]. But unhealthy snacking between main meals hurts an individual's health [35](Table 4).

Table 4: Importance of Eating Small and Frequent Meals in the Highlight of Research Studies

Study Design	Target Population	Results	References
Cross-sectional Study (Prospective study) Sample Size= 3009	Both male and female Aged between 47 and 68 years	Individuals with higher meal frequency (6 meals or >6 meals/day) had a lower risk of abdominal obesity than those with lower meal frequency (3 meals or < 3 meals/day).	[34]
Cohort Prospective Study (European Prospective Investigation into Cancer) Sample Size= 14666	Age 45-75 years Resident of Norfolk	Results highlighted that lower total cholesterol and LDL-C (low-density lipoprotein cholesterol) had a difference of 0.25 mmol/l in those who ate more than 6 meals/day than those who consumed 2 meals/day after adjusting for confounding variables.	[36]
Parallel Study (Iran) Sample Size= 66	Both Male and Female Mean age 51.8 years With type 2 diabetes	Participants who consumed 6 meals/d had decreased HbA1c (Glycated Hemoglobin) compared to those who consumed 5 meals/d. Results also highlighted that there was no significant difference observed in low-density lipoprotein, high-density lipoprotein, total cholesterol, and fasting blood glucose levels.	[37]
Research Study Sample Size= 91 Study Period 3 days	46 participants in the 2 main meals group (2MMG) and 45 participants in the 3 main meals group (3MMG) Age 18-64 years With overweight/obese.	3 MMG were found to have higher median values of body weight, Total body water, BMR, and hip circumference in men. Renal urea nitrogen and total cholesterol levels were higher in 2 MMG women than in 3 MMG.	[38]

Eating small and frequent meals spreads your whole day carbohydrate intake into smaller sections, which reduces the spike in insulin. Lower insulin spike requires fewer beta cells for insulin secretion [39]. A Small and frequent meal pattern brings less load to the liver and muscles and favors a more efficient oxidation process as compared to storing it in the form of fat. Hence, reduces ectopic lipid deposition [36]. Glucose metabolism in humans is under the control of the circadian rhythm.

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During the daytime, when a person usually consumes food, the body metabolizes glucose effectively, but the rate of metabolism of glucose is reduced during the night-dark hours. Various research studies on rodents and humans claimed that altered meal times can cause glucose intolerance because insulin and cortisol hormones (role in the metabolism of glucose) are regulated by the circadian system. Several studies have shown that glucose tolerance is more common in night shift workers [40]. The author Z Xie conducted a randomized controlled trial over a period of 5 weeks on healthy individuals. The author compared the insulin sensitivity response between two groups: one consisting of early-day eaters and the other a time-restricted feeding group. Their study results showed that improved insulin sensitivity was observed in the first group because, during the early part of the day, our body's biological clock aligns with our physiological mechanisms, causing muscles and adipose tissues to release more insulin in the morning than later in the day. Another author, Pavlou, conducted a randomized controlled trial for 6 months on adults with type 2 diabetes. Those who ate their meals earlier in the day (following a time-restricted eating regime without calorie counting) showed a comparable effect in reduction in body weight and HbA1c as the daily calorie restriction group [41]. Late-night snacking causes dysfunction of the circadian clock, disrupting lipid metabolism and leading to obesity. Various hormones, such as melatonin, leptin, and glucocorticoids, play a role in lipid metabolism and exhibit rhythmicity in a light/dark cycle. Mouse model research indicated that melatonin is considered a protective approach in the prevention of lipid metabolic disorders. Additionally, leptin regulates energy metabolism and its peak level at night [42]. The circadian cycle has an impact on protein metabolism. The anabolic activity of the protein is higher in the daytime; it might be effective to take protein in the daytime for muscle building. Taking protein close to bedtime might not be effective because of lower metabolic activity [43]. Yasuda performed 12 12-week randomized parallel trial on young adult that engaged in resistance training. There were two groups: one group (high protein intake in breakfast and also evenly distribution of protein all meals of day) and other group (on a skewed protein intake pattern: eating high protein in dinner) Their study outcomes showed that in group one where protein intake was evenly distributed in all day meals had increase muscle protein synthesis, increase muscle hypertrophy and its good for muscle building as compared to other group (skewed protein pattern) [44]. -Ghrelin: It was discovered in 1999 as a ligand for growth hormone. Ghrelin Hormone is released by the stomach. It is known as the "hunger hormone". It gives an appetite stimulus to the body. It releases more in the fasting state and decreases secretion after ingestion of food. The peak level of Ghrelin is in the evening according to the biological clock, so sleeping late at night, hunger sensation is at its peak level due to the ghrelin hormone [45]. Leptin: It was identified in 1994 as lipostat. The leptin hormone is secreted by white adipose tissues of the body. It is known as a "satiety hormone." It plays a key role in food intake and energy balance. The peak level of the leptin hormone is close to midnight. According to various intervention studies, the Concentration of leptin is higher in the morning in late eaters as compared to early eaters [45] (Table 5).

Table 5: Peak Time of Digestive Hormones according to the Circadian Clock

Hormones	Peak Time
Ghrelin	At Night
Leptin	At Night
PYY (Peptide YY)	At Morning
Insulin	At Morning
GIP (Glucose-Dependent Insulinotropic Polypeptide)	At Morning
GLP1 (Glucagon-Like Peptide 1)	At Morning

Vujovic conducted a controlled crossover lab study. There were two groups: early eaters and late eaters, but both groups followed the same iso-caloric eating pattern. Their research findings exhibited that in the late eating group increase in 24-hour ghrelin secretion and a decrease in 24-hour leptin secretion, hence, it increased hunger feeling and less feeling of satiety [46]. Another author, Manoogian, highlighted in his review that various clinical trials supported the same evidence that those who rely on a narrow feeding regimen (one meal/day) have higher ghrelin production and leads toward glucose intolerance [47].

Limitations and Future prospects

This review has certain limitations, including reliance on previously published studies with heterogeneous methodologies, variable sample sizes, and differing intervention durations, which may limit direct comparability. As a narrative review, it may also be subject to selection bias and does not provide quantitative pooled estimates. Additionally, most included studies were short-term and conducted in specific populations, reducing generalizability. Future research should prioritize large-scale, long-term randomized controlled trials and standardized chrononutrition protocols to establish evidence-based guidelines for meal timing in metabolic disease prevention and management.

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CONCLUSIONS

Recent studies have highlighted that intermittent fasting, time-restricted feeding, and eating small and frequent meals show different responses in individuals depending on their metabolism. Skipping breakfast or eating late at night has adversely affected hormonal regulation and increased the chances of obesity, which ultimately leads to metabolic diseases. Hence, lifestyle modification according to a circadian cycle result in preventing metabolic disorders.

Authors' Contribution

Conceptualization: SIT
Methodology: SIT, AM, IS
Formal analysis: SIT
Writing and Drafting: SIT, MH, IS, AR
Review and Editing: SIT, AM, MH, IS, AR

All authors approved the final manuscript and take responsibility for the integrity of the work.

Conflicts of Interest

All the authors declare no conflict of interest.

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Original Article

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The Prevalence of Iron Deficiency Anemia in Relation to Tea and Coffee Consumption Among Female University Students

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ABSTRACT

Tea consumption has been linked to iron deficiency anemia in several clinical trials. Lifestyle and dietary habits are important diagnostic considerations in this type of disease, and the consumption of caffeine-containing beverages can play a significant role. **Objectives:** To assess the prevalence of iron deficiency in relation to tea or coffee consumption among female university students. **Methods:** This cross-sectional study was conducted with 150 female students from the University of Lahore, selected via purposive sampling. Data were collected using a structured questionnaire comprising 25 items related to iron deficiency, dietary habits, and tea/coffee consumption patterns. **Results:** All participants reported regular consumption of tea or coffee. Key findings indicated that 88 (58.7%) participants experienced headaches when not consuming these beverages, 141 (94%) consumed them frequently, and 41 (27.3%) reported a constant desire to consume them. Notably, 49 (32.7%) felt tired without tea/coffee, and only 31 (20.7%) consumed these beverages with a meal. Furthermore, 27 (18%) participants reported feeling anemic, and 97 (64.7%) consumed dietary iron supplements less than 1-2 times daily. Common triggering factors after consumption included anxiety (71.3%), digestive issues (53.3%), and irregular menstrual cycles (98%). **Conclusions:** The study concludes that regular consumption of tea or coffee, particularly with meals, is a leading factor associated with iron deficiency risk among female university students. A notably low intake of dietary iron supplements further exacerbates the prevalence of iron deficiency anemia (IDA) in this population.

INTRODUCTION

Iron deficiency anemia (IDA) represents a major global health challenge, characterized by reduced hemoglobin levels and red blood cell counts that fail to meet physiological needs. As the most prevalent form of anemia, IDA arises when insufficient iron availability compromises hemoglobin synthesis, impairing oxygen transport and manifesting in symptoms such as fatigue, weakness, and shortness of breath [1, 2]. The global scope of iron deficiency is staggering, affecting approximately one-third of the world's population [3]. The World Health Organization estimates that nearly two billion people worldwide are anemic, with iron deficiency responsible for approximately 50% of these cases [4, 5]. Regional disparities are pronounced, with the highest prevalence rates observed in South Asia and Central and West Africa. In Pakistan, the National Nutrition Survey (2011-2012) revealed alarming rates of IDA, affecting 40-70% of children under five years and 18.1% of non-pregnant women of reproductive age [6, 7]. The consequences of untreated IDA are profound and far-reaching, including reduced

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cognitive function, compromised growth and development, diminished work capacity, and overall impaired quality of life [8]. While iron deficiency prevention strategies include consumption of iron-rich foods and vitamin C to enhance absorption [8], certain dietary components can significantly inhibit iron uptake. Among these, tea and coffee present particular concern due to their widespread consumption and potent inhibitory properties [9]. These beverages contain polyphenols, primarily tannins and oxalates, that bind dietary iron and form insoluble complexes, reducing absorption by up to 39% when consumed with meals. The effect is particularly pronounced for non-heme iron from plant sources, though heme iron from animal products is also affected [10, 11]. In Pakistan, tea consumption is deeply embedded in cultural practices, with approximately 91% of the population preferring tea over other beverages, while coffee consumption averages 0.8 kg per person annually [12, 13]. The tradition of consuming tea with meals is particularly concerning from a nutritional perspective, as this timing maximizes the inhibitory effect on iron absorption [14]. Recent evidence confirms that tea consumption with meals reduces iron absorption, while coffee consumption exceeding three cups daily has been linked to iron deficiency in pregnant women [15, 16]. Despite the high prevalence of both tea consumption and iron deficiency in Pakistan, limited research has explored this relationship within the specific context of female university students. This population represents a vulnerable group due to their reproductive age and potential for increased iron demands. With black tea consumption in Pakistan projected to increase from 172,911 tons in 2022 to 250,755 tons by 2027, understanding the impact of this dietary habit on iron status becomes increasingly urgent [13].

Despite the high prevalence of iron deficiency anemia (IDA) and the widespread consumption of tea and coffee in Pakistan, limited empirical research has examined their relationship among female university students. Most existing studies focus on pregnant women or general reproductive-age populations, with insufficient attention to young adult students who are at increased nutritional risk. Furthermore, local evidence exploring beverage consumption patterns alongside iron supplementation behaviors remains scarce. Therefore, there is a need to investigate this association within the university setting to generate context-specific data for targeted nutritional interventions. This study addresses this knowledge gap by investigating the prevalence of iron deficiency in relation to tea and coffee consumption patterns among female university students in Pakistan, providing evidence to inform targeted interventions and nutritional education programs. This study aimed to assess the prevalence of iron deficiency in relation to tea or coffee consumption among female university students.

METHODS

This cross-sectional study was conducted among 150 female students at the University of Lahore to assess the prevalence of iron deficiency in relation to tea and coffee consumption. Study was conducted from January 2023 to June 2023. Ethical consent was obtained from University of Lahore, and prior written informed consents were taken from all the study participants. The sample size was calculated using an online sample size calculator. Participants were selected using a purposive sampling technique, with inclusion criteria encompassing healthy females aged 18-30 years who consumed a minimum of half a cup of tea or coffee daily. Exclusion criteria included unwillingness to participate, non-consumption of tea or coffee, age outside the specified range, and presence of any diagnosed medical condition. Data were collected through a structured questionnaire designed to gather information on demographic characteristics, tea and coffee consumption patterns, dietary habits, and potential symptoms related to iron deficiency. The collected data were analyzed using SPSS version 24.0.

RESULTS

All of the participants consume tea or coffee regularly in their diet (Table 1).

Table 1: Consumption of Tea or Coffee Regularly in the Dietaracteristics of the Study Participants (n=460)

Sr. No.	Consumption of Tea or Coffee Regularly in the Diet of the Respondent	Frequency (%)
1	Yes	150 (100%)

Past 4 weeks, consumption of coffee or tea with a meal among female university students. Out of 150 participants, 31 consume tea or coffee with a meal, 71 sometimes consume tea or coffee with a meal, and 48 don't consume tea or coffee with a meal (Table 2).

Table 2: Consumption of Tea or Coffee with a Meal During the Past Four Weeks Among Female University Students

Sr. No.	Past 4 Weeks, Consumption of Coffee or Tea with a Meal	Frequency (%)
1	All of the time	31 (20.7%)
2	Some of the time	71 (47.3%)
3	None of the time	48 (32.0%)
4	Total	150 (100.0%)

Out of 150 participants, 27 participants feel anemic, 48 sometimes feel anemic, and 75 don't feel anemic (Table 3).

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Table 3: Frequency of Feeling Anemic During the Past Four Weeks Among Female University Students

Sr. No.	Past 4 Weeks, Feel Anemic	Frequency (%)
1	All of the time	27 (18.0%)
2	Some of the time	48 (32.0%)
3	None of the time	75 (50.0%)
4	Total	150 (100.0%)

Out of 150 participants, 106 know that excessive consumption of tea or coffee may lead to iron deficiency, whereas 44 don't think of it (Table 4).

Table 4: Awareness Regarding the Effect of Excessive Tea or Coffee Consumption Leading to Iron Deficiency Among Female University Students

Sr. No.	Excessive Consumption of Tea or Coffee May Lead to an Iron Deficiency in the Respondent	Frequency (%)
1	No	44 (29.3%)
2	Yes	106 (70.7%)
3	Total	150 (100.0%)

Out of 150 participants, 55 use iron supplements, whereas 95 don't use iron supplements (Table 5).

Table 5: Use of Iron Supplements Among Female University Students

Sr. No.	Use of an Iron Supplement	Frequency (%)
1	No	95 (63.3%)
2	Yes	55 (36.7%)
3	Total	150 (100.0%)

As the result shows, after consumption of tea or coffee out of 150, 107 respondents feel anxiety, 122 respondents feel insomnia, 80 respondents experience digestive issues, 79 respondents feel addiction, 127 respondents experience rapid heart rate, 98 respondents do not feel fatigue, 90 respondents do not experience headache, 76 respondents feel dehydrated, 147 respondents experience irregular menstrual cycle (Figure 1).

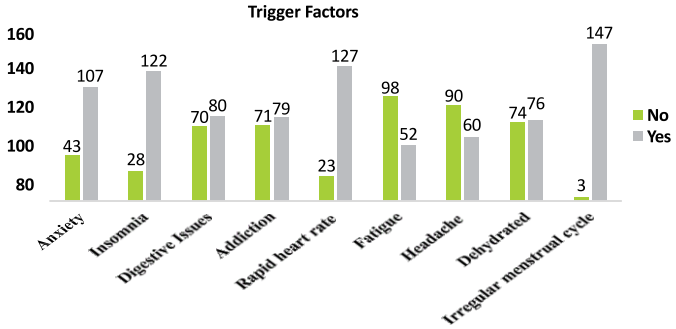

Figure 1: Distribution of Trigger Factor After Consuming Tea or Coffee Among Female University Students

Out of 150 participants, 97 consume a dietary supplement of iron less than 1-2 times daily, whereas 20 consume 2-3 times daily (Figure 2).

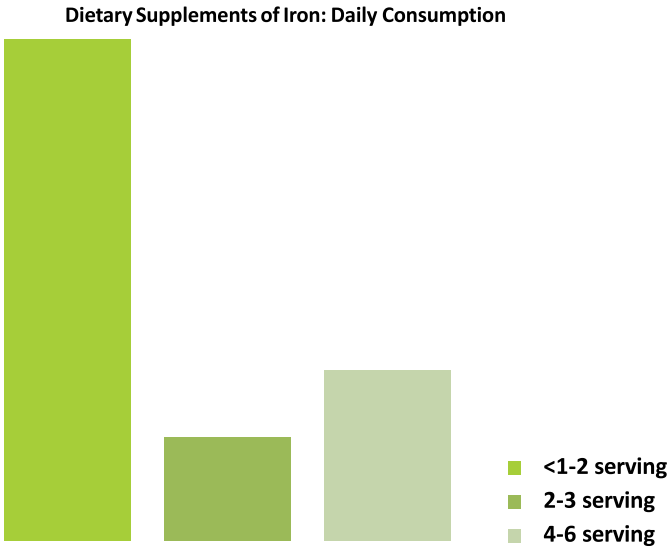

Figure 1: Daily Consumption of Dietary Supplement of Iron

DISCUSSION

The findings of this study demonstrate a strong association between regular tea and coffee consumption and indicators of iron deficiency among female university students in Pakistan. These results align with recent international evidence. A previous study reported that reproductive-aged women who regularly consumed tea or coffee had a 1.7-fold higher risk of iron deficiency compared to non-consumers [17]. Furthermore, our observation that 68% of participants consumed these beverages with meals (sometimes or always) is concerning, as an earlier study found that tea intake even one-hour post-meal significantly inhibits iron absorption, though less than concurrent consumption [18]. The low prevalence of iron supplement use (36.7%) among our participants is a major contributor to iron deficiency and mirrors and aligns with previous findings, where inadequate iron supplementation was also a key risk factor for anemia [19]. The high frequency of self-reported anemia symptoms (50%) corresponds with evidence of higher anemia prevalence among tea-drinking pregnant women compared to non-tea drinkers [20]. Moreover, the remarkably high rate of menstrual irregularities (98%) following tea or coffee consumption aligns with recent endocrinological research suggesting that elevated polyphenol intake may influence estrogen metabolism and menstrual cyclicity [21]. This study has some limitations. Self-reported data may be subject to recall and social desirability bias, and the cross-sectional design limits causal inference. Moreover, purposive sampling restricts generalizability to all female university students. Despite these limitations, the findings highlight the need for nutritional education on the timing of tea and coffee intake and the importance of iron supplementation. Future

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longitudinal and intervention studies using biochemical assessments are recommended to clarify the relationship between beverage consumption and iron status.

This study is limited by its cross-sectional design, which prevents causal inference, and reliance on self-reported data that may introduce recall bias. The use of purposive sampling from a single university also restricts generalizability to broader populations. Future research should incorporate longitudinal or interventional designs with biochemical assessments such as serum ferritin and hemoglobin levels to establish stronger evidence. Expanding studies across multiple institutions and regions would further strengthen understanding and support the development of evidence-based nutritional policies.

CONCLUSIONS

In conclusion, the high prevalence of regular tea and coffee consumption, particularly with meals, is significantly associated with iron deficiency risk among female university students. The widespread practice of consuming these beverages alongside meals, combined with insufficient iron supplementation, exacerbates the population's vulnerability to iron deficiency anemia. These findings underscore the critical need for targeted nutritional education programs to promote safer consumption habits and improve iron intake. Addressing these modifiable dietary factors is essential for reducing the burden of iron deficiency in this demographic.

Authors' Contribution

Conceptualization: NK
Methodology: MJ[1], MJ[2]
Formal analysis: MJ[1], HY
Writing and Drafting: MJ[1], NHS, YD, HY
Review and Editing: MJ[1], NHS, NK YD, HY, MJ[2]

All authors approved the final manuscript and take responsibility for the integrity of the work.

Conflicts of Interest

All the authors declare no conflict of interest.

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Original Article

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Integration of Nutrition into the Mathematics Curriculum of Primary Schools in Khyber Pakhtunkhwa: A Quasi-Experimental Study

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ABSTRACT

Malnutrition and unsuitable dietary habits remain an issue of high priority among the primary school students in Khyber Pakhtunkhwa (KP), Pakistan. Children in this region often lack adequate awareness about healthy eating patterns, and schools rarely integrate nutrition concepts into academic subjects. **Objectives:** To assess not only the role of nutrition education as a supplement in regular curricula (mathematics) but also their learning outcomes (mathematical and nutrition-related knowledge). **Methods:** A quasi-experimental design was implemented with a total of 240 participants (grades 3–5, ages 8–11 years) in eight primary schools (four interventions, four controls). The intervention group was exposed to mathematics with built-in nutrition material for 12 weeks, whereas the control group pursued the normal curriculum. Validated scales of mathematics achievement and nutrition knowledge were used in conducting pre- and post-tests. The data were analyzed with paired and independent t-tests. **Results:** The data did not show any differences between groups in terms of baseline. Nevertheless, after the intervention, analysis showed that both mathematics performance (mean change: 14.2 ± 5.1 in intervention vs. 5.1 ± 4.9 in control, $p<0.001$) and nutrition knowledge (mean change: 12.6 ± 4.3 in intervention vs. 2.3 ± 3.8 in control, $p<0.001$) had improved significantly. **Conclusions:** The inclusion of nutrition concepts into the mathematics curriculum was also a significant contributor to improvement in mathematics and nutrition awareness following the implementation of the concept in KP primary school students.

INTRODUCTION

Child nutrition is one of the most important aspects in cognitive development, concentration in the classroom, and general academic performance, and its relationship with education and health cannot be ignored [1, 2]. Nutrition in school children not only helps in physical development, but also in the optimum development of the brain, retaining skills and capacity to learn [3]. On the other hand, deficiencies and undernutrition of micronutrients have been demonstrated to affect attention, memory, and performance in academic work, resulting in end-of-term deficits in academic achievements. Malnutrition is one of the most long-standing public health issues in Pakistan and among school-going children in Khyber Pakhtunkhwa (KP). In the recent national nutrition survey reports [4, 5], stunting, underweight, and iron, vitamin A, and calcium deficiencies are alarmingly high. These nutritional deficiencies result in a vicious circle of underachievement in school, low productivity, and reduced human capital development in the province. Schools are a perfect and fair way of health promotion and avoiding malnutrition since they access children at a tender age and offer constant exposure to educational settings [6]. Nevertheless, in Pakistan (as in many other low- and middle-income countries), nutrition education is commonly taught as an independent, health-related course, without being linked to major academic courses. Such a disjointed method

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limits the support of essential health concepts and tends to be in conflict with other academic priorities, resulting in decreased student engagement and retention. Curriculum integration has been identified as a potential solution to these issues, in which the concepts of nutrition are incorporated into the normal school courses like mathematics, language, or social studies [7-9]. As an illustration, students can be taught fractions by ending up with food parts, percentages by nutrient composition, and data analysis by collecting food frequency surveys. This kind of combining gives a two-fold benefit of not only improving academic but also health literacy outcomes because it enhances contextual learning, interdisciplinary thinking, and good use of time within the current activities of the classrooms. International research demonstrates that cross-curricular integration enhances and improves knowledge retention, problem-solving skills, and higher-order thinking, while also increasing motivation and student engagement through practical application. This is particularly applicable in the case of the Education Sector Plan of Khyber Pakhtunkhwa, where the focus is on enhancing literacy and numeracy, as well as forming a multi-sectoral coalition to improve nutrition and health outcomes [10, 11]. The importance of the study is that it presents empirical data on the relationship between curriculum integration as a dual-purpose innovation in education in Pakistan as a means of improving the academic performance of students and increasing the necessary health knowledge. This study will contribute to the policy debate on integrated education and child development in low-resource contexts by showing a practical, contextually dynamic example.

Despite the high burden of malnutrition among school-aged children in Khyber Pakhtunkhwa, nutrition education remains inadequately integrated into core academic subjects, particularly mathematics. Existing school-based interventions in Pakistan primarily focus on standalone nutrition programs, with limited empirical evidence evaluating cross-curricular integration models. Moreover, there is a scarcity of quasi-experimental research assessing both academic and health literacy outcomes simultaneously in low-resource public school settings. Therefore, investigating the effectiveness of integrating nutrition concepts into the mathematics curriculum is essential to address both educational and nutritional challenges concurrently. The present study aimed to evaluate the effectiveness of teaching mathematics using a mathematics education curriculum with the inclusion of a nutrition education curriculum to determine mathematics achievement and nutrition literacy levels among primary school children in Khyber Pakhtunkhwa.

METHODS

A quasi-experimental study, following a pretest-posttest control group design, was conducted from September to December 2024 on eight randomly selected government primary schools located in Charsadda and Mardan districts of Khyber Pakhtunkhwa. The study included 240 students from grades 3—5 (aged 8—11 years), divided equally into intervention (n=120) and control (n=120) groups. Schools were selected using a cluster random sampling method. First, a list of all public primary schools in the two districts was obtained. From this list, schools were eligible if they were (a) mixed-gender, (b) located in a semi-urban area, and (c) not currently running any other formal nutrition program. Eight schools that passed these criteria were randomly chosen (with the aid of a random number sequence, which was generated on a computer) and randomly put in either the intervention or control group. The a priori determination of the sample size was conducted with the help of G*Power software. The power was calculated with 0.05 alpha (a), power (1- 5) = 0.80, and medium effect size (Cohen d = 0.5), which were based on the previous educational research done with interventions. This analysis meant that the necessary overall sample size was about 210, and it was planned to have a group of 240 so that the possibility of the loss of participants could be considered, as well as it would help to strengthen the results. Written informed consent was taken. The intervention group received 12 weeks of mathematics lessons, and nutrition education about fractions as portions, percentages, and measures as food volumes, and data analysis using food frequency surveys. The intervention schools involved the teachers in two days of training on the integrated pedagogy, as compared to the control schools that used the standard mathematics curriculum. The evaluation was conducted using two instruments that were previously tested and validated (30-item Mathematics Achievement Test (Cronbach 83) and 25-item Nutrition Knowledge Questionnaire (Cronbach 79)) and created and tested among primary school students in Pakistan. These scale instruments have been constructed and tested on a pilot sample of a different group of primary school students of the same demographic. Construct validity was determined by subjecting the items to a panel of five experts in education and nutrition, and making sure that the items were appropriate to measure the curriculum and important areas of knowledge. The instruments were then piloted, and a two-week test-retest reliability analysis showed strong stability (Intraclass Correlation Coefficient (ICC) = 0.85 for the Mathematics Test and ICC = 0.81 for the Nutrition Questionnaire). The changes in mathematical performance and nutrition knowledge were measured by the use of pre- and post-tests. Digital data on baseline

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demographics and diet were obtained via structured questionnaires. Analysis of data was done using SPSS version 26.0. The assumption of normality for the pre-post change scores was assessed and confirmed using the Shapiro-Wilk test (p>0.050 for both groups on both outcome measures). Changes were compared using paired t-tests between pre- and post-intervention change within the groups and independent t-tests between the groups. The residuals from the ANCOVA models were also checked and met the assumptions of normality and homogeneity of variances.

RESULTS

The framework for integrating nutrition into the primary school mathematics curriculum was analyzed (Table 1).

Table 1: Framework for Integrating Nutrition into Primary School Mathematics Curriculum

Mathematics Concept	Nutrition Theme	Classroom Activity Example	Expected Learning Outcome
Fractions (½, ¼, ⅓)	Portion sizes and balanced meals	Use a roti or an apple to show halves and quarters while discussing meal portions.	Students understand fractions and balanced meal portions
Percentages (%)	Daily nutrient requirements	Calculate % of daily calcium from a glass of milk	Students apply percentage concepts to real-life nutrition
Addition and Subtraction	Food groups and variety	Add or remove food items (lentils, vegetables, rice) to form balanced meals.	Students enhance arithmetic skills with nutrition awareness
Measurement (grams, kg)	Food quantities and weights	Weigh rice, lentils, or flour to learn measurement units	Students relate measurement to real food quantities
Geometry (shapes, area)	Food shapes	Identify shapes of roti, bread, or samosa and calculate area/perimeter	Students associate geometry with familiar food items
Data Handling (graphs, charts)	Dietary diversity	Survey favorite fruits and create bar/pie charts	Students learn data representation through dietary habits
Ratios & Proportions	Balanced diet plate	Express healthy plate in ratio (2:1:1 for veg: protein: grains)	Students understand ratios and balanced diet composition

Baseline characteristics of participants in the intervention (n=121) and control (n=119) groups. The p-value was determined as less than 0.050. No significant differences were observed between the two groups in terms of age, gender distribution, baseline mathematics scores, or baseline nutrition knowledge (p > 0.050), indicating that both groups were comparable at the start of the study (Table 2).

Table 2: Baseline Characteristics of Participants (n=240)

Variables	Intervention (n=121)	Control (n=119)	p-Value
Age (Years, Mean ± SD)	9.4 ± 0.8	9.3 ± 0.7	0.410
Gender (Male, %)	55 (45.8%)	58 (48.3%)	0.720
Baseline Math Score	48.6 ± 9.2	47.9 ± 9.5	0.630
Baseline Nutrition Knowledge	21.1 ± 5.6	20.7 ± 5.8	0.580

Post-intervention results showed significant improvement in both mathematics performance and nutrition knowledge among students taught with the integrated curriculum. ANCOVA, adjusting for baseline scores, age, gender, and socioeconomic status, confirmed a statistically significant effect of the intervention on both post-intervention mathematics scores (F (1,234) <0.001) and nutrition knowledge scores (F (1, 234) = 0.001). After 12 weeks, the intervention group demonstrated higher gains in mathematics (14.2 ± 5.1 vs. 5.1 ± 4.9) and nutrition knowledge (12.6 ± 4.3 vs. 2.3 ± 3.8) compared to the control group (p<0.001) (Table 3).

Table 3: Post-Intervention Outcomes

Outcomes	Intervention (n=121)	Control (n=119)	Mean Difference	p-Value
Math Score Improvement	14.2 ± 5.1	5.1 ± 4.9	9.1	<0.001
Nutrition Knowledge Improvement	12.6 ± 4.3	2.3 ± 3.8	10.3	<0.001

DISCUSSION

The intervention and control groups were similar at baseline in terms of demographics and academics. In the intervention group, 45.8% of the males and 48.3% in the control group (p=0.720). There were no significant differences observed in baseline scores of mathematics and nutrition knowledge, which means that there was comparability between the cohorts and minimal baseline confounding. Looking at the post-intervention scores of both groups in mathematics and nutrition knowledge, both groups demonstrated improved scores, but the intervention group demonstrated significantly better improvement. Table 2 revealed that the mathematics scores improved in the intervention group by 14.2 and 5.1 as compared to the control group by 5.1, respectively, with a mean difference of 9.1. Likewise, the difference in interventions between the intervention group and the control group in terms of nutrition knowledge improved by 12.6 and 2.3 points, respectively, and the difference is significant (p<0.001), indicating the effectiveness of the integrated cur riculum. Subgroup analyses, both gender-

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wise and grade-wise, did not find any significant differences, indicating that the intervention was effective with both boys and girls and Grades 3-5. This homogeneity favors the homogeneity of the integrated approach to other levels of primary schools in Khyber Pakhtunkhwa. The current investigation is a successful demonstration of the fact that incorporating mathematics classes with nutrition concepts will improve academic knowledge and health literacy. This observation is consistent with available global research on the topic, which has shown that cross-curricular integration leads to better student engagement, understanding, and retention [12-14]. Believing that active learning with real-life nutrition problems improved mathematical reasoning is plausible since the contextualized cases are easier to comprehend and solve problems [15, 16]. Similarly, repeated access to nutrition education throughout lessons will support the maintenance of knowledge and promote healthier attitudes, which is in line with the earlier educational interventions that associate classroom exposure with better health-related behaviors [17, 18]. The tools of the research, which were utilized in the study, were contextually valid and sound, including the Mathematics Achievement Test and the Nutrition Knowledge Questionnaire, with Cronbach 0.83 and 0.79 internal consistency respectively. Self-reported nutrition data, short intervention, and school participation were also weaknesses of it and had some implications in terms of generalizability. All in all, the findings suggest that mathematics lesson nutrition is a cost-effective, simple-to-use, and scalable intervention to enhance the academic performance and the health literacy level of primary students in Pakhtunkhwa. This study warrants the implementation of multi-sectoral nutrition policy at the policy level in line with integrated pedagogic practices in Khyber Pakhtunkhwa [19, 20]. The subsequent research must be aimed at measuring long-term results, professional training requirements, and institutional viability to make it sustainable.

This study has certain limitations, including its short intervention duration and restriction to semi-urban government schools in two districts, which may limit generalizability to other regions or private institutions. Additionally, nutrition outcomes were limited to knowledge assessment without evaluating long-term behavioral or nutritional status changes. Future research should examine the sustainability of integrated curricula over longer periods, assess dietary behavior and anthropometric outcomes, and explore teacher training scalability across diverse educational settings in Khyber Pakhtunkhwa and beyond.

CONCLUSIONS

The integration of nutrition education in the math curriculum enhanced both math achievement and nutrition knowledge among children in KP's primary schools. This innovative, low-cost strategy could be replicated throughout the province to address educational and health issues simultaneously.

Authors' Contribution

Conceptualization: IA
Methodology: IA
Formal analysis: IA
Writing and Drafting: IA
Review and Editing: IA

All authors approved the final manuscript and take responsibility for the integrity of the work.

Conflicts of Interest

All the authors declare no conflict of interest.

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Investigating Sustainable Food Habits by Using Novel Carbon-Footprint Method – A Cross-Sectional Analysis

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ABSTRACT

Environmental sustainability and nutrition are two emerging issues in public health that have become interdependent. The eating trends not only dictate the health outcomes of individuals but also the sources of greenhouse gases in the world. **Objectives:** To establish the relationship between food patterns and the carbon footprint of university students in Khyber Pakhtunkhwa, Pakistan. **Methods:** It was a cross-sectional study. Food intake data were measured using a semi-quantitative Food Frequency Questionnaire (FFQ) in 400 participants, and carbon emissions were estimated using the values of the life-cycle assessment (LCA). **Results:** Carbon footprints of meat-intensive diets were significantly higher compared to those of vegetarian diets. The Mean Adequacy Ratio (MAR) for meat-heavy was 72.6 ± 6.0 and for plant-forward diets was 72.6 ± 5.0, respectively. The emission of meat-based diets (5.2 ± 0.1 kg CO2-equivalent per day) was much higher than that of plant-based diets (2.9 ± 0.8 kg CO2-equivalent per day; p=0.001). Red meat generated 52% of all emissions in meat-based diets compared to the 0:14% in plant-based ones, with cereals and milk being moderate contributors and vegetables and legumes being minor contributors. Regression analysis revealed that higher emissions were predicted by the consumption of red meat (β = 0.44) and residing in urban areas (β = 0.54). **Conclusions:** The results suggest that simple changes to the diet, which decrease red meat consumption and increase the consumption of plant foods, can reduce carbon emissions but not decrease the nutritional sufficiency.

INTRODUCTION

The food system in the world is one of the largest causes of climate change, as it contributes to nearly one-third of the greenhouse gas emissions [1, 2]. Meanwhile, malnutrition in its various forms continues to be a burning issue in the world health. The crossroads of environmental sustainability and nutrition are becoming more acknowledged as the key to the fulfillment of the Sustainable Development Goals (SDGs), especially SDG 2 (Zero Hunger) and SDG 13 (Climate Action) [3, 4]. Carbon footprint assessment gives an estimate of greenhouse gas (GHG) emissions from food manufacture, transportation, and use [5]. Diets that are high in animal foods, especially red meat and milk, have unusually large carbon footprints compared to plant-based counterparts [6–8]. In contrast, eating patterns that are high in fruits, vegetables, whole grains, and legumes are associated with both better health consequences and fewer environmental effects [9–11]. In Pakistan, climate change and food security are urgent and connected challenges. Khyber Pakhtunkhwa (KP), with high undernutrition rates in the province, also witnesses high environmental stresses [12, 13]. However, there has been scant research on quantifying the environmental

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consequences of eating habits here.

Despite growing global evidence linking dietary patterns with environmental sustainability, there is limited empirical data from Pakistan quantifying the carbon footprint of habitual diets, particularly among young adults. Most available studies are based on Western populations and may not reflect the unique food systems, cultural practices, and production patterns of Khyber Pakhtunkhwa. Furthermore, few studies simultaneously evaluate both environmental impact and nutrient adequacy within the same population. This gap highlights the need for localized research integrating carbon-footprint estimation with nutritional assessment among university students. This research aims to measure the carbon footprints of dietary intakes among university students in KP and to simulate the consequences of dietary changes on nutrition as well as on the environment.

METHODS

A cross-sectional design was used to determine the association between dietary habits, nutrition adequacy, and their corresponding carbon footprint. The study conducted from September 2024 to December 2024 in Charsadda used a convenience sample of 400 students between the ages of 18–25 years. Sampling was performed by stratified random selection in a manner that provided proportional representation by gender, field of study, and socioeconomic status. Inclusion criteria were being enrolled as a full-time student, 18 to 25 years of age, and willingness to give informed consent. Students with self-reported chronic diseases (e.g., diabetes, kidney disease) or special diets (e.g., medical diets, veganism) were excluded to avoid confounding effects. Sample size was calculated from the basis of the anticipated medium effect size (Cohen's d =0.5) between groups based on diet, with 80% power and 5% significance level, which required a minimum of 352 participants. To provide for incomplete returns, 400 students were eventually recruited. Measured dietary intake was also measured through a semi-quantitative food frequency questionnaire (FFQ), which consisted of 85 locally adapted foods and beverages, which were grouped into major food categories. The FFQ was pilot-tested and validated for reliability in a sub-sample of 30 university students from the same population in Khyber Pakhtunkhwa. The tool demonstrated good test-retest reliability over two weeks (Intra-class Correlation Coefficient, ICC = 0.82) and acceptable internal consistency (Cronbach's α = 0.76). Participants were asked about the frequency of intake in the last month, and the portions were standardized using local food models and household measures. The reported intakes were calculated in grams per day with the help of standard conversion factors. FFQ interviews were done by trained nutritionists to reduce recall bias. The data were gathered and the nutrient adequacy calculated, and the dietary carbon footprints estimated. Carbon footprint of the diet (kg CO2-eq/day) was calculated by correlating the information of food frequency questionnaires with the life-cycle assessment (LCA) database emission factors (Poore and Nemecek [14], Our World in Data [15]). To enhance contextual accuracy, these base emission factors were adjusted to better reflect local production and transport patterns in Khyber Pakhtunkhwa using the formula: Adjusted EF = Base EF × (P_loc + T_loc), where P_loc (a factor of 0.9) accounts for less energy-intensive local production methods for certain crops, and T_loc (a factor of 1.1) accounts for the higher emissions from fragmented cold-chain transport for dairy and meats, based on local expert consultation and regional agricultural reports. Emission factors included production, processing, packaging, and distribution, but with the local food supply patterns of Khyber Pakhtunkhwa. The daily emissions of each participant were computed as the product of food quantities (g/day) and the respective factors of emission (kg CO2-eq/kg), then added up across all items. Types of meat (red meat, poultry, fish) were studied individually, and substitution modeling was used to determine emission cutting as a result of changes in the diet. The FFQ data on nutrient intake were transformed to nutrient and energy values by using the Pakistan Food Composition Tables with FAO data where needed. Protein, fat, carbohydrate, fiber, calcium, iron, vitamin A, and vitamin C were used as the key nutrients of interest and divided by the WHO/FAO recommended dietary allowance (RDA) of each food item at a specific age and sex to derive the nutrient Adequacy Ratios (NARs). The maximum percentage of the ratio of each participant was taken as one (1) to prevent overestimation. The Mean Adequacy Ratio (MAR), which is the mean of all truncated NARs, gave a total correlation of nutritional quality and environmental sustainability results. Data analysis was done using descriptive statistics, independent t-tests, substitution modeling, and multivariate linear regression.

RESULTS

The study gives the participant characteristics. The groups were similar in age, gender, and BMI (Table 1).

Table 1: Baseline Characteristics of Participants (n=400)

Variables	Meat-Heavy Diet (n=200)	Plant-Forward Diet (n=200)	p-value
Age (Years)	20.1 ± 1.4	20.1 ± 1.6	0.470
Gender (M/F)	96/84	105/95	0.230
BMI (kg/m^2)	22.5 ± 2.3	22.4 ± 2.5	0.380
Baseline kcal/day	2443 ± 311	2335 ± 421	0.130

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The study reveals mean dietary carbon footprints. Diets that were high in meat (n=180) contained considerably higher emissions (5.3 ± 1.3 kg CO_2-eq/day) than diets that were plant-based (n=200; 2.8 ± 1.7 kg CO_2-eq/day; p<0.001) (Table 2).

Table 2: Dietary Carbon Footprints and Nutrient Adequacy

Variables	Meat-Heavy Diet (n=200)	Plant-Forward Diet (n=200)	p-Value
Carbon Footprint (kg CO_2-eq/day)	5.3 ± 1.3	2.8 ± 1.7	<0.001
Protein Adequacy (%)	103 ± 9	99 ± 8	0.060
Fiber Intake (g/day)	18.5 ± 4.2	28.3 ± 5.6	<0.001
Mean Adequacy Ratio (MAR, %)	88 ± 6	91 ± 5	0.040

Nutrient adequacy analysis based on the Pakistan food-composition conversions showed distinct patterns across the two dietary groups. Protein adequacy was comparable between groups (meat-heavy NAR 102% vs plant-forward NAR 97%; p=0.060), indicating that both patterns generally met protein needs for the student population. Fiber intake differed markedly: the plant-forward group achieved substantially higher fiber intakes (29.1 ± 5.6 g) than the meat-heavy group (17.5 ± 5.3 g), corresponding to mean NARs of 97% and 58%, respectively (p<0.001). Calcium and iron showed shortfalls in both groups; calcium NARs averaged 56% (meat-heavy) and 53% (plant-forward), while iron NARs were 83% vs 70% (p=0.020). Vitamin A and vitamin C intakes were well below the recommended levels in both groups (NARs ~77–83%) and did not differ significantly. The composite Mean Adequacy Ratio (MAR), computed as the mean of the six truncated NARs, indicated overall diet adequacy of 72.60 ± 6.0% in the meat-heavy group and 78.1 ± 5.0% in the plant-forward group (difference p=0.040). This suggests that, while overall nutrient adequacy was reasonably high across the sample, plant-forward diets achieved marginally better balance across the assessed micronutrients and fiber, chiefly due to substantially higher fiber and somewhat improved micronutrient profiles (Table 3).

Table 3: Nutrient Intakes, Nutrient Adequacy Ratios (NARs) and Mean Adequacy Ratio (MAR) by Dietary Group (n=400)

Nutrient (RDA)	Meat-Heavy: Intake (mean ± SD)	Meat-Heavy: NAR	Plant-Forward: Intake (Mean ± SD)	Plant-Forward: NAR	p-Value
Protein (55 g)	56.3 ± 8.1	102 ± 8.0	53.5 ± 6.4 g	97 ± 9.0	0.060
Fiber (30 g)	17.5 ± 5.3	58 ± 12.2	29.1 ± 5.6 g	97 ± 21.9	<0.001
Calcium (1000 mg)	560 ± 312	56 ± 31.8	534 ± 190	53 ± 34.9	0.140
Iron (18 mg)	15.1 ± 4.1	83 ± 11.8	12.6 ± 4.3	70 ± 11.9	0.020
Vitamin A (700 µg)	543 ± 133	77.5 ± 22	544 ± 145	77.7 ± 31.1	0.380
Vitamin C (75 mg)	44.3 ± 19.4	59 ± 31.5	55.3 ± 21.1	73.7 ± 31.5	0.340
MAR (Mean of Truncated NARs)	—	72.6 ± 6.0	—	78.1 ± 11.0	0.040

RDA = reference daily allowance used for NAR calculation (adult reference values used for illustration). NAR (%) = (mean intake / RDA) × 100, truncated at 100. MAR (%) = mean of truncated NARs across the six nutrients shown. Values are mean ± SD. The p-values from independent t-tests comparing Meat-heavy vs Plant-forward groups. RDA values used for calculation: Protein = 55 g; Fiber = 30 g; Calcium = 1000 mg; Iron = 18 mg; Vitamin A = 700 µg RAE; Vitamin C = 75 mg.

The study separated aggregate dietary carbon footprints into large food-group contributors in order to determine where emissions are concentrated. Contributions are expressed as mean kg CO_2-eq/day (±SD) and as the percent contribution of the individual's aggregate diet carbon footprint. Disaggregation of carbon footprints from dietary sources indicated red meat as the prevailing source in meat-dense diets, responsible for around 52% of combined emissions and only ~14% in plant-based diets. Cereals and milk were significant secondary contributors; vegetables and legumes contributed very little to CF per gram but aided nutrient adequacy (Table 4).

Table 4: Mean Carbon Footprint by Food Group (kg CO_2-eq/day) and Percent Contribution, By Dietary Group

Food Groups	Meat-Heavy (Mean ± SD)	% of Total (Meat-Heavy)	Plant-Forward (Mean ± SD)	% of Total (Plant-Forward)
Red Meat (Beef, Mutton)	2.610 ± 0.70	52.0%	0.41 ± 0.24	13.8%
Poultry	0.44 ± 0.30	8.7%	0.34 ± 0.08	12.1%
Fish/Seafood	0.17 ± 0.10	3.1%	0.16 ± 0.07	4.8%
Dairy (Milk, Yogurt, Cheese)	0.93 ± 0.60	18.1%	0.57 ± 0.21	18.3%
Cereals and Grains (Wheat, Rice)	0.49 ± 0.28	10.0%	0.57 ± 0.13	20.0%
Legumes and Pulses	0.08 ± 0.03	1.7%	0.26 ± 0.10	8.6%
Fruits and Vegetables	0.11 ± 0.04	1.9%	0.26 ± 0.08	8.3%
Oils, Nuts, Misc	0.20 ± 0.08	3.80%	0.11 ± 0.04	3.4%
Total (mean)	5.16 ± 1.08	100%	2.59 ± 0.79	100%

Small rounding differences vs earlier reported totals arise from item-level aggregation; values remain consistent within reporting precision. Red meat is the dominant source of emissions in the meat-heavy group, contributing roughly half of total dietary carbon.

Multivariable regression identified red meat intake (β = 0.44 kg CO_2-eq per 50 g/day, 95% CI 0.35–0.53, p<0.001) and urban residence (β = 0.54, 95% CI 0.31–0.77, p<0.001) as strong predictors of higher dietary carbon footprints, while higher intakes of legumes and fruits/vegetables were associated with lower footprints. Sensitivity analyses varying LCA emission factors by ± 20% altered absolute estimates but did not change the core findings: meat-dominant diets have substantially higher carbon footprints, and modest dietary shifts can yield meaningful reductions in emissions (Table 5).

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Table 5: Selected Regression Coefficients Predicting Dietary Carbon Footprint (Adjusted)

Predictors	β (kg Co_2-eq/day)	95% CI	p-Value
Red Meat (per 50 g/day)	0.44	0.35 to 0.53	<0.001
Dairy (per 100 g/day)	0.16	0.07 to 0.21	<0.001
Poultry (per 50 g/day)	0.09	0.03 to 0.15	0.010
Legumes (per 50 g/day)	−0.08	−0.14 to −0.02	0.004
Fruits and Vegetables (per 100 g/day)	−0.07	−0.13 to −0.03	0.001
Total Energy (per 500 kcal/day)	0.1	−0.02 to 0.21	0.090
Urban (vs Charsadda)	0.54	0.31 to 0.77	<0.001
Female (vs Male)	−0.05	−0.23 to 0.13	0.510

DISCUSSION

The presented research has also pointed out that the food choice among the university students in KP leads to serious environmental consequences, where meat diets have a carbon footprint approximately twice the size of non-meat diets. This fact proves the developing global agreement that red meat consumption is closely associated with rising greenhouse gas (GHG) emissions and environmental pollution [16, 17]. Corresponding trends are also seen in South Asian and local settings where red meat contributes disproportionately to overall dietary carbon footprints [13]. Importantly, even comparatively minor dietary modifications (e.g., a 15% decrease in calories obtained through meat and an increase in calories obtained through legumes) had considerable positive effects regarding the environment, according to a free-living population sample of a developed country. In this analysis, red meat contributed about 52% of total emissions, while the vegetarian diets and plant-forward diets contributed 14% [14, 17]. Dairy and cereals were also considered as secondary contributors, and vegetables and legumes, although low in carbon intensity per gram, played an important role in enhancing nutrient adequacy. Such results are consistent with the worldwide life-cycle assessment (LCA), indicating that vegetarian food is generally linked to reduced GHG emissions, land-use, and water demand than animal foods [18, 19]. Our findings indicate that the concept of sustainability-based national dietary guidelines may have two advantages, whereby, in addition to cutting down on environmental footprints, mitigation of habitual micronutrient deficiencies may be experienced in Pakistan [13, 20]. Legume and pulse promotion and seasonal vegetable promotion could thus be an effective policy measure to enhance environmental performance and food security at the same time. Specifically, social awareness on sustainable dieting in schools and universities has the potential to develop sustainable dieting among young adults, a cohort in the early stages of developing a life-long food habit [16, 21].

This study has certain limitations, including its cross-sectional design, which restricts causal inference, and reliance on self-reported FFQ data that may be subject to recall bias. The use of adjusted life-cycle assessment emission factors, although contextually adapted, may not fully capture regional production variability. Future research should employ longitudinal designs, incorporate direct environmental indicators such as water use and biodiversity impact, and expand sampling to multiple provinces. Developing nationally representative sustainable diet models could further inform climate-responsive dietary guidelines in Pakistan.

CONCLUSIONS

Minor changes in food consumption, like cutting down the number of red meat meals and increasing the number of vegetarian meals, would significantly decrease the amount of carbon food-related emissions without posing any negative health impact on the population and contributing to maintaining the Pakistani climate. The above analysis needs to be applied to broader groups in the future, and other environmental variables, such as utilization of water and biodiversity effects, should also be included so as to develop a more comprehensive model of planning sustainable dietary patterns.

Authors Contribution

Conceptualization: SB[1]
Methodology: SB[2]
Formal analysis: FE
Writing and Drafting: FE
Review and Editing: FE, SB[1], SB[2]

All authors approved the final manuscript and take responsibility for the integrity of the work.

Conflicts of Interest

All the authors declare no conflict of interest.

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Voices Through Images: A Photo-Voice Study on Nutrition among Patients with Rheumatoid Arthritis, Cancer, and Type 2 Diabetes

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ABSTRACT

As a participatory visual approach, photovoice is increasingly becoming a powerful tool that is used to document and reflect lived experience regarding health and nutrition. **Objectives:** To determine the leadership nutrition-related issues, hindrances, and enablers that affect the eating habits of people with cancer, rheumatoid arthritis (RA), and type 2 diabetes in clinical and social settings. **Methods:** Photovoice was adopted to explore the diet-related issues in cancer, rheumatoid arthritis (RA), and diabetes patients, three chronic illnesses where dietary control is a critical part of the remedy. Patients were asked to capture some images of the food environment that they encounter daily, obstacles to obtaining appropriate nutrition, and encounters with nutritional education within the clinical setting. **Results:** The analysis resulted in four themes: (1) structural constraints, such as cost and limited access to food; (2) poorly written or standardized dietary prescriptions, which were incompatible with clinical management; (3) perceived need to have formal support provided by registered dietitians; and (4) patient coping strategies which exhibited their resilience and adaptation in the context of financial and clinical restrictions. Patients emphasized the inconsistency of nutritional advice provided by non-experts and its contradictory nature. The level of the problems was demonstrated by photo-stories of naked shelves, inappropriate proportions of portions, and self-designed diets. **Conclusions:** The findings suggest that the role of registered dietitians in multidisciplinary team care should be considered crucial to ensure the maximum patient outcome and the minimization of the risks of disease complications.

INTRODUCTION

Some of the most demanding health problems affecting people worldwide are cancer, rheumatoid arthritis (RA), and diabetes, which result in significant morbidity, mortality, and economic costs (World Health Organization [1]. The issue of nutrition plays a major role in the prevention, treatment, and prognosis of these illnesses, and normally, patients are faced with dire barriers to the provision of appropriate and individualized dietary data [2]. Despite nutrition being well-known as an essential, healthcare systems do not tend to engage a registered dietitian to do their best, resulting in patients being left to depend on generic-based recommendations, misinformation, or advice by the non-specialist [3]. In the case of cancer patients, the cases of side effects of cancer treatment, such as nausea, anorexia, or changes in taste, which make it hard to consume nutrients, can be addressed [4]. Rheumatoid arthritis spares the impact of appetite and nutrition, and patients are mostly left alone to handle conflicting information on the topic of anti-inflammatory diets [5]. Similarly, dietary control is also an essential component of diabetes management; however, patients often cite socioeconomic factors, adherence to traditional diets, and a lack of personalized attention as reasons for nonadherence to medical nutrition therapy [6, 7]. These

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complications are augmented by endemic gaps in healthcare delivery systems in which nutrition often has a low priority in contrast to pharmacological care. Participatory research methods are becoming more popular in health sciences to understand such lived experiences in a better manner. Photovoice, a visual technique that allows participants to document and describe their lives using photography, which was first developed by Wang and Burris in 1997, is a qualitative technique that is a visual technique in itself. Compared to fixed surveys or interviews, photovoice enables patients to share their hardships, strategies, and hopes in a narrative and visual format, providing researchers and policymakers with valuable insights [8, 9]. Photovoice has been utilized in food insecurity and adolescent nutrition research, but little has been done on the use of photovoice in identifying the nutrition barrier in cancer, RA, and diabetes patients [10]. The proposed study attempts to fill this knowledge gap by applying photovoice to understand the perceptions and experiences of patients with such chronic conditions in relation to nutrition barriers. Engaging patients as co-researchers enables the research to not only record structural and systemic encumbrances but also record patient demands for more predictable, evidence-based nutrition services by registered dietitians. It is expected that the findings (visual and narrative) generated by photovoice will be useful in informing clinical practice and policy to make nutrition a key pillar of chronic disease management.

Although nutrition plays a central role in the management of cancer, rheumatoid arthritis, and type 2 diabetes, limited research in Pakistan has explored patients' lived experiences of dietary challenges within real-life clinical and socioeconomic contexts. Existing studies largely rely on quantitative surveys and clinical outcomes, overlooking patient-generated visual narratives that capture structural and systemic barriers. Moreover, the integration of registered dietitians into chronic disease care remains underexamined from the patient perspective. Therefore, there is a need for participatory research to document these experiences and identify context-specific gaps in nutrition care. This study aimed to explore the lived nutrition-related challenges, barriers, and facilitators influencing dietary practices among individuals with cancer, rheumatoid arthritis (RA), and type 2 diabetes within clinical and social contexts.

METHODS

This study employed a qualitative photovoice design within a community-based participatory research framework. Photovoice was selected because it enabled patients to capture and comment on their visually and narratively described nutrition-related challenges. The research targeted cancer, rheumatoid arthritis (RA), and diabetes patients, where diet is crucial in the management of the diseases, but is underemphasized from the patient's point of view. The study was done in the period between January 2023 and April 2023. All of the participants had signed informed consent in writing. The participants were informed that they had the right to withdraw any penalty-free. In order to prevent the problems of confidentiality, the participants were reminded that they were not to take pictures of identifiable objects. Anonymity was applied to images and stories at analysis and dissemination. The adult patients (at least 18 years old) who had a diagnosis of one of the three chronic diseases (cancer, RA, or diabetes) during six months or longer were the participants of the study. Outpatient clinics and patient support groups in the Pakistani urban hospitals were used for recruitment. The sampling was purposive in order to be representative in terms of gender, age groups, and socioeconomic status. Data saturation was used as the sample size guide, which implied the interviews would go on until there was no new code or idea. The point of saturation was reached when the last two interviews did not yield more thematic information, and this fact proved that there was sufficiency in the sample. Out of the 35 people who were recruited, 24 were able to follow through with all the procedures of the study and were included in the final analysis; the other 11 did not in the end and failed to provide photos/interview information. The participants underwent an orientation workshop where they were oriented to the objective of the research, ethical concerns, and basic photography skills. Each of the participants was provided with a disposable or online camera (or used their personal smartphone) to shoot photos that would present their experiences of life during the period of two weeks. The overall study period was about four months, consisting of a one-week introduction workshop, two weeks of photography period, and the following focus group discussions and individual interviews that will be undertaken during the next six weeks. The participants were asked to capture the picture that was representative of: 1) Barriers to nutrition (e.g., food insecurity, cost, side effects of treatment); 2) Experiences with diet advice or medication (e.g., the obvious, confusing, or contradictory instructions); 3) Impressions of professional nutrition care (e.g., what might professional nutrition care do). The semi-structured focus group discussions (FGDs) and individual interviews followed the photography session to give the participants context for their photos. What is really happening? What is the relevance of this to our lives? Why does this problem exist? What can we do about it? Audio-recorded all interviews and

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FGDs were transcribed verbatim and analyzed along with photographs prepared by participants. Visual materials, symbolism, and contextual meaning were analyzed in each photograph, given the description by the participants in interviews. The visual data and the images were coded in order to form recurrent image patterns and stories. The thematic analysis has been undertaken based on a six-step model by Braun and Clarke [12]. Two researchers coded all transcripts independently to increase the level of reliability. Cohen's kappa (0.81) was used as a measure of inter-coder agreement, which showed high agreement. Variations were debated during consensus sessions and sorted out by repeated improvements to the definitions of codes up to the stage of complete consensus. The codes were inductively generated, and the visual, as well as the narrative codes, were coded by two independent researchers to achieve reliability. Similarities and specific differences to a specific condition were assessed by comparing emerging themes among the three groups of patients (diabetes, RA, cancer). To compute the co-occurrence frequency of key codes between participant accounts in the semantic analysis of the semantic network, the NVivo matrix query function was used. Nodes (themes) were connected using co-appearance in a particular participant response or narrative, and the strength of any connection was a measure of how often this co-appeared. The semantic network analysis was used to display connections among codes. The qualitative network research best practices were used to set the minimum threshold of co-occurrence of ≥3 to exclude chance code pairs and to emphasize relevant conceptual associations. In the resulting network, node size represented code frequency, edge thickness reflected the strength of co-occurrence between codes, and modularity clustering was used to identify communities of closely related concepts. For ensuring credibility, member checking was done through the provision of early findings to participants for their feedback. Triangulation of data sources (interviews, FGDs, and photographs) guaranteed validity. Dependability was maintained through audit trails of the analytic process. Transferability was ensured through the provision of thick descriptions of contexts and experiences of the participants.

RESULTS

Demographic and clinical profile of the participants is presented. Mean age in the three groups was 48.0 years (SD = 8.5). Most of them were female (75%), and the proportion was highest in the RA group (87.5%). Most (62.5%) had education at or below secondary level, and two-thirds (66.7%) had a monthly household income of less than PKR 30,000, suggesting socioeconomic vulnerability. Illness duration was inconsistent among groups, with the longest average duration reported by RA patients (7.2 years), followed by diabetes (6.1 years) and cancer (3.5 years) (Table 1).

Table 1: Sociodemographic Characteristics of Patients (n=24)

Variables	Cancer (n=8), n (%)	RA (n=8), n (%)	Diabetes (n=8), n (%)	Total (n=24), n (%)
Age	48.5 (9.2%)	45.1 (8.7%)	50.4 (7.8%)	48.0 (8.5%)
Female	6 (75%)	7 (87.5%)	5 (62.5%)	18 (75%)
Male	2 (25%)	1 (12.5%)	3 (37.5%)	6 (25%)
Education (≤Secondary)	5 (62.5%)	6 (75%)	4 (50%)	15 (62.5%)
Monthly Income < PKR 30k	6 (75%)	5 (62.5%)	5 (62.5%)	16 (66.7%)
Duration of Illness (Years)	3.5 (2.1%)	7.2 (3.4%)	6.1 (2.7%)	–

Four broad themes cut across all patient groups: barriers to nutrition, prescription issues with inappropriate or incomplete, lack of access to registered dietitians, and strategies for coping. Barriers caught affordability challenges, food insecurity, and physical constraints from illness. Prescription issues pointed out the incompatibility between medical guidance and realities on the ground. The absence of dietitian support arose prominently, as a majority of the participants depended on unqualified sources or general guidance. Coping strategies demonstrated resilience, where patients used such descriptions as dependence on family support, native remedies, or meal modification. Quotations given by the participants enhance the understanding of each theme (Table 2).

Table 2: Photovoice Data Thematic Analysis

Themes	Description	Illustrative Quote
Barriers to Nutrition	Treatment side effects, financial constraints, and limited food variety.	"Fresh fruits and vegetables are too expensive. I usually eat bread and lentils." (RA, female, 42)
Prescription Challenges	Unclear, vague, or impractical dietary instructions and advice.	"The doctor wrote 'avoid sugar' but didn't tell me what I could eat instead." (Diabetes, male, 50)
Lack of Dietitian Support	Absence of professional nutrition counseling, reliance on informal sources.	"In all my visits, I never met a dietitian." (RA, female, 39)
Coping Strategies	Family support, food substitutions, recipe modifications.	"I cannot afford meat every day, so I make lentil soup with extra vegetables." (Cancer, female, 47)

Findings show the interrelated themes that were derived from the photovoice study. Some central challenges were barriers to proper nutrition (e.g., affordability, access, and illness-related restrictions), improper or incomplete prescriptions, and inaccessibility to registered dietitians. These challenges intersected and supported each other, resulting in suboptimal eating habits. In spite of these limitations, patients explained coping mechanisms like

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family support, use of native remedies, and meal modification within financial constraints. The visual network also illustrates how these themes were connected and influenced patients' everyday nutrition experiences (Figure 1).

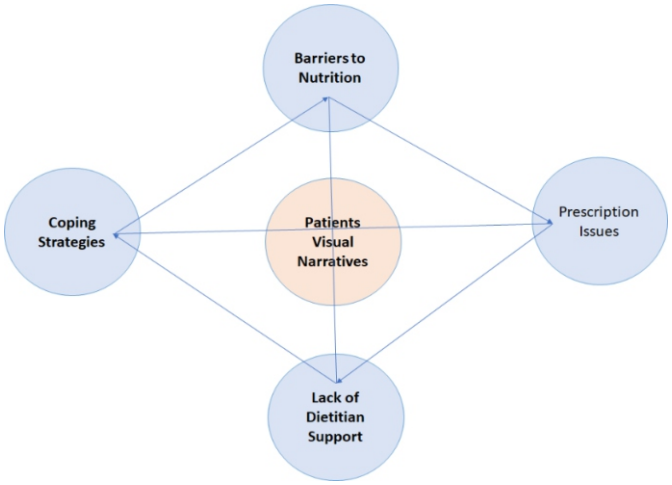

Figure 1: Thematic Network of Nutrition-Related Challenges and Coping Mechanisms among Cancer, Rheumatoid Arthritis, and Diabetes Patients(n=24)

Analysis of the photovoice stories and focus group discussions yielded four major themes: (1) Barriers to Optimal Nutrition, (2) Misdirected or Inadequate Prescription Practices, (3) Inadequate Access to Registered Dietitians, and (4) Patient Coping Strategies and Patient Advocacy. Theme 1: Obstacles to Ideal Nutrition: The patients have frequent exposure to limited and monotonous food, bare shelves, or inexpensive processed food. Such photos depicted economic hardships, adverse effects of treatment, and hunger. An illustration of this is a patient with cancer who said: "There are times when I can drink only tea and a biscuit; the chemotherapy makes everything taste of metal. One more diabetes patient photographed a shelf of white bread and sugar, and wrote: I understand that it is not good for me, but this is all I can afford most of the weeks. Theme 2: Improper or Lack of Proper Prescription Procedures: A few of the participants mentioned confusion about nutrition prescriptions that they had received at the hands of the general practitioners or non-specialist physicians. The scribbled notes and pictures of the generic supplements revealed a lack of customized dieting. One of the rheumatoid arthritis victims said that the doctor told him to eat more protein, but not how. I came out of the clinic even more confused. There were certain inconsistencies between what was written online and what had been prescribed: I was advised not to consume dairy, and another nurse appeared and mentioned that milk is fine. I don't know whom to trust." Theme 3: Inaccessibility to Registered Dietitians: Patients continued to make repeat photographs of empty diet offices in hospitals or referral slips with the term pending engraved on them. This imagery blankness highlighted structural deficits in nutrition. One of the patients with diabetes, in summary: I have never seen a dietitian in my hospital, only the doctor. Food is the major problem that I encounter. One of the participants said: They prescribed me insulin but did not tell me what to eat in relation to this. A dietician would have assisted in creating a difference. Theme 4: Resilience and Patient Advocacy: Despite the adversity, the patients were creative and flexible. Coping mechanisms were depicted in scenes of vegetable gardens, meals at the table with families, and self-help strategies of preparing meals. One cancer patient stated: When the hospital could not give me an opportunity, I formed a WhatsApp group consisting of other patients, and we share recipes we could actually afford. Similarly, an RA patient has described: I plant spinach in pots. It is cheap, fresh, and is something that I can do during bad pain days.

DISCUSSION

This photovoice research investigated patients with cancer, RA, and diabetes's lived experiences of nutritional concerns, and four superordinate themes were identified: barriers to proper nutrition, problems with inappropriately or inadequately prescribed treatments, inaccessibility of registered dietitians, and strategies to cope. The results built on existing research to present visual and narrative data from the patients themselves, noting that nutritional management in chronic disease was influenced as much by economic, social, and system limitations as by medical advice. Additionally, longer illness duration independently hindered adequate nutrition by contributing to financial strain, appetite loss, and fatigue that limited dietary adherence. Throughout all three groups of patients, barriers to proper nutrition were foremost. Respondents often captured images of bare fridges in kitchens or small servings, which they duplicated their comments on cost and lack of variety in food. These results were in line with other reports that food insecurity is very common among the chronically ill population whose dietary restrictions are worsened by the economic burden of disease [12]. Sixty-six-point seven percent of our sample had a monthly household income less than PKR 30,000 and this was directly correlated with the inability to adhere to dietary instructions. One of them stated that no matter what the doctor may recommend me to eat more fruits, how can I purchase them daily? These voices pointed out that nutritional recommendation that does not keep economic facts in consideration is always impractical [14]. Second, prescription issues emerged as one of the key challenges. Some patients also reported that diet instructions were either not provided in their check-up sessions or were not

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clear and as such. Unused prescription images with comments that the diet advice was only a leaflet, not specific, were indicative of dissatisfaction. This resonated with existing literature, which quotes that chronic patients are likely to be prescribed very specific or general nutritional plans, which leads to low adherence and misunderstanding [15]. The results stressed the necessity to offer dietary suggestions on a case-by-case basis and customized recommendations [16]. Third, the inefficiency of registered dietitians in the normal care was also mentioned in an acute way. Patients had noted dietary advice as the response of physicians or nurses, who confessed few facts on nutrition. One of the interviewees says that doctors consider medicine, however, no one informs me what to plan in the meals. This is in accordance with the results that incorporation of dietitians into the oncology, rheumatology and diabetes care teams enhances patient outcomes [17]. Insufficiency of professional nutrition education in the surrounding environment shows a failure in the system in respect of managing chronic illnesses [18]. Patients had innovative coping mechanisms in the presence of the barriers. There was resiliency in images of family-cooked meals, sharing in cooking, and traditional medicine. Some of them were found to modify recipes to suit their healthier budgets. Some employed peer recommendations or local community food practices. These processes were a reflection of outcomes in similar cases when patients cross structural and informational obstacles through the use of social networks and cultural information [19]. However, these interventions were normally insufficient to achieve optimal dietary goals. In general, the presented outcomes demonstrated the necessity of implementing a multi-dimensional solution to nutrition in patients with chronic illnesses. At the patient level, patient nutrition education should be both functional and culturally sensitive [20]. At the system level, it should be ensured that registered dietitians are included in interdisciplinary teams. At the policy level, subsidies or food stamps may help to reduce economic barriers. It is worth noting that the photovoice approach provided a voice to patients and developed the visual storytelling, which could have possibly become powerful tools for promoting a change in the health system. There were some limitations of this research. The size of the sample was limited and was in a single setting, so generalizability was an issue. This might have created bias in selection, as the patients may have chosen to highlight certain foods or difficulties using self-reported photographs and accounts. Nonetheless, the photovoice technique offered some advantage in narrating lived lives that would be readily missed in quantitative questionnaires.

This study has certain limitations, including a small sample size drawn from urban hospital settings, which may limit generalizability to rural populations. The reliance on participant-selected photographs and self-reported narratives may also introduce selection and recall bias. Future research should include larger, more diverse samples across multiple regions and healthcare settings. Longitudinal and mixed-method approaches could further evaluate the impact of integrating registered dietitians into multidisciplinary teams and assess measurable improvements in nutritional and clinical outcomes.

CONCLUSIONS

In this research, it has been concluded that cancer patients, RA patients, and diabetic patients all had overlapping nutritional problems, which were related to socioeconomic, medical, and systemic restrictions. The solutions to these difficulties will include the integration of professional nutrition counseling into routine care, improvement of prescription policies, and affordability of nutrition interventions, as well as their accessibility. With patient voices at the heart, future programs will be more focused on the lived experiences and aspirations of the hardest hit groups.

Authors' Contribution

Conceptualization: IAB
Methodology: IAB
Formal analysis: IAB, AM
Writing and drafting: IAB, AM
Review and editing: IAB, AM

All authors approved the final manuscript and take responsibility for the integrity of the work.

Conflicts of Interest

All the authors declare no conflict of interest.

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Association between Maternal Postpartum Vitamin A Supplementation and Child Vitamin A Vaccination Coverage in Pakistan: Evidence from the PDHS 2017–18

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ABSTRACT

Maternal postpartum vitamin A supplement is a necessary intervention for maternal and child health. It has not been properly investigated regarding its impact on the Pakistani child vaccination coverage of vitamin A. **Objectives:** To evaluate the maternal postpartum vitamin A supplementation and child vitamin A vaccination coverage, that is, first-dose, second-dose, and full two-dose coverage. **Methods:** A survey was carried out among 200 mother-child pairs. Information on the maternal postpartum vitamin A supplementation and the child's vitamin A vaccination status was available. Associations and the effect size were assessed on descriptive statistics, Spearman correlation, and ordinal and binary logistic regression. **Results:** There was a strong correlation between the child vaccination and the presence of maternal postpartum vitamin A supplementation. The children of supplemented mothers were much more likely to take 100% of vitamin A (100% vs. 51.3; OR = 84.39, p=0.001) and 25.9% vs. 0.8% coverage (OR = 41.30, p=0.001). The second dose effect was less but significant (odds ratio, OR = 2.64, p=0.004). In Spearman correlation, there was a strong positive correlation with the first dose (r = 0.733, p=0.001) and a weak positive correlation with the second dose (r =.204, p=0.004). **Conclusions:** Maternal postpartum vaccination with vitamin A is a key factor that determines child vaccination with vitamin A, especially the first dose. In Pakistan, continuity of postnatal care, the combination of supplementation with routine immunization, and better caregiver counseling are needed to realize full vaccination and the best preventive health care outcomes of children.

INTRODUCTION

Vitamin A deficiency (VAD) is a large-scale health problem in the low- and middle-income nations, especially in South Asia, where maternal and child under-nutrition has been playing a significant role in the prevalence of preventable morbidity and mortality [1]. Vitamin A is an important immunomodulator for vision, epithelial integrity, and child growth, and a lack of vitamin A in early life was linked to the predisposition to infections and an increased risk of death. To deal with this overload, massive vitamin A supplementation (VAS) programs of children aged 6 to 59 months have been extensively implemented, and in many countries, they are combined with regular immunization services [1]. Moreover, the past practice recommends that mothers should be provided with postpartum vitamin A supplementation to maximize the breast milk retinol concentration to improve infant vitamin A levels in the early postnatal period [2]. Although decades of use have passed, the coverage on both maternal postpartum vitamin A

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supplementation and child vitamin A vaccination coverage has not been homogeneous in most settings [3]. Recently, indicative evidence in the global sphere has shown that although there has been moderate adherence to the use of antenatal interventions like iron folic acid supplements, postnatal micronutrient interventions have often been overlooked and thus have gaps in the continuum of maternal and child care [4]. The research done in South Asia and sub-Saharan Africa indicates that maternal use of postnatal services is a robust predictor of child preventive health care use, such as immunization and intake of micronutrients [3, 5]. Nevertheless, there is little empirical data that links maternal postpartum-vitamin A supplementation to child-vitamin A vaccination. The current systematic reviews have cast doubt on the life-saving benefits of regular postpartum intake of vitamin A on infants, so the World Health Organization updated its guidelines. However, maternal supplementation is still being used in a number of countries where vitamin A deficiency is still endemic, mainly because it may have an indirect positive impact on maternal nutrition, breastfeeding quality, and healthcare compliance [4]. Notably, new research documents that maternal health practices throughout the postnatal life can be a proxy of the healthcare access, awareness, and adherence, which are critical predictors of child immunization uptake [2]. Findings on population-level surveys indicate that inequity in child vitamin A coverage remains high, and most cases of underdosing are often witnessed regardless of the first visit to health services. Literacies in opportunities to pursue follow-up, continuity of postnatal care, and the lack of awareness among the caregivers are recurrently named as obstacles in attaining complete coverage [1]. Although a number of studies have identified socioeconomic and healthcare determinants of child VAS uptake, few have used maternal-child association, specifically, whether maternal vitamin A postpartum vitamin A supplementation is associated with better child VAS vaccine uptake and completion. Even though in Pakistan, there is a national strategy to enhance maternal and child nutrition, the country has a dual burden of micronutrient deficiencies, as well as suboptimal immunization coverage. The information obtained in the Pakistan Demographic and Health Survey (PDHS) 201718 survey is a good chance to study these interrelationships on a population level [6]. Information on the relevance of maternal postpartum vitamin A supplementation to greater child vitamin A vaccination coverage could provide information about improving integrated maternal-child health prevention.

Despite the implementation of vitamin A supplementation programs in Pakistan, substantial gaps persist in both maternal postpartum supplementation and child vitamin A vaccination coverage. While previous research has explored socioeconomic and healthcare determinants of child immunization uptake, limited empirical evidence has examined the maternal–child linkage, particularly whether maternal postpartum vitamin A supplementation influences child vitamin A vaccination completion. Moreover, national-level analyses using PDHS data to assess this specific association remain scarce. Addressing this gap is essential to understand whether strengthening maternal postnatal nutrition interventions can improve child preventive health service utilization. This study aimed to evaluate the relationship between the use of prenatal vitamin A supplements among mothers and the coverage of child vitamin A vaccines in Pakistan based on the PDHS 2017-18. In addition, to investigate associations between the first dose coverage, second dose coverage, and full coverage of vitamin A vaccination, and hence provide evidence on the effectiveness of postnatal mother nutrition interventions in child preventive health care use.

METHODS

The research design adopted in this study was a retrospective cross-sectional design with secondary data from the Pakistan Demographic and Health Survey (PDHS) 2017-18. The analysis of data was done between April and May 2025. a national representative cross-sectional survey of households, carried out by the National Institute of Population Studies (NIPS) in partnership with ICF International [6]. All participants willingly gave their consent to data collection with the help of the original PDHS survey [7]. The stratified two-stage cluster sampling design was employed by the PDHS to gather data on maternal, child, and household health indicators in all the provinces and regions in Pakistan. The survey gives comparable and standardized information on maternal nutrition, child health, and immunization practices [7]. This sample study included women of reproductive age (1549 years) who had delivered within the reference period and had at least one child who was eligible to receive vitamin A supplements. In this analysis, a subsample of 200 mother-child pairs that had complete information on mother postpartum vitamin A supplementation and child vitamin A vaccination status was used. The records that lacked or contained inconsistent information regarding the key variables were not used in the analysis. Maternal postpartum vitamin A supplementation was used as the primary independent variable, which was measured as No or Yes based on self-reported vitamin A intake after childbirth. Child vitamin A coverage was identified as the main outcome variables, which included receiving the first

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dose, receiving the second dose, and overall coverage of vitamin A (receiving both doses). Child vitamin A status was categorized into ordinal levels to reflect none, partial, or complete coverage. Additional child health variables, including recent iron supplementation and deforming status, were included for descriptive purposes. Descriptive statistics were used to summarize maternal and child characteristics, reported as frequencies and percentages. Pearson's chi-square test was applied to examine associations between maternal postpartum vitamin A supplementation and categorical child vitamin A outcomes. Effect sizes were assessed using Cramer's V to evaluate the strength of associations. Spearman's rank correlation coefficient (ρ) was used to assess the relationship between maternal vitamin A supplementation and ordinal child vitamin A coverage variables due to their non-parametric nature. To further quantify the association between maternal postpartum vitamin A supplementation and child vitamin A outcomes, ordinal logistic regression models were fitted for child first-dose and second-dose vitamin A status. Odds ratios (ORs) with 95% confidence intervals (CIs) were reported to estimate the likelihood of higher child vitamin A coverage categories among mothers who received postpartum supplementation. In addition, binary logistic regression was performed to assess the association between maternal supplementation and complete child vitamin A coverage (both doses received). Model fit was evaluated using chi-square statistics and Nagelkerke's R^2. The sample of 200 mother-child pairs included in this analysis was determined to be sufficient based on a sample size calculation for a binary logistic regression. Using an anticipated odds ratio of 3.0, a significance level (alpha) of 0.050, a statistical power of 80%, and an estimated exposure prevalence (maternal supplementation) of 40% from prior data, the minimum required sample size was calculated to be 180 pairs. Our final sample of 200 pairs exceeds this threshold, providing adequate power to detect significant associations. All statistical analyses were conducted using standard statistical software, with significance set at $p<0.050$. As this study involved secondary analysis of anonymized, publicly available PDHS data, no additional ethical approval was required.

RESULTS

These results indicate that there is a major maternal-child health service gap: whereas the adoption of iron supplementation in pregnancy is relatively high, postpartum vitamin A coverage is relatively low, and is associated with poor child immunization coverage rates. This non-connection implies that the continuity of postnatal care might be a successful intervention to improve the overall vitamin A compliance in the maternal-child dyad, which is currently vastly under-covered (Table 1).

Table 1: Descriptive Statistics of Study Variables (n=200)

Variable	n (%)
Currently Pregnant	
No	160 (80.0%)
Yes	40 (20.0%)
Iron During Pregnancy	
No	77 (38.5%)
Yes	123 (61.5%)
Maternal Postpartum Vitamin A	
No	119 (59.5%)
Yes	81 (40.5%)
Child Vitamin A – First Dose	
No	58 (29.0%)
Yes	82 (41.0%)
Don't know	60 (30.0%)
Child Vitamin A – Second Dose	
No	40 (20.0%)
Yes	137 (68.5%)
Don't know	23 (11.5%)
Child Iron (Last 7 Days)	
No	80 (40.0%)
Yes	120 (60.0%)
Child Dewormed (Last 6 Months)	
No	78 (39.0%)
Yes	122 (61.0%)
Child Vitamin A Coverage Pattern	
Both doses	61 (30.5%)
First dose only	21 (10.5%)
Second dose only	76 (38.0%)
Other/unknown	42 (21.0%)

The analysis provides a statistically significant and strong relationship between the maternal postpartum intake of vitamin A supplements and child vitamin A immunization results. Mothers who underwent supplementation had a much higher probability of giving birth to a child who had been given that initial dose of vitamin A (100 percent vs. 51.3 percent) and had full coverage (25.9 percent vs. 0.8 percent). The large effect sizes also support the significance of an association between maternal preventive health services and childhood preventive health services (Table 2).

Table 2: Association Between Maternal Postpartum Vitamin A and Child Vitamin A Coverage

Child Vitamin A Outcome	Mothers Receiving Vitamin A (n=81)	Mothers Not Receiving Vitamin A (n=119)	χ^2 (df)	p-value	Effect Size (Cramer's V)
Vitamin A1 Coverage	81/81 (100%)	61/119 (51.3%)	2 (112.39)	<0.001	0.750
Vitamin A2 Coverage	64/81 (79.0%)	96/119 (80.7%)	2 (34.61)	<0.001	0.416

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Any Vitamin A Coverage	81/81 (100%)	119/119 (100%)	1 (7.22)	0.007	–
Complete Coverage (Both Doses)	21/81 (25.9%)	1/119 (0.8%)	1 (28.47)	<0.001	–

Maternal Vitamin A supplementation appears to have a limited direct influence on ensuring children receive their second Vitamin A dose. Other healthcare access, follow-up systems, or community-level factors may be more critical for completing the two-dose Vitamin A schedule (Table 3).

Table 3: Correlation between Maternal Postpartum Vitamin A Supplementation and Child Vitamin A Coverage

Variable 1	Variable 2	Correlation Coefficient (ρ)	95% CI	*p* -value
Maternal Vitamin A	Child Vitamin A1 (Dose 1)	.733	[0.67, 0.79]	<0 .001
Maternal Vitamin A	Child Vitamin A2 (Dose 2)	.204	[0.07, 0.34]	0.004

Note. Spearman's ρ was used due to the ordinal nature of the child's Vitamin A variables. Maternal Vitamin A coded as 0 = No, 1 = Yes; Child Vitamin A coded as 0 = None, 1 = Partial, 2 = Complete

Mothers who received postpartum Vitamin A supplementation were **84 times more likely** to have children with better first-dose Vitamin A coverage, showing an extremely strong association. For the second dose, the effect was much smaller but still significant. Mothers who received supplementation were **2.6 times more likely** to have children with better second-dose coverage (Table 4).

Table 4: Ordinal Logistic Regression: Predicting Child Vitamin A Status from Maternal Vitamin A Supplementation

Outcome Variables	Predictor	Coefficient	Odds Ratio (OR)	95% CI for OR	*p* -value
Child Vitamin A1	Maternal Vitamin A	4.435	84.39	[31.51, 296.19]	<0.001
Child Vitamin A2	Maternal Vitamin A	0.969	2.64	[1.39, 5.15]	-0.004

Children whose mothers received postpartum Vitamin A were **41 times more likely** to receive both Vitamin A doses, showing a very strong link to complete child coverage (Table 5).

Table 5: Binary Logistic Regression: Predicting Complete Child Vitamin A Coverage from Maternal Vitamin A Supplementation

Outcome Variables	Predictor	Odds Ratio (OR)	95% CI for OR	*p* -value
Complete Child Vitamin A Coverage	Maternal Vitamin A	41.30	[8.33, 749.26]	<0.001

Note. Complete coverage is defined as Child_VitaminA1 = 2 AND Child_VitaminA2 = 2. Model $\chi^2(1) = 28.47$, *p* <0.001, Nagelkerke $R^2 = 0.45$.

There is a strong relationship between the mother and child vitamin A. The comparative analysis of the maternal and child Vit-A relationship (Figure 1).

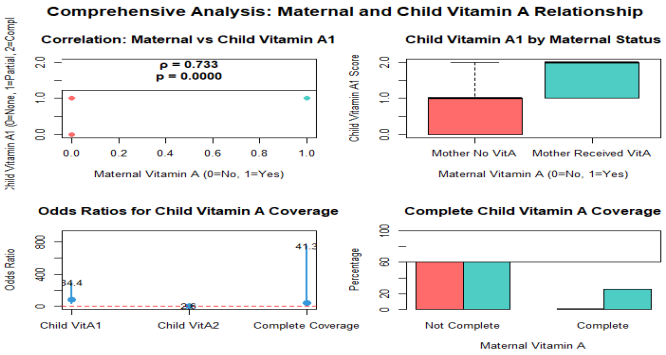

Figure 1: Comparative Analysis of Maternal and Child Vit-A Relationship

DISCUSSION

The present study demonstrates a strong and statistically significant association between maternal postpartum vitamin A supplementation and child vitamin A vaccination outcomes in Pakistan. Mothers who were given vitamin A during the postpartum period had a significantly higher likelihood of giving birth to children who had been given the initial dose of vitamin A and full coverage with the two doses. These results demonstrate the importance of postnatal maternal health interventions as an important point of entry to enhance child preventive care use, especially in such a country as Pakistan, where continuity of care is still disjointed. The fact that the correlation between maternal postpartum vitamin A supplementation and child first-dose coverage is very high (OR=84.39) is corroborated by and adds significantly to current literature. It supports the hypothesis that maternal, postnatal health service interaction is a strong facilitator of early child immunization practices [7, 8]. This is known as the gateway effect; research in the same low and middle-income country setting has repeatedly found that maternal linkage to the healthcare system in the postnatal stage is a robust predictor of the acceptance of first child health services, including vaccinations and micronutrient supplementation [9, 10]. It was observed that almost all children of supplemented mothers received the first dose, indicating that the very process of supplementation is an opportunity to contact the health system, and the maternal health-seeking behavior is seized immediately and used on behalf of the child [11]. The key and policy-relevant implication of this analysis is the significant difference between the impact of the first dose and the second dose of vitamin A coverage. Although there was an overwhelming association between maternal supplementation and first-dose uptake, the effect of maternal supplementation on second-dose coverage was significantly smaller (OR=2.64). This trend is not exclusive to vitamin A but indicates a greater problem in child health programming, the so-called drop-off in coverage between

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the first and subsequent health contacts [12]. This gap is described by previous studies as a result of systemic issues, such as the ineffectiveness of postnatal follow-up systems, the geographical problems faced by individuals caring for infants, and the ineffectiveness of reminder systems, instead of the absence of intentions initially [10, 13]. The lesser correlation of the second dose is a strong indication that maternal postpartum supplementation, though superior in the initiation of care, cannot be efficient alone in ensuring schedule adherence. It highlights the importance of overshadowing such interventions at the individual level with enhanced mechanisms of the health system aimed at fostering retention and follow-up [14]. The observation that children born to supplemented mothers had more than 40 times the likelihood of full vitamin A coverage also supports the conclusion that the intervention of maternal postnatal nutrition is a strong proxy of greater healthcare access, maternal agency, and health literacy [7, 15]. Such an association probably indicates a group of favorable conditions, such as increased maternal education, increased household wealth, improved geographic access to facilities, and enhanced trust in health services that allow the maternal supplementation uptake as well as regular vaccination of children [16]. The recent multi-country study, in particular, had shown that institutional delivery and timely postnatal care (both are close correlates of maternal supplementation) were strongly related to increased chances of full childhood vaccination [13]. This point of view changes the emphasis on the direct biological connection to the interpretation of maternal interventions as parts of the integrated service delivery platforms. Specifically, the data provided by us are descriptive; therefore, the reported iron supplementation during pregnancy is 61.5%, and postpartum vitamin A is 40.5%. This is a steep drop in national and regional reports that show that antenatal services are always more covered compared to postnatal interventions, mainly because of the reduction in healthcare contact in the postpartum period [17, 18]. This is a missed opportunity and a critical antenatal- postnatal connection. It postulates that the maternal-child health programs might not be successful in terms of bridging this care continuum and thus restricting an important time frame to encourage a full immunization and supplementation schedule of the child [8, 14]. Findings of this study also add a touch to the current debate on whether postpartum vitamin A supplementation is programmatic or not. Although the recent WHO guidelines have redirected attention to the fact that their suggestion was to decrease infant mortality, our results indicate that in endemic VAD regions such as Pakistan, infant maternal supplementation can still have considerable indirect worth by enhancing the maternal-child health services connection and serving as an incentive to alleviate child preventive health outcomes [19, 20]. This favors new views that highlight the non-textual advantages of maternal interventions, behavioral, service-delivery, and program-synergy, in contrast to the limited emphasis on the biological efficacy itself.

This study has several limitations, including its cross-sectional design, which limits causal inference, and reliance on self-reported PDHS data that may be subject to recall bias. The relatively small subsample of 200 mother–child pairs may also limit generalizability to the entire national population. Additionally, unmeasured confounding factors such as maternal education, socioeconomic status, and healthcare accessibility may influence the observed associations. Future research should employ larger nationally representative samples, longitudinal designs, and multivariable-adjusted models to better clarify causal pathways and inform integrated maternal–child health policy interventions in Pakistan.

CONCLUSIONS

This paper offers solid reasoning that maternal postpartum vitamin A supplementation has a significant correlation with child vitamin A vaccination coverage, especially the first dose, in Pakistan, which can be an important indicator of maternal health-seeking behavior and effective health system utilization. Programmatic initiatives to maximize child health outcomes must combine maternal postnatal nutrition with child immunization services, and intensify postnatal follow-up systems in ensuring that vaccination schedules are enforced to provide a strategic platform in sealing endemic gaps in maternal and child health service coverage.

Authors' Contribution

Conceptualization: IF
Methodology: MI, NS
Formal analysis: MI
Writing and Drafting: IF, AM, AGK, MI
Review and Editing: IF, MI, AM, AGK, MI, NS

All authors approved the final manuscript and take responsibility for the integrity of the work.

Conflicts of Interest

All the authors declare no conflict of interest.

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