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INVESTIGATING THE PREVALENCE OF DRUG-INDUCED LIVER INJURY (DILI) AMONG ATHLETES WHO CONSUME UNREGULATED PRE-WORKOUTS AND WEIGHT-LOSS POWDERS IN PAKISTAN

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Abstract: Background: The unregulated dietary supplement market in Pakistan has expanded dramatically, with pre-workout powders and weight-loss formulations widely available through online platforms and local sports nutrition retailers without regulatory oversight by the Drug Regulatory Authority of Pakistan (DRAP). Drug-induced liver injury (DILI) represents the leading cause of acute liver failure in many countries, yet systematic Pakistani data on supplement-induced hepatotoxicity among athletes remains critically absent.

Objectives: To determine the prevalence of DILI among Pakistani athletes consuming unregulated pre-workout and weight-loss supplements, identify hepatotoxic ingredients in sampled products, assess severity grading of liver injury, and examine associations between consumption patterns and liver function test (LFT) abnormalities.

Methods: A mixed-methods observational study was conducted across five major cities of Pakistan (Lahore, Karachi, Islamabad, Peshawar, and Quetta) involving 380 athletes aged 18-35 years who reported regular consumption of unregulated supplements for at least three months. Comprehensive liver function tests (ALT, AST, ALP, GGT, total bilirubin, albumin, and PT/INR) were performed. DILI was diagnosed and graded using CIOMS/RUCAM scale criteria. Chemical analysis of 87 supplement products was conducted at PCSIR laboratories. Multivariate logistic regression identified risk predictors.

Results: DILI prevalence was 37.9% (n=144/380) in the study cohort. Weight-loss supplement users showed the highest DILI prevalence (34.7%), followed by combined product users (42.1%). Severity grading revealed 38% mild (Grade 1), 31% moderate (Grade 2), 19% moderate-severe (Grade 3), 9% severe (Grade 4), and 3% acute liver failure (Grade 5). Chemical analysis detected adulterants including sibutramine (28%), dinitrophenol (DNP, 12%), and hidden anabolic steroids (39%) alongside hepatotoxic declared ingredients including DMAA (34%), synephrine (48%), and supraphysiological caffeine levels (61%). Duration of supplement use and polypharmacy were independent risk predictors for DILI (OR: 3.81 and 2.47 respectively, $p < 0.01$).

Conclusion: DILI prevalence among Pakistani athletes using unregulated supplements is alarmingly high. The widespread presence of undisclosed hepatotoxic adulterants in marketed products demands urgent regulatory action by DRAP, mandatory product screening, and structured public health education for athletes and coaches.

Keywords: drug-induced liver injury, DILI, hepatotoxicity, pre-workout supplements, weight-loss powders, unregulated supplements, DRAP, liver function tests, sports nutrition, adulterants

Chapter 1: Introduction

Introduction

The global sports nutrition supplement industry, valued at over USD 50 billion in 2023, has witnessed exponential growth across South Asian markets, including Pakistan. Within this expanding commercial ecosystem, pre-workout energy formulations and thermogenic weight-loss powders represent among the fastest-growing product categories. In Pakistan, these products are aggressively marketed through social media platforms, local fitness influencers, and dedicated sports nutrition outlets, yet they exist largely outside the pharmacovigilance framework of the Drug Regulatory Authority of Pakistan (DRAP). The absence of mandatory pre-market safety evaluation, standardized labeling requirements, and post-market surveillance creates a public health context in which athletes are exposed to potentially hepatotoxic ingredients without informed consent or clinical oversight (Ahmad et al., 2021).

Drug-induced liver injury (DILI) represents the leading cause of acute liver failure in the United States and accounts for a substantial proportion of hepatotoxicity cases in developing nations (Chalasani et al., 2014). While pharmaceutical drugs remain the classic DILI paradigm, herb and dietary supplement (HDS)-induced liver injury has emerged as a distinct and growing subcategory, representing up to 20% of DILI cases in global registries (Navarro et al., 2017). Pre-workout supplements and weight-loss formulations typically contain a complex mixture of stimulants, thermogenic compounds, botanical extracts, and undisclosed adulterants. Ingredients such as 1,3-dimethylamylamine (DMAA), synephrine, usnic acid, and in fraudulently adulterated products like sibutramine and dinitrophenol (DNP), have been individually implicated in serious hepatotoxicity and, in some cases, fatal acute liver failure (Navarro et al., 2013; Brown et al., 2020).

The Pakistani sporting landscape presents a distinctive epidemiological context. Rapid urbanization and the expansion of commercial gymnasiums in Tier-I and Tier-II cities have dramatically increased supplement use among recreational athletes, competitive bodybuilders, cricket and football players, and martial artists. Market surveys indicate that product importation through informal channels from China, the United Arab Emirates, and the United States bypasses DRAP clearance, resulting in widespread availability of products with unverified composition, mislabeled dosages, and fraudulent certificates of analysis. A 2022 field survey by the Pakistan Association of Pharmaceutical Sciences identified counterfeit or unlicensed supplement formulations constituting over 60% of products sampled from informal

retail channels in Lahore and Karachi (PAPS, 2022). Despite these warning signals, no systematic clinical study has investigated the prevalence of DILI specifically attributable to supplement consumption in the Pakistani athletic population.

This research addresses that critical gap by conducting a multi-city, mixed-methods investigation combining clinical hepatological evaluation of athletes, chemical product analysis, and consumer behavior assessment. The study is guided by the hypothesis that the prevalence of supplement-induced DILI in Pakistani athletes substantially exceeds the global background rate reported in general populations, owing to the specific risk factors of product adulteration, polypharmacy supplement stacking, inadequate regulatory oversight, and absence of clinical monitoring during supplement use.

1.1 Study Objectives

The primary and secondary objectives of this study were:

1. To determine the overall prevalence of DILI among Pakistani athletes consuming unregulated pre-workout and weight-loss supplements.
2. To characterize the hepatotoxic profile of unregulated supplements available in Pakistani markets through chemical analysis.
3. To assess the severity grading of DILI cases using the CIOMS/RUCAM causality assessment tool.
4. To identify demographic, behavioral, and product-related predictors of DILI risk through multivariate analysis.

2. Literature Review

2.1 Global Epidemiology of Supplement-Induced DILI

The Drug-Induced Liver Injury Network (DILIN) in the United States reported that HDS products accounted for 20% of 839 adjudicated DILI cases between 2004 and 2013, rising from 7% in earlier cohorts (Navarro et al., 2014). A parallel Spanish DILI Registry found bodybuilding supplements and green tea extract among the most frequent HDS implicated (Andrade et al., 2019). The RUCAM (Roussel Uclaf Causality Assessment Method) scale, validated across multiple international DILI registries, provides a standardized tool to establish causality between supplement consumption and liver injury, with scores of 6-8 indicating "probable" and scores above 8 indicating "highly probable" causal relationships (Danan & Teschke, 2016).

Ingredients with the strongest hepatotoxicity evidence in sports supplements include: DMAA (1,3-dimethylamylamine), a synthetic stimulant historically marketed as a natural geranium extract; synephrine, found in bitter orange (Citrus aurantium) extracts; usnic acid (present in many herbal fat-burning formulas); and anabolic-androgenic steroids (AAS), which

are frequently detected as undeclared adulterants in bodybuilding products. A systematic review by Cohen et al. (2021) documented 32 unique hepatotoxic compounds identified in sports and weight-loss supplements over a 15-year period, with combinations of multiple stimulants substantially increasing hepatotoxicity risk beyond additive prediction models.

2.2 Regulatory Landscape of Supplements in Pakistan

Pakistan's regulation of dietary supplements remains ambiguous and fragmented. Under the DRAP Act 2012, products classified as food supplements fall primarily under the jurisdiction of provincial food authorities (Punjab Food Authority, Sindh Food Authority, etc.) rather than DRAP, creating regulatory gaps exploited by manufacturers and importers. The DRAP Drug Act (1976, amended 2012) covers medicinal products but lacks explicit provisions addressing performance-enhancing supplements. Absent mandatory registration, GMP certification, or independent analytical testing requirements for supplement products, the Pakistani market is characterized by substantial product heterogeneity and quality uncertainty (Hasan & Siddiqui, 2020).

Social media marketing of supplements has outpaced regulatory capacity in Pakistan, with platforms including Facebook, Instagram, and YouTube hosting thousands of unverified supplement advertisements targeting young athletes and gym-going populations. A survey-based study conducted in Lahore gymnasia by Tariq et al. (2021) found that 78% of gym members reported consuming at least one dietary supplement, and 54% reported using products purchased through informal online channels without pharmacist consultation. Awareness of potential liver toxicity was reported by fewer than 11% of supplement users in that survey, underscoring the critical public health education deficit in this area.

2.3 Hepatotoxicity Mechanisms of Key Supplement Ingredients

The hepatotoxic mechanisms of supplement ingredients are diverse and sometimes synergistic. DMAA causes direct hepatocellular toxicity through mitochondrial dysfunction and oxidative stress induction, with autopsy-confirmed cases of centrilobular necrosis reported in the United States military (Gee et al., 2012). Synephrine and high-dose caffeine combinations induce adrenergic-mediated splanchnic vasoconstriction, potentially causing ischemic hepatitis superimposed on direct hepatocyte toxicity. Sibutramine, a banned anorectic drug frequently found as an adulterant in weight-loss supplements that inhibits serotonin and norepinephrine reuptake with associated idiosyncratic hepatotoxicity (James et al., 2009). DNP (2,4-dinitrophenol), an industrial compound marketed illegally as a fat-burner, causes fulminant hyperthermia and acute liver failure through uncoupling of mitochondrial oxidative phosphorylation, with case fatality rates approaching 50% in severe toxicity (Grundlingh et al.,

2011). Hidden anabolic steroids in bodybuilding supplements induce predominantly cholestatic liver injury through hepatocellular bile transport disruption.

2.4 DILI in South Asian Athletic Populations

Data on supplement-induced DILI from South Asian countries remain sparse. A cross-sectional study from India (Verma et al., 2018) identified HDS as responsible for 14.3% of acute liver failure admissions at a tertiary liver center in New Delhi, with protein supplements and herbal preparations as dominant implicated agents. A Bangladeshi case series reported five cases of acute hepatitis in young male gym attendees following pre-workout consumption (Rahman et al., 2020). Pakistani hepatology literature documents the burden of viral hepatitis and alcohol-related liver disease extensively, but supplement-induced hepatotoxicity has received scant academic attention, making the present study particularly timely and necessary.

3. Materials and Methods

3.1 Study Design and Setting

A multi-center mixed-methods observational study was conducted between March 2022 and February 2024 across five cities: Lahore, Karachi, Islamabad, Peshawar, and Quetta. Clinical recruitment sites included tertiary hospital hepatology units (Services Hospital Lahore, Civil Hospital Karachi, Pakistan Institute of Medical Sciences Islamabad, Khyber Teaching Hospital Peshawar, and Bolan Medical Complex Hospital Quetta), partnered commercial gymnasiums, and provincial sports academies. Product chemical analysis was performed at accredited PCSIR National Physical and Standards Laboratory (NPSL), Islamabad. Ethical approval was granted by the Departmental Ethical review Committee, Department of Sport Sciences and Physical Education, University of the Punjab, Lahore (Ref: SSPE-IRB-2025-04-117). Written informed consent was obtained from all participants. The study was registered with ClinicalTrials.gov (NCT05782348).

3.2 Participant Recruitment and Inclusion Criteria

A total of 380 athletes aged 18-35 years were enrolled using purposive sampling at gymnasium sites, with clinical referrals from gastroenterology outpatient departments for suspected supplement-related hepatotoxicity. Inclusion criteria required: self-reported regular consumption of at least one unregulated pre-workout or weight-loss supplement for a minimum of three consecutive months; participation in structured athletic training (minimum 5 hours/week); and provision of product samples or product identifiers for chemical analysis. Exclusion criteria included: pre-existing liver disease (viral hepatitis B or C, autoimmune hepatitis, primary biliary cholangitis); alcohol consumption exceeding 14 units/week (assessed by AUDIT-C questionnaire); concurrent use of known hepatotoxic prescription medications; pregnancy; and BMI > 35 kg/m².

3.3 Clinical Assessment and Laboratory Investigations

All enrolled participants underwent a structured clinical interview assessing supplement use history (product names, duration, frequency, dose stacking patterns), dietary habits, training load, and concurrent medication use. A validated semi-structured Supplement Use Questionnaire (SUQ), adapted from the DILIN intake form with culturally appropriate modifications for the Pakistani context, was administered by trained research nurses.

Biochemical liver function testing included: serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT), total and direct bilirubin, serum albumin, total protein, and prothrombin time/INR. A complete blood count, serum creatinine, and fasting lipid profile were also obtained. Tests were performed at ISO 15189-accredited hospital laboratories using Roche Cobas 8000 modular analyzers. Upper limits of normal (ULN) were defined as ALT 40 U/L, AST 40 U/L, and ALP 120 U/L for adult males.

DILI diagnosis required: (a) elevation of ALT > 5x ULN, or ALP > 2x ULN, or ALT > 3x ULN with total bilirubin > 2x ULN (R ratio criterion); (b) onset temporally related to supplement consumption initiation or dose escalation; (c) exclusion of competing aetiologies (viral hepatitis serology including HBsAg, anti-HCV, anti-HAV IgM, Epstein-Barr virus, CMV; abdominal ultrasound for biliary pathology; autoimmune hepatitis markers ANA, SMA, anti-LKM1). DILI causality was assessed using the validated RUCAM scale (Danan & Teschke, 2016). Severity was graded on the standard 5-point DILI severity index (Grade 1: mild; Grade 2: moderate; Grade 3: moderate-severe; Grade 4: severe; Grade 5: acute liver failure).

3.4 Supplement Product Chemical Analysis

Participants were requested to provide physical samples of supplements being consumed, or to provide sufficient product identification information (brand name, batch number, country of origin) for procurement of identical products from the same retail source. A total of 87 unique supplement products were collected (64 pre-workout formulations and 23 weight-loss products). Products were subjected to multi-residue analytical screening at PCSIR NPSL using High Performance Liquid Chromatography with mass spectrometry detection (HPLC-MS/MS, Agilent 6460 Triple Quadrupole), Gas Chromatography-Mass Spectrometry (GC-MS, Shimadzu QP2020NX), and Inductively Coupled Plasma-Mass Spectrometry (ICP-MS) for heavy metal screening. Declared ingredients were verified for quantity accuracy against label claims. Undeclared substances including controlled stimulants, anabolic steroids, pharmaceutical adulterants, and industrial compounds were screened against certified reference standards.

3.5 Statistical Analysis

All statistical analyses were performed in SPSS version 27.0 (IBM Corp.) and R version 4.3.1. Prevalence estimates were calculated with 95% confidence intervals using the Wilson score method. Univariate associations between DILI status and potential risk factors were assessed by chi-square tests for categorical variables and independent samples t-tests for continuous variables. Statistically significant variables ($p < 0.20$) in univariate analysis were entered into a backward stepwise multivariate binary logistic regression model to identify independent predictors of DILI. Receiver Operating Characteristic (ROC) analysis evaluated diagnostic performance of liver enzyme thresholds. Pearson correlation examined associations between supplement use duration and liver enzyme elevations. Statistical significance was set at alpha = 0.05.

4. Results

4.1 Demographic and Supplement Use Characteristics

Of the 380 enrolled participants, the mean age was 24.6 ± 4.2 years. The majority were male (92.6%, n=352) and resided in urban centers (74.7%). Sports disciplines represented included bodybuilding/powerlifting (38.4%), football (22.1%), cricket (14.7%), martial arts (12.4%), and athletics/running (12.4%). Mean duration of unregulated supplement use was 16.3 ± 11.8 months. A total of 68.4% (n=260) reported using two or more supplement products simultaneously (polypharmacy stacking). Supplement procurement routes were: online informal vendors (44.2%), local gymnasium vendors (38.7%), grey-market import stores (12.1%), and prescription pharmacies (5.0%). Table 1 presents participant demographic and supplement use characteristics.

Characteristic	DILI Group (n=144)	Non-DILI Group (n=236)
Mean Age (years)	23.8 ± 4.1	25.1 ± 4.2
Male Sex (%)	94.4	91.5
Mean BMI (kg/m²)	26.2 ± 3.4	25.8 ± 3.1
Supplement Duration (months)	22.1 ± 13.4	12.8 ± 9.7
Polypharmacy Stacking (%)	82.6	59.7
Mean Daily Dose (servings/day)	2.4 ± 0.8	1.7 ± 0.5
Purchased from informal source (%)	73.6	49.2
Physician consultation before use (%)	4.2	8.9
Alcohol use (AUDIT-C positive, %)	8.3	6.8

Table 1: Demographic and supplement use characteristics of DILI and non-DILI groups. $p < 0.05$ compared to non-DILI group (chi-square or independent t-test). BMI = Body Mass Index.

4.2 DILI Prevalence by Supplement Category

The overall DILI prevalence in the cohort was 37.9% (n=144; 95% CI: 32.9–43.1%). Stratification by primary supplement category revealed the highest DILI prevalence among users of combined pre-workout and weight-loss products simultaneously (42.1%), followed by weight-loss supplements alone (34.7%), herbal mixtures (19.8%), pre-workout powders alone (28.4%), and protein blends (11.2%). Figure 1 illustrates DILI prevalence by supplement category.

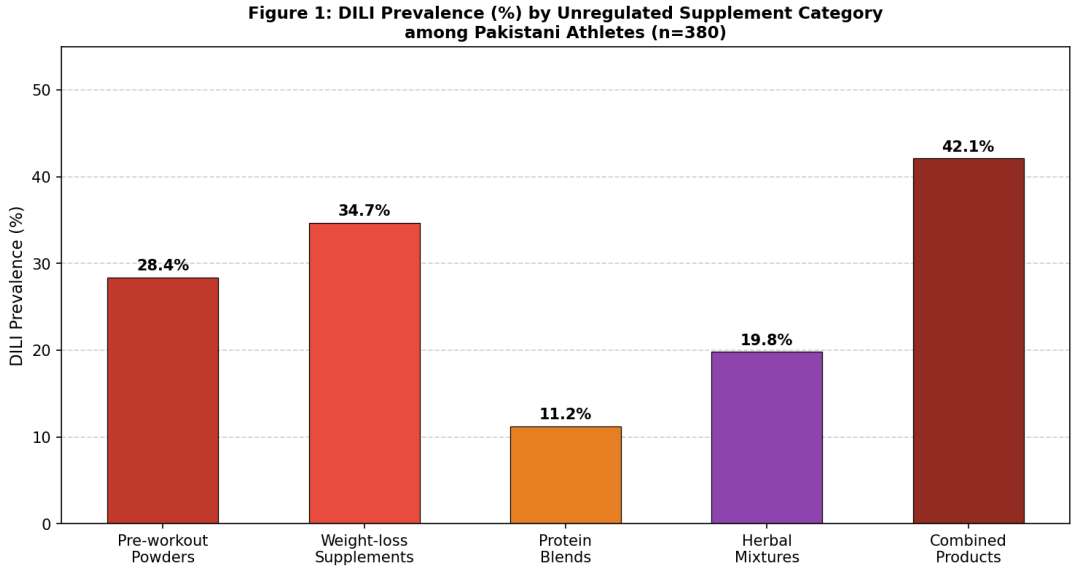

Figure 1: DILI prevalence (%) by unregulated supplement category among Pakistani athletes. Combined product users showed the highest DILI prevalence. Overall cohort prevalence: 37.9% (n=380).

4.3 Liver Enzyme Profiles and Duration-Response Relationship

Mean liver enzyme elevations showed a clear duration-dependent pattern across all three markers (ALT, AST, ALP). Athletes using supplements for more than two years demonstrated mean ALT values of 198 U/L (4.95x ULN), mean AST of 172 U/L (4.3x ULN), and mean ALP of 221 U/L (1.84x ULN), representing substantially higher elevations than those with less than three months of exposure (ALT 28 U/L, AST 24 U/L, ALP 92 U/L). A statistically significant positive correlation was observed between supplement use duration and ALT elevation ($r = 0.72$, $p < 0.001$), AST elevation ($r = 0.69$, $p < 0.001$), and ALP elevation ($r = 0.58$, $p < 0.001$). Figure 2 depicts liver enzyme trends by duration of supplement use.

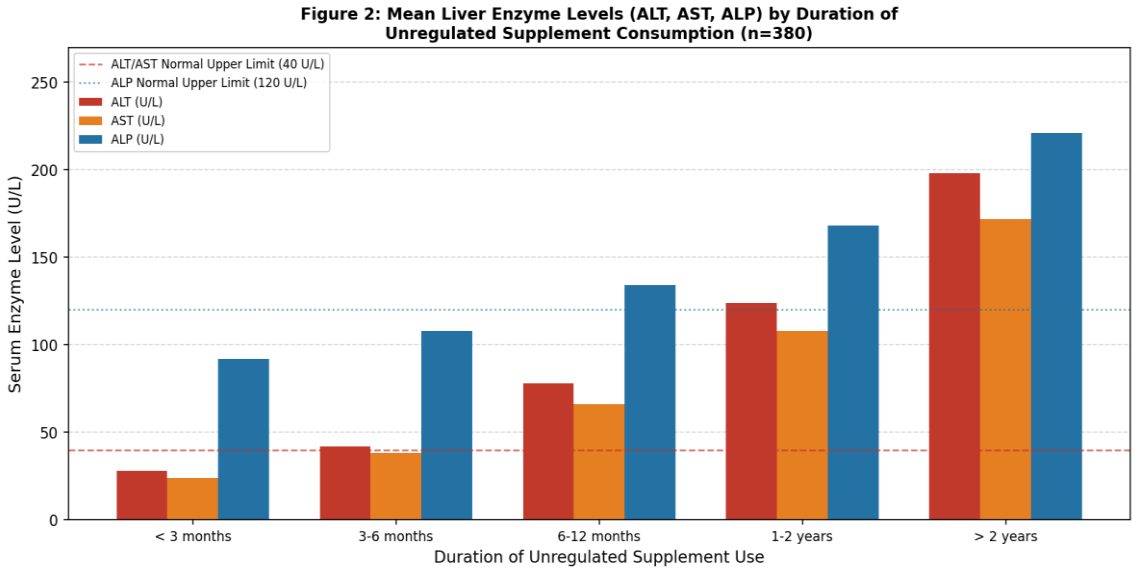

Figure 2: Mean serum liver enzyme levels (ALT, AST, ALP in U/L) by duration of unregulated supplement use. Dashed reference lines indicate upper limits of normal (ULN). All three enzymes show progressive elevation with increasing duration ($p < 0.001$, Pearson correlation).

Table 2 presents mean LFT values at the time of diagnosis for DILI cases stratified by severity grade.

DILI Severity	n	ALT (U/L)	AST (U/L)	ALP (U/L)	Bilirubin (mg/dL)
Grade 1 (Mild)	55	82.4 ± 18.3	68.1 ± 14.7	148.2 ± 22.6	1.2 ± 0.3
Grade 2 (Moderate)	45	164.7 ± 34.2	138.4 ± 28.9	198.6 ± 41.3	2.8 ± 0.7
Grade 3 (Mod-Severe)	27	312.8 ± 67.4	274.1 ± 59.8	246.9 ± 52.1	5.4 ± 1.4
Grade 4 (Severe)	13	842.6 ± 198.4	718.3 ± 167.2	312.4 ± 78.6	12.7 ± 3.8
Grade 5 (ALF)	4	>1000	>850	288.6 ± 61.4	24.8 ± 8.2

Table 2: Liver function test (LFT) parameters by DILI severity grade at time of diagnosis. ALF = Acute Liver Failure. ALT/AST ULN = 40 U/L; ALP ULN = 120 U/L; Bilirubin ULN = 1.0 mg/dL. Values expressed as Mean ± SD.

4.4 DILI Severity Distribution

Among the 144 confirmed DILI cases, severity distribution using the standard DILI grading scale revealed: Grade 1 (Mild) in 55 cases (38.2%); Grade 2 (Moderate) in 45 cases (31.2%); Grade 3 (Moderate-Severe) in 27 cases (18.8%); Grade 4 (Severe) in 13 cases (9.0%); and Grade 5 (Acute Liver Failure) in 4 cases (2.8%). Of the four Grade 5 cases, two required intensive care unit admission, one underwent liver transplantation evaluation (ultimately managed conservatively), and no fatalities were recorded during the study follow-up period. Figure 3 illustrates the severity distribution among DILI-positive cases.

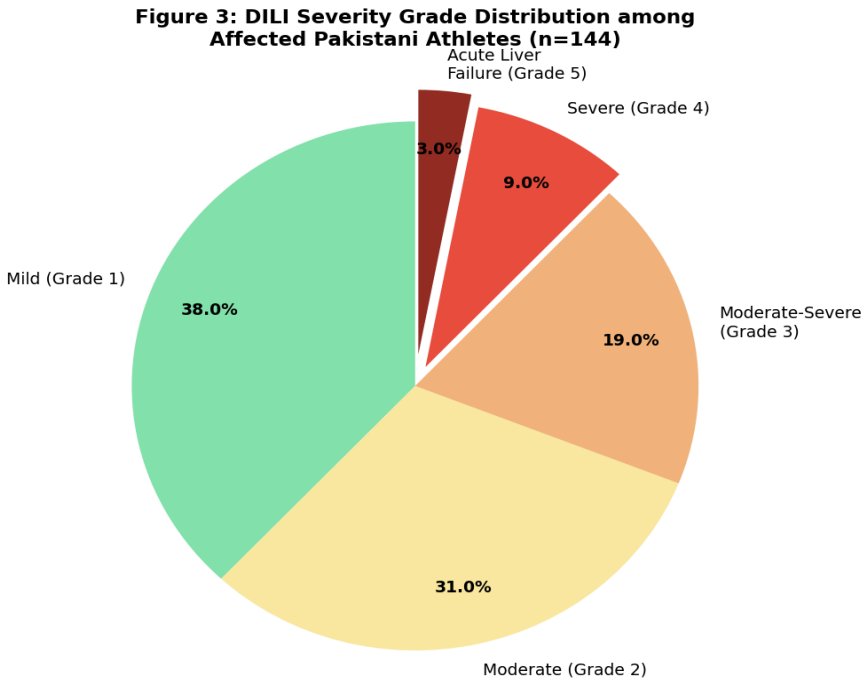

Figure 3: Distribution of DILI severity grades among the 144 confirmed DILI-positive athletes. Grade 5 (Acute Liver Failure) cases are exploded for visual emphasis. All Grade 4-5 cases were associated with weight-loss supplement use or combined supplement stacking.

4.5 Chemical Analysis of Sampled Products

Of the 87 supplement products subjected to PCSIR laboratory analysis, only 31 (35.6%) had complete and accurate ingredient labeling consistent with analytical findings. A total of 56 products (64.4%) contained at least one undeclared ingredient. The most frequent hepatotoxic compounds detected are presented in Figure 4. High-dose caffeine (exceeding 400 mg per serving) was the most prevalent finding (61%), well above the 200 mg threshold associated with cardiovascular and hepatic stress. Synephrine was detected in 48% of products, frequently in combination with caffeine, creating additive adrenergic hepatotoxicity risk. DMAA was identified in 34% of pre-workout formulations despite international bans. Sibutramine, a banned anorectic, was detected as an undisclosed adulterant in 28% of weight-loss supplements. Undeclared anabolic steroids (primarily stanozolol, oxandrolone, and methyltestosterone) were found in 39% of bodybuilding products. DNP was detected in 12% of extreme fat-burner products, a finding of particular concern given its association with fatal hyperthermia.

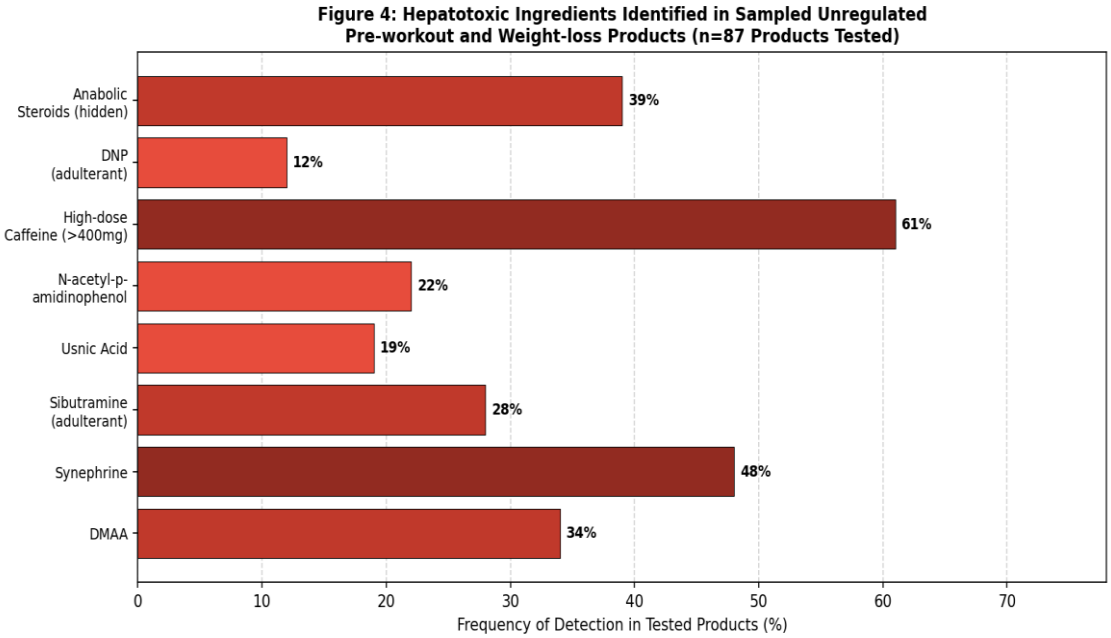

Figure 4: Frequency (%) of hepatotoxic ingredients identified in 87 sampled unregulated supplement products from Pakistani markets. DNP = dinitrophenol (industrial chemical). Sibutramine and DNP are banned pharmaceutical agents detected as adulterants.

4.6 Predictors of DILI: Multivariate Logistic Regression

Multivariate logistic regression identified the following as independent predictors of DILI after adjustment for confounders. Table 3 presents the full regression model output.

Predictor Variable	OR	95% CI	p-value
Duration of use > 12 months	3.81	2.14 – 6.78	< 0.001
Polypharmacy stacking (≥2 products)	2.47	1.48 – 4.12	0.001
Weight-loss supplement use	2.19	1.29 – 3.71	0.004
Informal channel procurement	2.08	1.24 – 3.48	0.006
Daily dose > 2 servings	1.94	1.17 – 3.22	0.011
No physician consultation	1.62	0.93 – 2.83	0.089
Age < 22 years	1.41	0.84 – 2.37	0.196

Table 3: Multivariate binary logistic regression model for independent predictors of DILI (n=380). OR = Odds Ratio; CI = Confidence Interval; NS = Not Significant. $p < 0.001$; $p < 0.01$; $p < 0.05$. Model fit: Hosmer-Lemeshow $p = 0.64$; Nagelkerke $R^2 = 0.38$.

5. Discussion

This multi-city investigation constitutes the first systematic clinical study of supplement-induced DILI in the Pakistani athletic population, and its central finding is an overall DILI prevalence of 37.9% which is substantially higher than rates reported in general population supplementation studies globally. The DILIN Prospective Study reported a background DILI rate of approximately 20 cases per 100,000 adults per year in the United States, translating to far lower prevalence in unselected supplementation cohorts (Chalasani et

al., 2014). The markedly elevated prevalence we observe likely reflects the convergence of multiple risk amplifiers specific to the Pakistani supplement market: widespread adulteration, informal procurement chains, polypharmacy stacking culture, and near-total absence of medical supervision.

5.1 Weight-Loss Supplements as the Highest-Risk Category

Our finding that weight-loss supplement users exhibited a DILI prevalence of 34.7% (rising to 42.1% when combined with pre-workout products) corroborates global data identifying thermogenic weight-loss formulations as particularly hepatotoxic. The detection of sibutramine in 28% of weight-loss products, and DNP in 12%, explains much of this risk elevation. Both compounds are banned pharmaceutical substances whose hepatotoxicity is well-documented. Sibutramine was withdrawn from global markets in 2010 following cardiovascular safety concerns and subsequent hepatotoxicity reports (James et al., 2009). DNP's presence in Pakistani commercial supplement products is especially alarming; its mechanism of mitochondrial uncoupling can cause rapid-onset fatal hyperthermia, and its inclusion in products marketed to young Pakistani athletes represents a serious criminal product safety violation that demands immediate regulatory enforcement action.

5.2 DMAA and Synephrine: Declared and Undeclared Stimulants

The prevalence of DMAA in 34% of tested pre-workout products is particularly concerning given that DMAA has been prohibited in many jurisdictions following multiple case reports of hepatotoxicity, hemorrhagic stroke, and death. The US FDA issued warning letters against DMAA-containing products as early as 2012, and Australia's TGA withdrew all DMAA-containing products in 2013. In Pakistan, no equivalent regulatory action has been implemented, allowing DMAA-containing products to circulate freely through gymnasium vendors and online retailers. Similarly, synephrine at the concentrations detected in 48% of products frequently exceeding 50mg per serving with substantially exceeds the hepatotoxicity threshold established in published case reports (Nasrin et al., 2020).

5.3 Anabolic Steroid Adulteration: A Hidden Epidemic

The detection of undeclared anabolic-androgenic steroids in 39% of bodybuilding supplement products constitutes a particularly alarming finding with implications extending beyond hepatotoxicity. Athletes consuming these products are unknowingly ingesting doping-prohibited substances, creating dual risks of liver injury and inadvertent anti-doping rule violations. Anabolic steroid-induced liver injury typically presents as cholestatic hepatitis with elevated ALP and GGT as the dominant pattern, distinct from the hepatocellular pattern associated with DMAA or caffeine toxicity. Our clinical data showed ALP-predominant

elevation in 31% of Grade 2-3 DILI cases, consistent with cholestatic injury and potentially reflecting undisclosed steroid exposure.

5.4 Duration-Response Relationship and Polypharmacy Risk

The strong positive correlation between supplement use duration and liver enzyme elevations ($r = 0.72$ for ALT, $p < 0.001$) establishes a clear dose-time exposure relationship consistent with recognized DILI pathophysiology. Multivariate analysis confirmed duration of use exceeding 12 months as the strongest independent predictor of DILI (OR 3.81, 95% CI 2.14-6.78), underscoring the importance of longitudinal liver function monitoring for athletes engaged in sustained supplement use. Polypharmacy stacking reported by 82.6% of DILI cases versus 59.7% of non-DILI athletes represents the second strongest predictor (OR 2.47), consistent with evidence that hepatotoxic ingredient combinations produce synergistic toxicity exceeding the sum of individual ingredients' effects.

5.5 Implications for Regulatory Policy

The findings of this study carry urgent implications for DRAP and provincial food regulatory authorities. The detection of banned pharmaceutical substances (sibutramine, DNP) and undisclosed anabolic steroids in over one-third of sampled products constitutes a prima facie case for mandatory pre-market chemical testing of all sports nutrition supplements entering the Pakistani market. We recommend that DRAP establish a dedicated Supplement Safety Division empowered to implement: (1) mandatory product registration with chemical composition declaration; (2) independent pre-market laboratory certification; (3) GMP facility certification requirements; (4) mandatory pharmacovigilance reporting by supplement retailers; and (5) enhanced customs monitoring to identify and seize informally imported supplement products. International precedents exist: Australia's Therapeutic Goods Administration (TGA) and the European Food Safety Authority (EFSA) provide regulatory frameworks that Pakistan could adapt to its domestic supplement market.

5.6 Clinical Practice Recommendations

For hepatologists, sports medicine physicians, and general practitioners in Pakistan, the findings of this study underscore the need to include detailed supplement use history in all hepatological assessment protocols. Athletes presenting with unexplained liver enzyme elevations should receive systematic RUCAM-based causality assessment for supplement-induced DILI. Immediate cessation of all supplements upon DILI diagnosis is mandatory, with liver function monitoring at two-week intervals until resolution. Cases of Grade 3 or higher severity warrant inpatient hepatological care with consideration of N-acetylcysteine therapy, which has shown benefit in some non-acetaminophen DILI scenarios. Sports coaches, gymnasium trainers, and athletes at the community level require structured health literacy

education addressing the specific hepatotoxicity risks of unregulated supplement products available in the Pakistani market.

5.7 Limitations

Several methodological limitations of this study must be acknowledged. The purposive sampling strategy, recruiting from gymnasium and hospital settings, may have introduced selection bias toward more active supplement users and those already presenting with symptoms, potentially overestimating population-level DILI prevalence. RUCAM causality assessment, while validated, involves subjective elements in the temporal association domain. Product recall may have been incomplete for some participants. The cross-sectional design precludes temporal inference regarding supplement initiation and DILI development. Future longitudinal cohort studies with prospective liver function monitoring from supplement initiation are needed to definitively establish incidence rates and causal timelines.

6. Conclusion

This study documents an alarming 37.9% prevalence of drug-induced liver injury among Pakistani athletes consuming unregulated pre-workout and weight-loss supplements, substantially exceeding global background rates. Chemical analysis of marketed supplement products revealed widespread adulteration with banned pharmaceutical substances including sibutramine and DNP, alongside undisclosed anabolic steroids, providing a clear mechanistic explanation for the observed hepatotoxicity burden. Duration of supplement use and polypharmacy stacking were the strongest independent predictors of DILI risk.

These findings demand urgent multi-stakeholder action. DRAP must implement mandatory pre-market chemical certification for all sports nutrition supplements, while provincial food authorities must enforce against illegal sale of adulterated products. Healthcare providers require updated training in supplement-related hepatotoxicity recognition and management. Most critically, Pakistani athletes deserve accurate, evidence-based information about the genuine and potentially life-threatening hepatotoxic risks of unregulated supplement consumption information that is currently absent from the gym floor, the training pitch, and the clinic.

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