ESSENTIAL AND TOXIC TRACE METALS STATUS IN CHRONIC HASHISH CONSUMERS: EVIDENCE FROM A PAKISTANI COHORT

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ABSTRACT

OBJECTIVES

Chronic hashish consumption may influence trace metal homeostasis and promote oxidative stress. This study aimed to evaluate serum concentrations of essential and toxic trace elements-iron (Fe), copper (Cu), zinc (Zn), lead (Pb), and cadmium (Cd)-in long-term hashish users.

METHODOLOGY

A cross-sectional study was conducted in Peshawar, Pakistan, involving 84 adult males aged 25–60 years, divided into chronic hashish users (n = 53) and non-users (n = 51). Venous blood samples were collected and analyzed for trace metal content using flame atomic absorption spectrophotometry (FAAS) following nitric and perchloric acid digestion.

RESULTS

Mean serum concentrations of Fe (2.6 ± 0.3 µg/dL) and Cu (0.5 ± 0.1 µg/dL) were significantly elevated in hashish users compared to non-users (2.4 ± 0.2 µg/dL and 0.3 ± 0.02 µg/dL, respectively; $p \leq 0.05$). No significant differences were observed in Zn, Pb, or Cd levels. Pearson's correlation revealed no significant association between trace metal levels and either duration or quantity of hashish use.

CONCLUSION

Elevated iron and copper levels in hashish users suggest potential disturbance in redox-active metal regulation, potentially contributing to oxidative stress. Further mechanistic studies are warranted to explore cannabis-associated metal exposure and its toxicological consequences.

KEYWORDS: *Hashish, Trace Elements, Metalloenzymes, Cell Injury, Free Radicals, Oxidative Stress*

INTRODUCTION

Metals are indispensable for maintaining physiological homeostasis, playing critical roles in enzymatic activity, signal transduction, and protein stabilization. They serve as essential cofactors for metalloenzymes, which catalyze numerous metabolic reactions.[1,2] Trace amounts of essential elements, such as iron, calcium, magnesium, copper, manganese, and zinc, are vital for growth, immune function, and neurological development.[3,4] The regulatory balance of these metal ions is tightly controlled; however, dysregulation, whether due to environmental exposure or physiological disorders, can result in excessive metal accumulation, aberrant binding to non-target proteins, and ensuing cellular damage.[5] Such disruptions impair protein conformation and mitochondrial integrity, ultimately provoking apoptosis or necrosis.[6] Human exposure to abnormal metal levels originates from multiple sources, including industrial processes (such as mining, metalworking, and leather tanning), contaminated groundwater, agricultural runoff, and environmental pollution.[7] Numerous studies have confirmed heavy metal toxicity in these settings, linking it to neurodegenerative, renal, and reproductive disorders.[8,9] Central among the mechanisms of metal-induced toxicity is oxidative stress. Overabundant essential metals (e.g., Fe, Cu, Mn) can trigger Fenton and Haber-Weiss reactions, generating ROS such as hydroxyl radicals, hydrogen peroxide, and singlet oxygen.[10,11] Meanwhile, non-essential metals such as lead, cadmium, and mercury disrupt mitochondrial electron transport and deplete antioxidants, including glutathione, thereby further increasing ROS levels.[12,13] This oxidative imbalance damages cellular lipids, proteins, and DNA, contributing to the development of over 50 diseases-including cancer, cardiovascular disease, diabetes, neurodegenerative disorders, and autoimmune conditions. Specifically, ROS-induced genomic instability and lipid peroxidation drive chronic inflammation, mutagenesis, and tissue degeneration.[14,15,16] Metallothioneins and metallochaperones are intracellular proteins that bind metals, regulating their distribution and buffering against oxidative stress within cells.[17,18] However, excessive metal exposure can overwhelm these protective systems, leading to metal dysregulation and oxidative stress. Cannabis plants-especially in

unregulated harvests-frequently accumulate heavy metals from soil and cultivation processes, potentially exposing consumers to lead, cadmium, chromium, nickel, and mercury. For example, seedlings grown in metal-rich soils have shown concentrations of cadmium and lead exceeding WHO limits.[19,20] A meta-analysis has confirmed that cannabis's high bioaccumulation potential poses risk through consumption, particularly in smoking forms, which offer high pulmonary absorption.[21] Epidemiological data from NHANES (>7,000 adults) indicates that exclusive marijuana users have 22–27% higher blood lead and cadmium levels compared to non-users-after adjusting for confounding factors.[22] Reddit users analyzing similar data noted that "marijuana users … had statistically higher levels of lead and cadmium in their blood and urine than people who do not use weed".[21,22] In Pakistan, the UNODC–Ministry of Narcotics survey reported ~4% cannabis consumption nationwide, with the highest rates in Khyber Pakhtunkhwa ^31%. Given this, populations in that region may face elevated oxidative stress and trace metal imbalance due to chronic hashish use.[23] Past studies have demonstrated that substance abuse-including tobacco, alcohol, opioids, and cannabis-is linked to altered serum trace element profiles and weakened antioxidant defenses.[24,25,26] The present study aims to quantify serum concentrations of trace elements central to redox metabolism in chronic cannabis users versus matched controls, and to assess biomarkers of oxidative stress, thus elucidating the biochemical correlates of cannabis-related trace metal exposure.

METHODOLOGY

This cross-sectional study was conducted in rural communities near the University of Peshawar. Data on socio-demographic characteristics and baseline clinical parameters were obtained using a structured questionnaire. The questionnaire included variables such as age, body weight, blood pressure, duration of hashish consumption, and the average quantity of hashish used per day, calculated based on self-reported lifetime usage. A non-probability convenience sampling technique was employed for participant recruitment. The study was approved by the Advanced Study and Research Board, University of Swabi, vide Notification No. 1(3)-Dy.Reg-3/UOS/2017/170, dated: 14-09-2017. Ethical Approval was provided by the Ethics Committee of the Department of Pharmacy, University of Swabi, vide letter No. UOS/Pharm-Ethl20 181/223. All study participants signed the informed consent form to participate in the study. All protocols adhered to the ethical principles outlined in the Declaration of Helsinki (1964) and its subsequent amendments. Each participant provided written informed consent after being briefed on the objectives, procedures, and confidentiality measures of the study. A total of 113 adult male participants were recruited and categorized into two groups: (a) chronic hashish users and (b) non-users. Nine individuals declined to participate due to concerns about the confidentiality of their personal information. Hashish users were identified through direct interviews conducted with the assistance of local focal persons nominated from each village. These individuals were selected to facilitate participant identification while ensuring privacy and maintaining the confidentiality of participants substance use history. Inclusion criteria for the hashish user group were: males aged 25–60 years, with a self-reported history of continuous hashish consumption for at least the past five years, and no current or prior diagnosis of major chronic illnesses such as hypertension, diabetes mellitus, or asthma. The control group consisted of individuals from the same geographic area and within the same age range (25–60 years), with no history of chronic diseases or substance use (including tobacco, alcohol, or illicit drugs). Participants with any prior or current use of hashish or other psychoactive substances were excluded from the control group to ensure clear differentiation between groups.

Reagents and Standards preparation

Analytical-grade reagents were used throughout the study. Nitric acid (65% purity) was obtained from Riedel-de Haën-Sigma-Aldrich, USA, while perchloric acid (70% purity) was procured from Scharlau Chemie, S.A., Spain. A certified 2% standard solution of heavy metals in nitric acid was sourced from PerkinElmer, Inc., USA. High-purity deionized water was prepared using a water still (Model P-21701, Favorit, Malaysia). Working standards were freshly prepared prior to each analysis by diluting the stock solution to the desired concentration. A 10% stock solution was prepared by adding 10 mL of the certified standard to deionized water, resulting in a final volume of 100 mL. All glassware and equipment used in the analysis were thoroughly cleaned by soaking in 2% nitric acid, followed by multiple rinses with deionized water to eliminate contamination. To ensure analytical precision and accuracy, all samples were analyzed in triplicate. The instrument was calibrated regularly using certified reference standards. Metal analysis was performed at the Centralized Resource Laboratory (CRL) at the University of Peshawar.

Serum Sample preparation and analysis

Approximately 3 mL of venous blood was collected aseptically from the antecubital vein of each participant using sterile disposable syringes. The samples were transferred into gel clot activator tubes and centrifuged at 3500 revolutions per minute (rpm) for 10 minutes at 4°C to obtain serum. The serum was carefully separated

and transferred to pre-cleaned, acid-washed polypropylene tubes for further analysis. For the digestion of serum samples prior to heavy metal analysis, 1 mL of serum was mixed with 5 mL of concentrated nitric acid (HNO_3) in a digestion tube and heated in a water bath at 60°C for 2 hours. Subsequently, 5 mL of perchloric acid ($HClO_4$) was added to each sample, followed by an additional 30-minute heating period at the same temperature to complete the digestion process. The final volume was brought up to approximately 10 mL with deionized water. All reagents used were of analytical grade, and high-purity water was employed throughout the procedure to minimize contamination risk. Centrifugation was performed using a Centurion Centrifuge (Centurion Scientific Ltd., UK). Quantification of serum trace metal concentrations was carried out using a flame atomic absorption spectrophotometer (AAS), Model A Analyst 700 (PerkinElmer Inc., USA), equipped with an automatic background correction system and metal-specific hollow-cathode lamps (HCLs). Instrumental parameters, including wavelength, lamp current, slit width, and detection limits for each metal, are presented in Table 1.

Table 1: Atomic Absorption Spectrophotometric Specifications

METAL	Parameter					
	Air (L/min)	Acetylene(L/min)	Lamp Current (A)	Slit Width (nm)	Wavelength (nm)	Detection Limits (mg/L)
Iron	17	02	30	0.2	248.3	0.0150
Zinc	17	02	15	0.7	213.9	0.0015
Copper	17	02	15	0.7	324.8	0.0015
Lead	17	02	10	0.7	283.3	0.0150
Cadmium	17	02	04	0.7	288.8	0.0008

Statistical analyses were conducted using GraphPad Prism (version 5.01; GraphPad Software Inc., San Diego, CA, USA). Descriptive statistics were calculated and presented as mean ± standard deviation (SD). Group comparisons between hashish users and non-users were performed using an unpaired, two-tailed Student's t-test to determine statistically significant differences in serum metal concentrations. A p-value ≤ 0.05 was considered indicative of statistical significance. To evaluate the relationship between the quantity and duration of hashish consumption and serum metal levels, Pearson's correlation coefficient (r) was computed. All tests were conducted under the assumption of normal distribution and homogeneity of variance unless otherwise stated.

RESULTS

The demographic and clinical characteristics of the study participants are outlined in Table 2, while serum concentrations of selected trace metals are summarized in Table 3. The biochemical analysis showed significant differences in serum trace metal levels between chronic hashish users and non-users. Chronic hashish users had elevated serum iron (2.6 ± 0.3 µg/dL) and copper (0.5 ± 0.1 µg/dL) compared to non-users (2.4 ± 0.2 µg/dL for iron, P = 0.05; 0.3 ± 0.02 µg/dL for copper, P < 0.05). No significant differences were noted in zinc, lead, and cadmium levels, with zinc at 0.1 ± 0.007 µg/dL (P = 1.0), lead at 0.5 ± 0.01 µg/dL (P = 0.8), and cadmium at 0.3 ± 0.03 µg/dL (P = 0.34) in both groups. Moreover, Pearson correlation analysis revealed no significant relationships between the duration or amount of hashish use and serum levels of iron, zinc, copper, lead, or cadmium. This indicates that while chronic hashish use may elevate iron and copper levels, the relationship does not scale with consumption intensity or duration. Detailed correlation values can be found in Table 4.

Table 2: Characteristics of the Study Subjects in the Current Study

Parameter	Non-users (N=53)	Hashish users(N=51)	P-Value
	Mean ± SD		
Age (Years)	36.8 ± 1.4	36.2 ± 6.3	0.63
BMI (Kg/m^2)	22.3 ± 3.7	21.7 ± 4.8	0.11
FBS (mg/dl)	91.8 ± 8.8	90.5 ± 9.5	0.59
T. Cholesterol (mg%)	180.2 ± 22.1	211 ± 40.2	< 0.05*
CrCl (ml/min)	105.4 ± 19.2	152.2 ± 39.6	< 0.05*
Duration of Hashish Use (Years)	N/A	19.1 ± 8.8	-
Quantity of drug consumption per day (gms)	N/A	3.2 ± 1.5	-

*Statistically significant at p < 0.05

Table 3: Trace Metal Concentrations in Serum Samples of the Study Groups in the Current Study

Serum trace metals (µg/dl)	Non-users (N=53)	Hashish users (N=51)	P-Value
	Mean ± SD		
Iron (Fe)	2.4 ± 0.2	2.6 ± 0.3	0.05*
Zinc (Zn)	0.1 ± 0.01	0.1 ± 0.01	1
Copper (Cu)	0.3 ± 0.02	0.5 ± 0.1	< 0.05*
Lead (Pb)	0.5 ± 0.01	0.6 ± 0.1	0.80
Cadmium (Cd)	0.3 ± 0.03	0.3 ± 0.05	0.34

*Statistically significant at p < 0.05

Table 4: Pearson Correlation between Serum Concentration of Metals and (a) Duration of Hashish use in Years, (b) Quantity of Hashish Use Per Day

Serum trace metals (µg/dl)	Correlation with	R-value	P-value
Iron	Duration of Hashish use (Years)	0.970	0.891
	Quantitiy of Hashish use per day (g)	0.120	0.423
Zinc	Duration of Hashish use (Years)	-0.015	0.912
	Quantitiy of Hashish use per day (g)	-0.251	0.055
Copper	Duration of Hashish use (Years)	0.173	0.169
	Quantitiy of Hashish use per day (g)	0.029	0.826
Lead	Duration of Hashish use (Years)	0.033	0.981
	Quantitiy of Hashish use per day (g)	0.182	0.165
Cadmium	Duration of Hashish use (Years)	0.210	0.221
	Quantitiy of Hashish use per day (g)	0.219	0.093

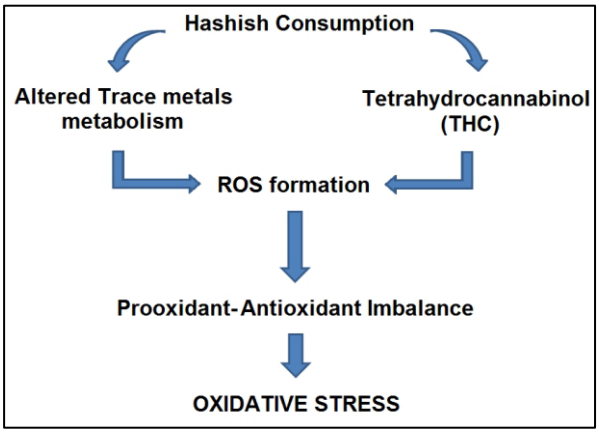

Figure 1: A Proposed Mechanism of Hashish-Induced Oxidative Stress

DISCUSSION

The present study demonstrated a notable increase in serum concentrations of iron and copper among chronic hashish users compared to non-user controls. This finding is suggestive of altered trace metal homeostasis in individuals with long-term cannabis exposure. In addition, statistically significant changes were observed in creatinine clearance and serum cholesterol levels, which may reflect early signs of impaired kidney function and disrupted lipid metabolism. While these changes could be directly attributed to the pharmacological effects of hashish, it is also plausible that they result from secondary disturbances in the regulation of essential metal ions. Nevertheless, this hypothesis remains speculative and warrants further investigation through controlled experimental studies. To our knowledge, this is the first study in Pakistan to assess serum levels of biologically relevant trace metals involved in oxidative stress pathways among chronic hashish users. The findings offer preliminary biochemical insights into the redox-related toxicological burden associated with cannabis use, an area largely underexplored in existing literature, especially in low-resource or high-prevalence regions such as Khyber Pakhtunkhwa. Trace metals play indispensable roles in human physiology, particularly in enzymatic catalysis, maintaining redox balance, regulating the immune system, and mediating cellular signaling. However, dysregulation of these metals-whether from environmental, occupational, or lifestyle exposures-can have adverse consequences. Natural processes, such as volcanic activity, soil erosion, and microbial cycling, introduce metals into ecosystems. However, anthropogenic sources-including fossil fuel combustion, industrial effluents, agricultural runoff, and waste incineration-significantly increase their bioavailability and exposure risk.[27,28] These metals can enter the human body via inhalation, ingestion, dermal contact, or contaminated food and water, accumulating in tissues over time. Once absorbed, heavy metals may bind to proteins, nucleic acids, and membranes, disrupting normal biochemical processes. Several of these metals, particularly lead, cadmium, mercury, and arsenic, are known to induce oxidative stress, cause mitochondrial dysfunction, and impair organ systems. Chronic exposure has been linked to neurotoxicity, nephrotoxicity, hepatotoxicity, and even carcinogenicity.[24,25,29] Long-term accumulation has also been implicated in neurological degeneration, impaired physical development, endocrine disruption, and reproductive toxicity through DNA damage, hormonal mimicry, and gene mutation.[26,30] In biological systems, many trace metals are part of metalloproteins, which play key catalytic, structural, and regulatory roles. Disruption in their balance-particularly of redox-active metals such as iron and copper-can shift the cell into a pro-oxidative state. Iron, present as Fe^{2+} and Fe^{3+}, is central to oxygen transport and cellular respiration. However, in excess, it can participate in the Fenton reaction, producing reactive oxygen species (ROS) such as hydroxyl radicals, which damage lipids, proteins, and DNA. Iron overload, which is reported to cause organ damage when levels exceed 3-5 times the physiological norm, has been linked to conditions such as hemochromatosis and cardiomyopathy.[31,32] Similarly, copper is a vital cofactor in enzymes such as cytochrome c oxidase, superoxide dismutase, and lysyl oxidase. However, elevated copper levels can induce oxidative stress via Fenton-like mechanisms or by depleting intracellular glutathione, a major antioxidant that also facilitates copper detoxification. In the context of hashish, elevated serum copper and iron levels

observed in this study may stem from environmental contamination during the cultivation, processing, or packaging of cannabis products. Cannabis plants are known to be hyperaccumulators of heavy metals from soil and water, which can lead to significant metal transfer to users, especially when smoked or ingested in concentrated forms.[30,31] In contrast, no statistically significant differences were observed between the two groups in the serum concentrations of lead, cadmium, and zinc. This may suggest limited contamination of these specific metals in the cannabis products used or a threshold effect not reached in the exposed group. Zinc levels, in particular, were comparable between users and non-users. Given zinc's essential role in the antioxidant enzyme superoxide dismutase (SOD), its stability across both groups may indicate a preserved zinc-dependent antioxidant response. Nonetheless, further studies are required to assess enzyme-specific activity and to explore whether subtle alterations in zinc bioavailability exist at the functional level. Interestingly, no statistically significant correlation was found between either the quantity or duration of hashish use and serum metal concentrations. This finding suggests that the alterations in trace metal levels observed in hashish users may be more influenced by product-specific contamination rather than the intensity or frequency of use. However, the cross-sectional design and modest sample size of the study may limit the ability to detect dose-response relationships.

LIMITATIONS

Two notable limitations of this study must be acknowledged. First, female participants were excluded due to cultural and religious sensitivities within the target population, which limited the generalizability of the results to male users only. Second, the study focused solely on trace metal quantification and did not incorporate direct biochemical markers of oxidative stress, such as malondialdehyde (MDA), 8-OHdG, or glutathione peroxidase activity. Inclusion of such markers in future research would provide more definitive evidence of oxidative damage associated with hashish consumption.

CONCLUSIONS

This study demonstrated significantly elevated serum concentrations of iron and copper among individuals with chronic hashish use compared to non-users. These alterations may result from the direct introduction of metal contaminants through the consumption of hashish or may occur indirectly via the generation of reactive oxygen species (ROS) that disrupt the body's oxidant-antioxidant balance. However, the observed differences may also be influenced by other confounding factors, including environmental exposures, dietary habits, or genetic variability, which were not fully accounted for in this study. Currently, there is a paucity of data exploring the molecular and biochemical mechanisms through which hashish use alters trace metal homeostasis. The potential link between metal dysregulation, oxidative stress, inflammation, and tissue toxicity remains poorly understood and warrants further investigation. Future studies should incorporate larger, more diverse populations, include functional oxidative stress markers, and aim to elucidate mechanistic pathways through which chronic cannabis exposure may contribute to metal-induced cellular damage.

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AUTHORS CONTRIBUTION

Asmat Ullah - *Concept & Design; Data Acquisition; Data Analysis/Interpretation; Drafting Manuscript; Final Approval*

Abad Khan - *Concept & Design; Data Acquisition; Data Analysis/Interpretation; Drafting Manuscript; Critical Revision; Supervision; Final Approval*

Asif Ali - *Concept & Design; Data Acquisition; Data Analysis/Interpretation; Drafting Manuscript; Final Approval*

The authors accept responsibility for all aspects of the work and will ensure that any concerns regarding the accuracy or integrity of any part are properly investigated and resolved.