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Original Research Article

Assessing immune parameters among cow urine consumers in diverse Indian settlements: An observational study

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Abstract

Background: Indigenous cow urine (ICU) is a revered agent in traditional Indian medicine with purported immunomodulatory properties. However, systematic observational data comparing its effects across diverse populations is lacking and further study among the Indian population is recommended.

Aims & Objectives: This study aimed to investigate the association between habitual ICU consumption and immune system parameters in two distinct Indian populations: traditional rural inhabitants and core urban dwellers for their immune body functionalities.

Materials and Methods: A comparative, cross-sectional observational study was conducted in Village Bhadoriya (rural) and Indore city (urban) in Madhya Pradesh. A total of 240 participants were stratified into four groups: Rural Consumers, Rural Non-Consumers, Urban Consumers, and Urban Non-Consumers (n=60 each). Fasting blood samples were analysed for innate and adaptive immune markers, cytokine profiles, and oxidative stress markers. A subset (n=20/group) provided faecal samples for 16S rRNA microbiome sequencing.

Results: Significant positive associations were found between ICU consumption and enhanced immune markers, with more pronounced effects in the rural cohort. Rural consumers showed higher NK cell activity and IgG levels. Urban consumers showed a higher CD4/CD8 ratio and lower IL-6. Both consumer groups exhibited improved antioxidant status (higher SOD/catalase, lower MDA) and rural consumers showed greater gut microbial diversity.

Conclusion: Habitual ICU consumption is associated with favourable immunomodulatory and antioxidant profiles, particularly in a traditional rural setting. These findings support the ethnopharmacological basis for ICU's use and highlight the need for further mechanistic research.

Keywords: Cow urine, Immune modulation, Traditional medicine, Ayurveda, Rural health, Urban health.

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1. Introduction

The therapeutic use of indigenous cow urine (ICU) from *Bos indicus* breeds, known as 'Gomutra,' is deeply rooted in ancient Indian traditional health systems, particularly Ayurveda, where texts like the *Charaka Samhita* and *Sushruta Samhita* allude to its medicinal properties.[1,2] Traditionally, ICU has been acclaimed for a wide spectrum of benefits, including antimicrobial, antioxidant, anti-inflammatory, cytoprotective, and specifically, immunomodulatory effects.[3-6] Rural populations in India often continue to integrate ICU into their health and wellness regimens, utilizing various preparations such as raw, distilled ('arka'), or fermented forms.[7] In contrast, urban populations generally exhibit lower awareness or more skepticism, partly due to sociocultural shifts and a greater reliance on modern scientific validation for health practices.[8]

The immune system is essential in maintaining homeostasis, defending against pathogens, and reducing susceptibility to infections and chronic diseases. Several studies have explored the role of natural compounds in modulating immune function, with recent attention being drawn toward CU due to its bioactive components. Indigenous CU contains essential minerals, volatile fatty acids, phenolic compounds, and immunomodulatory agents,

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which are believed to enhance both innate and adaptive immunity. However, a systematic investigation of its immunological impact in different population groups is lacking.[9,10]

Urban and rural populations exhibit variations in immune status due to differences in environmental exposures, dietary habits, stress levels, and lifestyle factors. Urban individuals are often exposed to higher pollution levels, processed food consumption, and deskbound lifestyles, which may contribute to compromised immune function.[11] In contrast, rural populations, with their relatively higher exposure to natural environmental antigens and traditional dietary practices, might display a different baseline immune response. Understanding how indigenous CU affects these two distinct populations could provide valuable insights into its immunomodulatory potential.[12] Given the limited scientific studies available, this research aims to validate the immunomodulatory effects of indigenous CU by assessing its impact on immune parameters in urban and rural populations. This study evaluates the differences in immune response between these two groups, hypothesizing that urban individuals may experience a more pronounced immune enhancement due to a relatively weaker baseline immune system. By comparing pre- and post-intervention immune markers, the study seeks to provide objective evidence on the efficacy of ICU as a natural immunomodulator.

Despite a rich history of anecdotal evidence and increasing pre-clinical research indicating potential biological activities of ICU,[13-15] there is a paucity of systematic observational studies comparing the immune profiles of individuals who habitually consume ICU versus those who do not, especially across diverse environmental and lifestyle contexts. This study aimed to address this gap by characterizing and comparing key immune parameters in individuals from a traditional rural setting in Village Bhadoriya, Indore District, Madhya Pradesh, and a core urban environment in Indore city, Madhya Pradesh, based on their self-reported patterns of ICU consumption. Understanding these associations is crucial for evaluating the contemporary relevance of this traditional practice within public health and personalized wellness frameworks.

2. Materials and Methods

A comparative, cross-sectional observational study was conducted. Participants were recruited from two distinct settings within Madhya Pradesh:

1. A traditional rural area: Village Bhadoriya, Indore District, characterized by farming lifestyles, close proximity to livestock, and prevalent use of traditional remedies.
2. A core urban area: Indore city, characterized by higher population density, different dietary patterns, and greater exposure to urban pollutants.

Participants were categorized based on their detailed, self-reported ICU consumption habits, verified where possible through household confirmation:

1. Regular ICU Consumers (ICU-C): Individuals reporting consumption of ICU (from *Bos indicus* cows, typically sourced locally) at least 3-4 times per week for a minimum of the preceding 6 months.
2. Non-Consumers (ICU-NC): Individuals reporting no consumption of ICU in the past 12 months or a lifetime history of no consumption.

A total sample of 240 participants was enrolled, stratified as follows:

1. Rural ICU Consumers (from Village Bhadoriya, n=60)
2. Rural Non-Consumers (from Village Bhadoriya, n=60)
3. Urban ICU Consumers (from Indore city, n=60)
4. Urban Non-Consumers (from Indore city, n=60)

Groups were matched for age (range 18–60 years) and sex distribution as closely as possible.

2.1. Participant recruitment and selection

Recruitment was facilitated through community health workers, Anganwadi centres in Village Bhadoriya and urban health posts in Indore city, local Ayurvedic practitioners, and community announcements. A preliminary screening questionnaire assessed eligibility.

2.2. Inclusion criteria

1. Age 18–60 years.
2. Resident of the designated rural or urban area for at least 5 years.
3. Provided written informed consent.
4. Clearly identifiable as a regular ICU consumer or a non-consumer based on detailed interview.

2.3. Exclusion criteria

1. Current smokers.
2. Diagnosed chronic immune-compromising illnesses (e.g., HIV, active tuberculosis, autoimmune diseases, cancer).
3. Use of immunosuppressive medications, corticosteroids, or immunomodulatory supplements (other than ICU for the consumer group) within the last 3 months.
4. Acute illness or antibiotic use within the last 30 days.
5. Pregnancy or lactation.

2.4. Data collection

2.4.1. Questionnaire

A pre-validated, structured questionnaire administered by trained field investigators collected data on: Sociodemographic (age, sex, education, occupation);

Medical history, lifestyle (diet, physical activity, alcohol); and for ICU-C: source of ICU (*Bos indicus* breed if known), collection/processing (e.g., fresh, filtered, distilled as 'arka'), frequency, dosage (approx. 10-20 ml typical), duration, reasons for use, perceived benefits, and any adverse effects.

2.4.2. Biological sample collection

Fasting (8-10 hours) venous blood samples (10 ml) were collected by certified phlebotomists. Samples were processed within 2 hours for serum, plasma, and isolation of Peripheral Blood Mononuclear Cells (PBMCs).

2.5. Parameters assessed

2.5.1. Innate immunity markers

Neutrophil phagocytic activity: Assessed by quantifying the uptake of fluorescein-labeled *E. coli* by neutrophils in whole blood via flow cytometry.[16]

NK cell activity: Measured by CD107a degranulation assay on PBMCs stimulated with K562 target cells, analyzed by flow cytometry for CD3-/CD56+/CD107a+ cells.[17]

2.5.2. Adaptive immunity markers

Lymphocyte subsets: CD3+, CD4+, CD8+ T cells (for CD4/CD8 ratio), and CD19+ B cells quantified from PBMCs by multi-color flow cytometry.[18]

Serum Immunoglobulin levels: IgG, IgM, IgA quantified by nephelometry (e.g., using Siemens BN™ II System).[19]

2.5.3. Cytokine profile

Serum levels of IL-2, IL-6, TNF-α, and IFN-γ measured using a high-sensitivity multiplex bead-based immunoassay (e.g., Milliplex MAP, Millipore).[20]

2.5.4. Oxidative stress markers

Plasma Malondialdehyde (MDA): Measured by thiobarbituric acid reactive substances (TBARS) assay.[21]

Erythrocyte Superoxide Dismutase (SOD) and Catalase (CAT) activity: Measured by established spectrophotometric methods.[22,23]

2.5.5. Microbiome sequencing (Subset Analysis)

Fecal samples were collected from a subset (n=20 per group) for 16S rRNA gene V3-V4 region sequencing on an Illumina MiSeq platform to assess gut microbial diversity and composition.[24]

2.6. Statistical analysis

Data were analyzed using SPSS Statistics v.25 (IBM Corp.). Descriptive statistics were calculated. Independent samples t-tests or Mann-Whitney U tests were used to compare continuous variables between ICU-C and ICU-NC groups within rural and urban cohorts. Chi-square tests were used for categorical variables. ANCOVA was used to compare immune markers, adjusting for potential confounders (age, BMI, socioeconomic status). Pearson/Spearman correlations assessed relationships between ICU consumption details and immune markers. P-values <0.05 were considered statistically significant. Microbiome data were analyzed using QIIME2 and R packages (phyloseq, vegan).

3. Results

3.1. Immune marker associations

1. *Rural Cohort:* Rural ICU-C exhibited significantly higher NK cell activity (+23% mean activity index, p=0.008) and serum IgG levels (+18% mean concentration, p=0.02) compared to rural ICU-NC. Neutrophil phagocytic capacity was also significantly elevated in rural ICU-C (p=0.015).
2. *Urban Cohort:* Urban ICU-C demonstrated a significantly higher CD4/CD8 T cell ratio (+12% mean ratio, p=0.03) and significantly lower levels of the pro-inflammatory cytokine IL-6 (-15% mean concentration, p=0.04) compared to urban ICU-NC.
3. *Inter-Cohort Comparison:* The magnitude of positive associations with NK cell activity and IgG levels was more pronounced in the rural ICU-C group than in the urban ICU-C group when compared to their respective non-consuming counterparts.

3.2. Oxidative stress

Both rural and urban ICU-C groups showed significantly higher antioxidant enzyme activities (SOD: rural p=0.005, urban p=0.02; Catalase: rural p=0.002, urban p=0.01) and lower MDA levels (rural p=0.001, urban p=0.018) compared to their respective ICU-NC groups. The effect sizes for improved antioxidant status were notably greater in the rural ICU-C group.

3.3. Microbiome impact (Subset Analysis)

Preliminary analysis of the subset data indicated that rural ICU-C had significantly greater gut microbial alpha diversity (Shannon index, p=0.04) and a higher relative abundance of *Lactobacillus spp.* (p=0.03) and *Bifidobacterium spp.* (p=0.045) compared to rural ICU-NC. Urban ICU-C showed a trend towards increased *Lactobacillus spp.* but it did not reach statistical significance compared to urban ICU-NC.

3.4. Safety and acceptability

No serious adverse events attribuFIg to ICU consumption were reported by any participants in the ICU-C groups. Mild, transient gastrointestinal discomfort (e.g., nausea, loose stools) during the initial days of consumption was reported by 7% of urban ICU-C and 3% of rural ICU-C. Self-reported acceptability and perceived general health benefits were high among rural ICU-C (87% reporting satisfaction) and moderate among urban ICU-C (42% reporting satisfaction).

4. Discussion

The findings of this observational study demonstrate significant positive associations between habitual indigenous cow urine consumption and several markers of enhanced immune function and reduced oxidative stress, particularly within the traditional rural population. The elevated NK cell activity and IgG levels observed in rural ICU consumers suggest a bolstering of both innate and humoral adaptive immunity, consistent with some preclinical studies that have reported immunopotentiation effects of cow urine distillates.[13-15] The improved CD4/CD8 ratio and lower IL-6 in urban consumers point towards a better-regulated T-cell compartment and reduced systemic inflammation, respectively.

The more pronounced positive immune associations in the rural cohort compared to the urban one could be multifactorial. Rural dwellers often have lifelong or long-term exposure to a wider range of environmental microbes and a diet richer in traditional fermented foods, potentially leading to a baseline gut microbiome that interacts more synergistically with ICU constituents.[25] The observed higher gut microbial diversity and abundance of beneficial genera like *Lactobacillus* and *Bifidobacterium* in rural ICU consumers supports this notion, as these bacteria are known to play crucial roles in immune maturation and regulation.[26] Furthermore, the components of ICU, which include volatile fatty acids, minerals, and potentially other unidentified bioactive molecules[4,27,28] might exert direct immunomodulatory effects or act indirectly via microbiome modulation.

The enhanced antioxidant status (higher SOD, catalase; lower MDA) in both consumer groups aligns with reports on the antioxidant properties of cow urine, attributed to constituents like uric acid and allantoin.[5,6] This reduction in oxidative stress can itself contribute to improved immune cell function.

Differences in the source, processing, and freshness of ICU between rural (often fresh, locally sourced) and urban settings (potentially more processed or from varied sources) might also contribute to differential effects, although our study attempted to capture these details. The higher acceptability in rural areas likely reflects deeper cultural integration and potentially fewer preconceived notions compared to urban settings.

5. Limitations

Despite the significant associations observed, this study has limitations inherent to its observational, cross-sectional design:

1. *Causality:* The study establishes associations, not definitive cause-and-effect relationships. It's possible that individuals with inherently more robust immune systems or specific lifestyle choices are more inclined to consume ICU.
2. *Confounding Variables:* While we adjusted for key confounders, unmeasured variables (e.g., detailed dietary micronutrient intake, psychosocial stress, specific genetic polymorphisms, other un-reported traditional practices) could have influenced the outcomes.
3. *Recall Bias:* Data on ICU consumption patterns and lifestyle were self-reported and subject to recall bias, although efforts were made to corroborate information.
4. *Selection Bias:* Participants volunteering for the study might not be fully representative of all ICU consumers or non-consumers in their respective communities.
5. *Heterogeneity of ICU:* The biochemical composition of ICU can vary based on cow breed, diet, age, health status, and collection/processing methods. While *Bos indicus* was specified, standardizing this "exposure" across participants is a challenge in observational research.
6. *Microbiome Subset Size:* The microbiome analysis was conducted on a subset, limiting the statistical power to detect more subtle microbial shifts.

6. Conclusions

This comparative observational study provides evidence of significant positive associations between habitual indigenous cow urine consumption and favorable immune profiles, including enhanced innate and adaptive immune markers and improved antioxidant status, particularly in the traditional rural Indian population of Village Bhadoriya, Indore District, Madhya Pradesh. These findings support the ethnopharmacological basis for ICU's traditional use in promoting health and suggest its potential as a complementary immunomodulatory agent. The observed differences between rural consumers in Village Bhadoriya and urban consumers in Indore city highlight the importance of considering host and environmental contexts in the effects of traditional remedies.

7. Recommendations

1. *Prospective Cohort Studies:* Long-term prospective studies are needed to better establish the temporal relationship between ICU consumption and immune changes, and to minimize recall bias.
2. *Randomized Controlled Trials (RCTs):* To confirm causality and efficacy, well-designed RCTs using standardized and characterized ICU preparations (e.g., 'arka' from specific *Bos indicus* breeds fed controlled diets) are warranted.
3. *Mechanistic Studies:* Further in-vitro and in-vivo animal studies are essential to elucidate the specific active constituents of ICU responsible for immunomodulation and their precise mechanisms of action on immune cells and the gut microbiome.

4. *Standardization:* Research into methods for standardizing ICU collection, processing, and quality control is crucial if its use is to be considered more broadly.
5. *Gut Microbiome Interactions:* Larger-scale studies specifically investigating the interplay between ICU, gut microbiota, and host immunity are highly recommended.

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10. Conflict of Interest

The authors declare no conflict of interest.

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