Review Article

BIOMEDICAL Journal of Scientific & Technical Research

ISSN: 2574 -1241

DOI: 10.26717/BJSTR.2025.62.009801

Advances in Chain of Evidence in Medicine

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ARTICLE INFO

Received: May 20, 2025

Published: August 01, 2025

Citation: Yanan Bai, Wanying Wang, Jiajia Duan, Xue Wang, Mingjing Jiang, Han Ni, Ding Li and Chuanxin Liu. Advances in Chain of Evidence in Medicine. Biomed J Sci & Tech Res 62(5)-2025. BJSTR. MS.ID.009801.

ABSTRACT

The theory of chain of evidence originates in the field of jurisprudence, emphasizing the elimination of reasonable doubt through mutual corroboration of evidence and the construction of a complete system of facts, which is highly consistent with the core needs of the current practice of evidence-based medicine. This paper reviews the theoretical transmutation and practical evolution of the chain of evidence, and clarifies the internal logic of the chain of evidence's migratory application from jurisprudence to medicine. Focusing on the evidence-based dilemmas in the three major fields of forensic toxicology, modern medicine and traditional Chinese medicine, we systematically sort out the research progress of the chain of evidence in the corresponding fields of medicine, and emphasize the unique advantages of toxicological evidence chain (TEC) in the safety evaluation of traditional Chinese medicine. Interdisciplinary exploration of the chain of evidence is reshaping the standards of medical facts determination with the logic of jurisprudence, which is expected to elevate the level of evidence for medical research findings and accelerate the transition of medical practice to an evidence-based closed-loop.

Keywords: Chain of Evidence; Forensic Toxicology; Modern Medicine; Traditional Chinese Medicine; Toxicological Evidence Chain

Abbreviations: TEC: Toxicological Evidence Chain; LC-HRMS: Liquid Chromatography-High Resolution Mass Spectrometry; SAP: Single Amino Acid Polymorphism; IEC: Integrated Evidence Chain; MS: Multiple Sclerosis; TEC: Toxicological Evidence Chain; HIE: Hazardous Ingredients Evidence; AOE: Adverse Outcomes Evidence; IPE: Injury Phenotype Evidence; GTEA: Grades of Toxicological Evidence Assessment; TMT: Tandem Mass Tags; WB: Western Blot

Introduction

"Chain of evidence" uses the "evidence" as the core, based on the mutual corroboration in the content of the evidence, complementary or aggregation in the structure of a complete collection of evidence that can prove a relevant fact. "Evidence" has a wide range of applications in daily life. It supports the factual content relies on the "evidence" tends to have a high degree of authenticity and credibility. The theoretical discussions on "evidence" and "chain of evidence" are mostly found in the field of research about jurisprudence, for example, the evidence law for criminal, civil, administrative and other litigation about determination of fact in the research. The common sense understanding of the term "evidence" is also mostly based on the concepts of the legal field [1]. As for the medical field, there are also some practical needs for "evidence", especially in pathology, toxicology, pharmacology and other issues in the medical investigations. The research in "evidence" and "chain of evidence" in the field of jurisprudence, including related results and theoretical discussions, are also of great reference significance to the medical practice. In more details, the popularization and application of the "chain of evidence" in the medical field is mostly manifested in the practical process of exploring the mechanism of pathology, toxicology and pharmacology, etc., and obtaining the corresponding material bases through medical examination and testing, etc., which are used to identify and evaluate the cognitive methods of relevant medical facts. The method of constructing the chain of evidence plays a decisive role in the scientific use of evidence materials to determine the medical facts, and the current research progress of the chain of evidence in the field of medicine also needs to be traced back to the field of research about jurisprudence. This paper depicts the scientific usage of the chain of evidence in the field of medicine through an interdisciplinary research approach.

The Theoretical Transmutation and Concrete Application of Chain of Evidence

Initially, the chain of evidence is a practical product of the judicial proceedings, aimed at harmonizing the relevant evidence in order to ascertain the truth of the case, which is an important method or tool for fact-finding. The chain of evidence is formed in the case of the linkage between the evidence, and this linkage is based on the mutual corroboration of the content of the evidence will often form different structural relationships. The development of corroboration theory has led to a convergence of theories of evidence from atomism to holism or structuralism, where the interconnection of evidence is the important role of the chain of evidence.

Traceability and Development of Research on the Concept of the Chain of Evidence

As we all know, legality, relevance and authenticity are the basic attributes of the concept of evidence in the field of law. "Relevance" refers to the link between the content of the evidence and the facts of the case, as well as the often complementary and corroborative links between the evidence and the evidence under the same facts of the case [2]. The latter emphasizes the "link" between the evidence, which is the essence of the "connecting point" that forms the chain of evidence. It is reported that the relevant theoretical elements of the chain of evidence can be traced back to the former Soviet Union, and were initially used mainly to solve the problem of the application and argumentation of circumstantial evidence in litigation activities [3]. The early concept of the chain of evidence was described and defined as "a collection of evidence consisting of two or more distinct links (or pieces of evidence), formed by the interconnection of the chain heads and the objective link between the chain heads and the chain body, the contents of which corroborate each other and embody or enhance the strength of the evidence to prove the facts of the case." [4] The use of chain of evidence in judicial practice often exists with a clear purpose-oriented approach, i.e., the discovery of the truth of the case. The standard of proof of "clear facts and solid and sufficient evidence" in judicial proceedings is reflected in the formal structure of the interlocking chain of evidence to reflect the facts of the case. The chain of evidence also exhibits characteristics such as hierarchy and completeness in describing the facts of the case [5].

Accompanied by the in-depth study of the concept of "chain of evidence" and the extensive use of judicial practice, "chain" and "links" and other content has gradually become the focus of theoretical research. Credibility or evaluation criteria also centre on the number of links in the chain and the hierarchical relationship of evidence [6]. The overall content of the facts of a case consisting of different kinds of evidence becomes the key to the shaping and portrayal of the chain of evidence, and litigation activities gradually begin to shift from the atomism which focuses on the analysis of individual pieces of evidence to the structuralism approach to the analysis of the chain of evidence [7]. It follows that the theory of corroboration, which emphasizes the cross-checking of evidence, combined with the chain of evidence, can be used to express the substantive relationship of the "points of connection" between evidence. The initial introduction of the concept of "chain of evidence" in the medical field was closely related to the emergence of evidence-based medicine in the 1990s, and has been standardized along with the development of evidence-based medicine. The basic aspects of the practice of evidence-based medicine include the formulation of clinical questions, retrieval of relevant evidence, assessment of the quality of evidence, application of evidence, and evaluation of posterior effect, the core of which is to search for the best evidence to be combined with specialized knowledge through rigorous and objective assessment of grading in order to guide clinical practice [8].

The concept of chain of evidence is closely related to the core of evidence-based medicine. By linking different levels and sources of evidence (e.g., basic research, clinical trials, observational studies, etc.) together in accordance with the logic of dynamic control to form

an interlocking chain of evidence materials, it ensures that medical decisions are based on high-quality and validated evidence, and significantly improves the scientific and reliability of decision-making. For example, the assessment of the efficacy of a new pharmaceutical requires the establishment of a complete system of evidence covering four parts: laboratory studies, early clinical trials, large-scale randomized controlled trials and long-term real-world studies. This system of evidence is layered in a progressive manner, and this type of integration and demonstration of relevant materials essentially reflects the characteristics of mutual corroboration between materials.

Presentation of the Theory of Corroboration in the Chain of Evidence

How to determine the criteria and methods of evaluation of evidence? This is a question that cannot be avoided when adopting, using, and determining the fact. In evidence jurisprudence, although the common law system and the civil law system have various rules to select evidence and to evaluate the power of proof, they all recognize the adjudicator's personal free will essentially. The ability of the adjudicator to form a subjective and objective inner conviction in the face of the evidence and to make factual judgments as a result is the main method of evaluating the evidence under the personal free will. However, purely free will is often influenced by the personal subjective factors of the adjudicator, making the overall determination process of the case at risk of subjective error [9]. In order to avoid this, Professor Long Zongzhi put forward the theory of corroboration in the chain of evidence, the theory pays more attention to the articulation and coordination between the evidence, is to review and evaluate the evidence of a more objective way and method [10]. He advocates that the corroborative proof should be the dominant of evidence review and the determination of the process [11]. The same certification content, fitness and convergence are three basic methods in the theory of corroboration, on the other hand, the complementary, internal evidence and similarity are the auxiliary methods in the theory of corroboration [12]. To be specific, corroborative proof in the chain of evidence contains the points of corroboration and the chain corroboration.

The points of corroboration between evidence are mutual, and the linking points between this and that evidence form a more stable structural state based on the relationship of corroboration, while at the same time further accomplishing each other, increasing the proof of strength of each and playing a role in proving the content of the same point of fact. The chain corroboration, on the other hand, is based on the correlation between the intermediate facts to be proved, and the corroborative relationship between different chains of evidence is formed by the collection of evidence at different points of fact, which results in a larger collection of evidence or proof system to prove the content of the final facts to be proved. "The number of linking points depends on how much the contents corroborate each other, and there can be more than one link between a pair of pieces of evidence. The chain of evidence of the whole case is linked together to form a closure, which creates a closed loop of evidence." [13] The theory of corroboration in the chain of evidence focuses on the structuralism that the evaluation of evidence depends on the overall contours of the evidence assemblage. The proposed theory of corroboration for evidence jurisprudence about the combined use of evidence, evaluation criteria, comprehensive review provides a specific methodological path, which is an important element in the development of the history of evidence. As for the medical field, although there are no clearer criteria and methods for evaluating evidence, the clinical confirmation of pharmacological, pathological and toxicological effects actually involves judgement and understanding that the materials corroborate each other.

For example, in addition to its use in the treatment of type 2 diabetes mellitus, some studies have focused on its pharmacological effects in the treatment of ovarian cancer: metformin administration significantly inhibited the proliferative clonal activity and reduced angiogenesis of SKOV3 and A2780 ovarian cancer cell lines in in vitro assays, while monotherapy significantly reduced the size of tumours in a mouse transplantation tumour model of ovarian cancer. In addition some clinical data and epidemiological evidence suggest that metformin users have a reduced risk of ovarian cancer of about 34% and prolonged overall survival, confirming in various ways that metformin may have anti-ovarian cancer activity [14].When patients with Alzheimer's disease come to the clinic, clinicians need to combine the results of the Neuropsychological Assessment Scale, the presence of hippocampal atrophy on imaging tests, the presence of elevated levels of the cerebrospinal fluid biomarker Tau protein, as well as the patient's medical history and family history, etc., in order to issue a definitive pathological diagnosis [15]. Similarly, in the process of confirming the patient 's poisoning, it is also necessary to combine medication history, serum test, protein biopsy, histopathological examination, and the patient 's clinical manifestations to make a comprehensive judgment: for example, in suspected aconitine poisoning cases, patients may have numbness of the lips and mouth, weakness of the limbs, palpitations and hypotension, laboratory tests found that the blood contains benzoyl aconitine and other related metabolites, troponin levels abnormally high suggesting that cardiomyocyte damage, combined with the patients and their relatives oral history of aconitine-based Chinese medicines or folk prescriptions, basically able to confirm the aconitine poisoning [16].

Criteria for Closure in the Application of the Chain of Evidence

The closure of the chain of evidence is based on mutual corroboration, requiring evidence to be verified by internal and external evidence and other verification methods to achieve a high degree of conformity and to eliminate the contradictory elements of reasonable doubt. Once the chain of evidence is closed, an interlocking

system-like pattern can be achieved, where the links are mutually reinforcing but organically unified. The criteria for the closure of the chain of evidence can be summed up as in five main features: relevance, consistency, completeness, reliability and traceability. Relevance of both evidence and evidence of corroboration between the correlation, including evidence and the correlation between the facts. Consistency is the evidence is unified in the case within the scope of the facts, based on the objective content of the corroboration reflects the same factual content. Completeness of the evidence in the interlocking basis, describing the complete factual appearance. Reliability is the corresponding evidence have a high degree of credibility, although individual evidence cannot achieve absolute certainty, but the linkage between each other can form a reliable corroboration effect. Traceability is the description of the content of the chain of evidence, that is to say, the content of the chain of evidence can be traced back to the past facts. The criterion of closure of the chain of evidence is a reliability requirement aimed at avoiding bias in descriptions. It has been argued that rather than exploring factual certainty through the chain of evidence, the basis for shaping the theory of corroboration should be grounded in the idea of reliability.

"In scientific terms, 'reliability' is a better safeguard than 'certainty' because reliability is based solely on realistic conditions, whereas certainty must be satisfied by adding assumptions based on imagination." [17]. The rationale for the interlocking of corroborating evidence to increase its credibility lies in the stacking of probabilities at the probabilistic level. However, since the factual content described by the chain of evidence is not absolutely objective, "in the process of finding facts, many times the evidential facts converted by the evidence or the 'intermediate facts to be proved' which are further transformed need to be summarized in order to explore the 'ultimate facts to be proved'." [3] From the nature of cognitive activity, it is inevitable that people need to make summary about the content of the object of cognition when they are aware of objective facts. Therefore, cognitive bias is an unavoidable and inevitable event, and the closure of the chain of evidence requires that errors and biases caused by summary be avoided to the maximum extent possible.

The Application of Chain of Evidence from Jurisprudence to Medicine

Up to now, "chain of evidence" has become an essential term in the field of law, and it is also widely used in daily life. In the process of exploring the mechanism of pharmacology, toxicology and pathology in the medical field, the thinking method and cognitive path of evidence chain and corroboration theory also play an important role. For example, scholars have classified the types of structures within the evidence chain system and concluded that the evidence chain can form topological structures such as the simple structure, the sequence structure, the convergence structure, and the coordination structure after integration. Based on the relation state of mutual corroboration between the evidence chain, sometimes an evidence is premised on the existence of another, or more than one evidence, at this time there is a simple structure, sequence structure, convergence structure and coordination structure and other related state. This leads to the fact that what is reflected in one piece of evidence is often linked to one or more other pieces of evidence in a variety of ways based on corroboration. In the medical field, take the diagnosis of leukemia as an example, in the process of determining that a patient is suffering from leukemia, the doctor, in addition to making a preliminary judgment based on the patient's clinical symptoms, also needs to combine the routine blood tests, bone marrow aspiration, cytotyping, immunophenotyping and other medical tests. On the basis of mutual corroboration of these test reports, the patient is identified as belonging to acute myeloid leukemia or granulocytic leukemia, etc., so as to realize the targeted treatment for the patient's disease.

The comprehensive judgment of medical testing and examination data, in essence, needs to clarify the mutual corroboration relationship of the contents of the medical report to evaluate the specific condition of the patient. In addition to exploring the pathology and comprehensive judgment, in the process of treating leukemia, in order to explore the mechanism of action and effect of drugs, it is also necessary to determine the recovery status of the patient's condition during the period of receiving treatment through the process mentioned above. And this in turn needs to draw on the " chain of evidence " and the aforementioned theory of evidence utilization to clarify the specific operation process and determination method.

Advances in Chain of Evidence in Medicine

With the growing demand for scientific identification of medical facts and integration of relevant data to make the best clinical decisions in today's medical research and clinical practice, it has become a trend for the chain of evidence theory to penetrate from the field of law to the diversified scenarios of medicine. In the following article, we will focus on the three major fields of forensic toxicology, modern medicine and traditional Chinese medicine, and systematically review the research progress and innovative applications of the chain of evidence in the corresponding fields, to show how this interdisciplinary fusion can standardize the evaluation system of medical evidence, and then lay the foundation for the construction of a more complete and feasible model of evidence-based practice.

Advances in Chain of Evidence in Forensic Toxicology

In recent years, research in the field of forensic toxicology has faced serious challenges: the number of cases of deaths due to drug abuse has continued to rise, and new types of toxicants have shown explosive growth, resulting in a serious lack of basic toxicological information on their chemical structure, metabolic pathways and corresponding products, pharmacokinetic parameters, etc. in the short term; In the process of forensic identification, the pretreatment process of blood, urine and other drug-related biological matrices is cumbersome, and the concentration of the target substances is often low

(often at trace level) and cross-metabolizable with existing substances of abuse, while the lack of cross-reactivity of traditional detection reagents leads to insufficient specificity in identification, which puts forward a higher requirement for the setting of the toxicological screening procedure; The sources of toxicants cover a wide range of categories, including licit drugs, illicit drugs, industrial chemicals and environmental pollutants, some of which may undergo complex processing and disguise and enter the human body through different means, such as oral administration, injection, inhalation and transdermal absorption. These factors together lead to ambiguous source of exposure, difficulty in determining the dose of poisoning, complex analysis of mass spectrometry characteristics, and insufficient interpretation of toxicological mechanisms in poisoning cases, which impede the transformation of forensic toxicology identification results into reliable judicial evidence [18].

Therefore, the introduction of liquid chromatography-high resolution mass spectrometry (LC-HRMS) and other emerging high-precision means of analysis, integrated to form a complementary technical application system, to build a closed chain of evidence covering the signs of the deceased, on-site investigation, pathological examination, biological sample toxicology screening, drug history and family history, etc. It can effectively make up for the gap of basic toxicology data, enhance the timeliness of detection, optimize the allocation of judicial resources, and at the same time, complete the enhancement of the evidence effect of the results of forensic toxicology research. Castro et al. reported a case of fatal poisoning by a mixture of novel psychoactive substances (3-MeO-PCP and o-desmethyltramadol), which demonstrates in full the important value of a multidimensional chain of evidence in the determination of the cause of death: the study synthesized the use of broad-spectrum analytical techniques (e.g., GC-MS, UPLC-MS/MS) to confirm and determine the composition and content of the mixture of the powders, spoon extracts, and injection extracts, respectively, which were obtained from the site investigation collection; Cadaveric whole blood samples were also collected and analyzed in accordance with current laboratory toxicology screening procedures, and 3-MeO-PCP and o-desmethyltramadol were detected in the submitted material at concentrations exceeding the threshold for toxicity; this, combined with anatomo-pathological evidence of pulmonary congestion and mild cerebral edema, further validated the conclusion that the mixture was lethal in overdose [19].

From the collection of suspicious poisons at the scene, the quantification of poisons in the body to the verification of toxicity characterization, the multi-module evidence is closely related and cross-corroborated, forming a clear and typical, logical closed-loop forensic toxicology chain of evidence, eliminating the reasonable doubt of determining the facts, and enhancing the level of evidence for determining the cause of death in the same kind of complex poisoning cases. From point to surface, Tabarra et al.in view of the huge threat to public health caused by the abuse of Novel Synthetic Opioids (NSOs), based on various death reports related to NSOs, summarized the chemical structure, pharmacological effects, toxic threshold, metabolic characteristics and toxic reactions of NSOs such as tetrahydrofuran fentanyl, acetyl fentanyl, butyl fentanyl, allyl fentanyl and U-47700. The significant advantages of LC-HRMS technology in the quantitative analysis of NSOs are highlighted: increasing the molecular mass resolution to 0.001 atomic mass units, deducing the correct molecular formula from an accurate mass database, and accomplishing the detection and identification of compounds with unknown mass spectrometric information at concentrations below 1ng/ml [18]. The toxicological evidence chain of NSOs constructed based on the support of this technology is expected to accelerate the transformation process from toxicology traceability to the determination of judicial relationship, and provide a new method of evidence chain perspective for the accurate determination of cases involving NSOs deaths.

Notably, Adeola et al. focused on the great potential of human hair proteomics in forensic toxicology practice, stating that human hair proteins based on single amino acid polymorphism (SAP) can provide molecular forensic evidence similar to DNA fingerprinting. Moreover, hair proteins can enable longer (more than 24-48h) drug abuse or toxicity tracking compared with blood and urine. It is emphasized that the improvement of the hair proteome database is the key to promote the evidence-based application of this ideal non-invasive sample in forensic toxicology, and to significantly improve the compliance of the acquisition of biological evidence, which is the core link in the construction of the evidence chain in drug-related cases [20].

Advances in Chain of Evidence in Modern Medicine

As the core methodology of integrating multi-source data and strengthening causal association, the chain of evidence theory breaks through the traditional framework of evidence-based medicine through the systematic coupling and dynamic verification of relevant evidence, and demonstrates its unique value in enhancing the scientific and practical efficacy of modern clinical decision-making. Based on the clinical value appeal in the information transmission of drug product positioning, Shen and others pioneered the construction of a medical literature evidence chain centered on product positioning, systematically combing basic research, clinical research, quality control and other sources of evidence system to provide a powerful tool for accurate clinical decision-making, improving the degree of consensus between doctors and patients and the adherence to medication, as well as ensuring the effectiveness of information transfer [21]. He et al focused on the application value of Integrated Evidence Chain (IEC) in the diagnosis of drug-induced liver injury based on the results of prospective cohort study : The integrated causal evidence chain constructed on the basis of patients' medication history, clinical manifestations and chemical test results breaks the limitations of traditional methods and significantly improves the sensitivity of clinical diagnosis of drug-induced liver injury, especially in the assessment of liver injury of traditional Chinese medicines, which is complicated and difficult to trace back [22].

Meanwhile, in the clinical decision-making and prognostic management of Multiple Sclerosis (MS), the demographic characteristics of patients, clinical signs, MRI indexes, and biomarkers such as neurofilament light chain levels are correlated and mutually corroborated. The complete chain of evidence is the key to building a personalized treatment framework, dynamically adjusting treatment strategies, and realizing precise diagnosis and treatment of MS [23]. In addition, in the face of the increasing complexity of public health problems and the new challenge of vague association between suspected causative factors and diseases (showing multiplicity and non-linearity), Rang et al. proposed that public health professionals should be trained to develop core decision-making ability based on the analysis of the evidence chain. They advocate the use of multivariate statistical tools (such as SPSS, R, Stata, etc.) to support the in-depth mining and decision-making analysis of the medical evidence chain, which can help to accurately identify the core chain of causality among the multiple disease-causing factors, and provide methodological innovations for the scientific development of prevention and control strategies [24].

Advances In Chain of Evidence in Traditional Chinese Medicine

Driven by the background of modernization of traditional Chinese medicine, the interdisciplinary cross-fertilization of evidence-based medicine, metabolomics, microbiomics and other disciplines has given rise to the innovative practice of multidimensional evidence chain construction methods, which is aimed at perfecting the scientific research method system of traditional Chinese medicine, and providing an innovative solution for the breakthrough of the bottleneck of traditional research methods. Taking the academic theory of "Spleen deficiency causes arthralgia syndrome" as the starting point, Wen and others constructed a multifaceted and integrated evidence chain of "treating arthralgia syndrome from the spleen", and explained the science and effectiveness of Xin 'an Jianpi Tongbi prescription through the multi-dimensional paths of disease-syndrome combination research, clinical efficacy validation, multi-omics experimental analysis, and the exploration of the mechanism of action. It has established a complete evidence chain research paradigm for the modernization of Chinese medicine, from theoretical innovation to technical evidence, and has also pointed out the direction for the future in-depth integration of multi-omics and exploration of therapeutic targets [25]. Gao et al. used evidence-based medicine research methods to systematically evaluate the clinical efficacy and treatment mechanism of Guizhi Fuling Capsule in various diseases, and integrated the evidence chain with the core pathogenesis "blood stasis" as a clue, not only to compare and demonstrate the superiority of the indications of Guizhi Fuling Capsule in the treatment of related diseases, but also to construct an innovative system of precise drug use and decision-making based on the evolution law of syndromes.

At the same time, they verified the modern guiding value of the theory of "treating diseases with syndromes and treating different diseases with the same method" in traditional Chinese medicine with cross-disease research data, highlighting the unique role of TCM syndromes and pathogenesis changes in disease diagnosis and treatment [26]. In addition, in order to break through the limitations of traditional randomized controlled trials in evaluating the effectiveness of traditional Chinese medicine's multi-component and multi-target system, Xiao et al. pioneered the integrated evidence chain-based effectiveness evaluation of traditional Chinese medicine (iEC-Eff), which is based on clinical experience and organically integrates experimental research and clinical trials to construct a systematic and evidence-based efficacy evaluation system. On the one hand, it provides scientific evidence to verify the effectiveness of classical prescriptions and significantly enhances their level of evidence; on the other hand, it promotes the transformation of Chinese medicine research from traditional empirical medicine to modern evidence-based medicine, and injects a strong impetus for the modernization and internationalization of Chinese medicine [27]. Coincidentally, Liu et al. proposed a new paradigm for the safety evaluation of traditional Chinese medicines, which is constitution-based toxicology, based on the core principle of "constitutional-herb mutual exclusion".

The toxicological evidence chain (TEC), as its central methodological support, closely follows the holistic principle of "constitution differentiation-disease differentiation-syndrome differentiation" in Chinese medicine diagnosis and treatment, and through the integration of multi-level toxicological evidence, solves the pain point of isolation of evidence and ambiguity of mechanism in traditional toxicity research and embeds the constitution characteristics into the whole link of toxicity evaluation. At the same time, a cross-scale data support system is constructed by network toxicology, metabolomics and other emerging technologies, which will help to further clarify the dynamic relationship of "evidence-quantity-toxicity" of toxic Chinese medicines [28].

The Connotation and Advances in Toxicological Evidence Chain

Focusing on key issues such as insufficient analysis of the complex toxicity mechanisms of traditional Chinese medicines, unclear signal transduction networks, and fragmentation of evidence in traditional toxicological analysis, our research group proposes a new strategy—TEC, that is more applicable to the study of toxic Chinese medicines. TEC is composed of five core modules: clinical risk (CRE), injury phenotype evidence (IPE), hazardous ingredients evidence (HIE), toxic events evidence (TEE), and adverse outcomes evidence (AOE). By anchoring the source of toxicity exposure, observing the signs of toxicity, tracking the basis of toxicity, locating the key inflection points of the toxicity process, and focusing on the substantial damage to the target organ, the strategy connects the complete process of toxicity transmission of traditional Chinese medicines step by step and completes the causal determination of toxicity events [28]. Based on the theoretical framework of TEC, Liu et al. comprehensively utilized UP-

LC-Q-TOF/HRMS, molecular docking, and network toxicology to orderly elucidate the HIE, TEE, and AOE of hepatotoxicity induced by the traditional Chinese medicine, dysosma versipellis (DV), and to constructed a rigorous and clear chain of evidence for toxicology. The study preliminarily screened, isolated and identified the potential toxic components of DV, and clarified the core roles of targets such as PAH, SOD1, SOD2 and their associated metabolic pathways, which provided a replicable TEC-based research paradigm for the safety evaluation of traditional Chinese medicine and ethnomedicine [29].

On this basis, Kong et al. introduced the grades of Toxicological Evidence Assessment (GTEA) system to quantitatively assess the quality of evidence for each dimension of TEC to further refine the chain of evidence for Nephrotoxicity Of Podophyllotoxin (PPT): After PPT administration, SD rats showed signs of toxicity such as weight loss, skin erythema, and rhinorrhea, accompanied by behavioral abnormalities such as arching of the back and curling up and Inhibition of Activity (IPE); metabolomics analysis revealed that differential metabolites such as L-methionine, phosphatidylethanolamine, and lysophosphatidylethanolamine triggered disturbances in amino acid metabolism and glycerophospholipid metabolism pathways, as well as significant upregulation in the expression of oxidative stress markers such as indoxyl alcohol sulphate and paracresol sulphate and inflammatory factors (TEE); biochemical tests showed abnormally elevated levels of serum urea nitrogen and creatinine, and pathology confirmed the presence of substantial renal injury such as edema of renal tubular epithelial cells and tubular lumen dilatation (AOE). Combined with network toxicology validation, it reveals that PPT inhibits the autophagy process mediated by PI3K/Akt/mTOR signaling pathway on one hand, and activates the Nrf2/HO1 signaling pathway to trigger oxidative stress on the other hand, which synergistically induces renal dysfunction [30,31]. In the cardiotoxicity study of PPT, Jiang et al. jointly applied targeted metabolomics and tandem mass tags (TMT) quantitative proteomics techniques, and found that the abnormal expression of PPAR pathway-related proteins in cardiac tissues induced metabolic toxicity events (TEE), such as tricarboxylic acid cycle disorders and enhancement of glycolytic processes.

At the same time, the degree of myocardial injury (AOE) was assessed by histopathological examination and myocardial enzyme activity test, combined with the changes in body weight, phenotype, behavior, and organ coefficients in SD rats (IPE) and the results of western blot (WB) validation, to target the key regulatory nodes in the toxicity process, and systematically demonstrated that PPT triggers energy metabolism disruption and inflammation in cardiomyocytes through the modulation of the SIRT1/PPAR/NF-κB signaling axis, which in turn leads to cardiac injury [32,33]. Similarly, Sun et al. strictly followed the TEC research model and integrated microbiomics and metabolomics analyses to add that PPT exposure induces structural dysregulation of the intestinal flora, disrupts the metabolic homeostasis of the intestinal flora, and in turn interferes with cardiac metabolism, emphasizing the key role of the "gut-cardiac" axis in the cardiotoxicity of PPT [34]. In addition, based on the concept of TEC, our group has carried out active scientific practice in the exploration of multi-target organ toxicity mechanisms, such as PPT neurotoxicity, ovarian toxicity, and enterotoxicity, etc. The research ideas and technical paths of TEC can provide standardized references for the analysis of complex toxicity mechanisms of other traditional Chinese medicines and ethnomedicines in the following years, and we expect that this innovative evaluation strategy will be applied in a wider range of toxicological studies, which will provide assistance for the continuous improvement of the safety evaluation system of traditional Chinese medicines [35-39].

Conclusion and Prospect

This paper centers on the theory of "chain of evidence", reviews the theoretical transmutation and evaluation system of the chain of evidence in the field of jurisprudence, and elucidates the basic connotations of the core concepts of "linkage point", "corroboration theory" and "closure of chain of evidence" one by one and clarifies the theoretical basis and deep logic of the migration of the chain of evidence theory to the medical field. In view of the precise identification needs of forensic toxicology in complex poisoning cases in the context of drug abuse, the decision-making challenges of multi-source data integration in modern medicine, and the evidence-based dilemmas in the modernization process of traditional Chinese medicine, this paper systematically combs through the research progress of the chain of evidence in the corresponding fields of medicine, and demonstrates the unique advantages of the closed chain of evidence in the aspects of quickly finding the key causative relationship, enhancing the level of factual evidence, improving the efficacy of the judicial transformation, simplifying the diagnostic process, and optimizing the clinical decision-making. It is worth noting that our group has focused on the safety evaluation of traditional Chinese medicine for a long time, internalized the chain of evidence theory in the toxicity study of traditional Chinese medicine, and proposed a new paradigm of toxicity study of TEC, integrating metabolomics, microbiomics, transcriptomics, and network toxicology to construct a five-dimensional systematic corroboration system of "CRE-IPE-AOE-TEE-HIE" to analyze the cascade toxicity pathway of toxic Chinese medicine DV inducing different target organ damages at multiple levels, with the hope of providing a new methodological breakthrough for the longstanding problems of imperfect research on toxicity mechanism of traditional Chinese medicines and fragmentation of evidences.

In summary, the theory of chain of evidence shows great potential in the medical field, but there are still some limitations in its research practice: Compared with the field of law, the types of evidence in the medical field (clinical symptoms, intervention outcomes, integrated multi-omics data, biomarkers, etc.) are complex and changeable, the standardized procedures and evaluation system of the evidence chain has not yet been completed, and there is a lack of recognized stan-

dards for the division of evidence levels, weight distribution and quality assessment, which can easily lead to subjectivity and arbitrariness in the construction of the evidence chain; at the same time, Chinese medicine emphasizes syndrome differentiation, and the evidence forms such as symptoms and disease mechanisms are ambiguous, complex, and dynamic, making it difficult to adapt to the linear causal logic of the existing evidence chain models; in addition, the acquisition of high-quality evidence puts forward higher requirements for the construction of routine laboratory screening system, such as LC-HRMS technology has been proved to be the most effective method for toxicological analysis of NSOs, but due to its high equipment cost and technical threshold requirements leading to low configuration rate, it is difficult to promote the realization of routine application [40]; despite the long-term monitoring advantages of hair as an ideal non-invasive sample in toxicological evidence tracking, conventional proteomics methods are not applicable due to its physicochemical nature [20].

The TEC strategy is centered on forensics and combines molecular docking techniques to achieve the screening of potentially toxic ingredients, but as a computational method, the results may have false-positive situations, etc [29]. In view of the above shortcomings of the evidence chain in medical research, it is urgent to integrate the characteristics of medical evidence, pay attention to the special characteristics of evidence in Chinese medicine, formulate unified grading standards and develop quantitative evidence assessment tools and models, and then build a standardized framework for the medical evidence chain. Based on the empowerment of artificial intelligence and big data technology, accelerating the research and development of highly accessible equipment such as microfluidic chips, miniaturization, portable mass spectrometry, and at the same time making full use of shared laboratory platforms and cloud-based data analysis and other means will offer the possibility of effectively bridging the gap of high-quality evidence acquisition. In addition, novel techniques such as surface plasmon resonance (SPR) have been introduced into the toxicity studies of traditional Chinese medicines, combined with multi-dimensional validation means such as cell and animal experiments, with a view to providing more accurate information on molecular interactions, and significantly enhancing the level of evidence for the TEC strategy. The interdisciplinary exploration of the chain of evidence theory is promoting the transformation of medical research from empiricism to evidence-based closed-loop, and reshaping the medical facts determination standard with jurisprudential logic is expected to lead the medical field to break through the predicament of fragmentation, which urgently needs to draw more scholars' attention to explore the new paradigm of logically self-consistent evidence-based medical practice.

Author Contributions

All of the authors participated in the discussion and development of consensus management approaches, contributed to editing of the draft manuscript and providing additional recommendations, and have read and approved the final manuscript.

Funding

This study was supported by the National Natural Science Foundation of China (82404774), and Henan Province Excellent Young Scientists Program for Health Science and Technology Innovation (Young and Middle-aged) (YQRC2023003).

Conflicts of Interest

The authors declare no conflict of interest.

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ISSN: 2574-1241

DOI: 10.26717/BJSTR.2025.62.009801

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