JOURNAL OF ANIMAL BEHAVIOUR AND BIOMETEOROLOGY

RESEARCH ARTICLE
Published Online: May 8, 2025
https://doi.org/10.31893/jabb.2025007

Protective effect of herbal medicine on murine spermatogenesis under heat stress and other injury models: Evidence from meta-analyses

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Abstract The incidence of male infertility has increased dramatically in recent decades, and herbal medicine is a potential complementary treatment. Animal studies are essential to gain insights into the mechanisms involved and refine the development of herbal medicine in human trials. This study aimed to systematically review the protective effects of herbal medicines on murine spermatogenesis. A literature search was conducted using databases such as EMBASE, PubMed, and Google Scholar, as well as scientific journals indexed in the Web of Science, until October 21, 2024. The studies included in the analysis focused on the effects of herbal medicines on murine models, specifically examining physical and organ weights, reproductive parameters, metabolic and biochemical markers, oxidative stress, and antioxidant markers. The analysis included 48 studies from 44 articles involving 1597 murine subjects, 27 plant species, and seven formulations. Herbal medicine significantly increased male murine reproductive organ weights, including body weight (SMD = 1.83; 95% CI: 1.24, 2.42), testis weight (SMD = 1.94; 95% CI: 1.42, 2.46), relative testis weight (SMD = 2.01; 95% CI: 1.27, 2.75), epididymis weight (SMD = 1.22; 95% CI: 0.79, 1.65), relative epididymal weight (SMD = 1.93; 95% CI: 0.93, 2.93), seminal vesicle weight (SMD = 1.25; 95% CI: 0.75, 1.75), and relative seminal vesicle weight (SMD = 1.67; 95% CI: 0.81, 2.54). Additionally, treatment increased the seminiferous tubule diameter (SMD = 9.10; 95% CI: 4.93, 13.28) and Johnson's score (SMD = 6.40; 95% CI: 3.73, 9.06). In the injury models, the semen parameters, including sperm count (SMD = 3.47; 95% CI: 2.72, 4.21), motility (SMD = 3.57; 95% CI: 2.70, 4.44), viability (SMD = 2.04; 95% CI: 1.21, 2.87), and abnormal sperm morphology (SMD = -2.28; 95% CI: -3.65, -1.72), improved. Although no significant changes were observed in FSH and LH levels, the serum testosterone levels increased favorably. Moreover, blood serum biochemical parameters and oxidative stress markers in the injured mouse model were improved following herbal treatment. Herbal medicines have been shown to enhance spermatogenesis in murine models. These in-depth findings provide a foundation for optimizing future human studies.

Keywords: herbal medicine, male murine, sexual dysfunction, fertility impairment, male reproductive system, spermatogenesis

1. Introduction

Male infertility has increased over the past few decades, with over 55 million cases reported worldwide, an alarming 76.9% increase since 1990 (Huang B. et al., 2023). This increase may be partly linked to the ongoing decline in the male sperm count over the last 50 years (Sengupta et al., 2018). An increase in some of these factors, such as genetic abnormalities, environmental toxins, unhealthy lifestyle choices, and chronic medical conditions, can contribute to this increasing trend. Genetic mutations, such as those in AZF, are strongly associated with conditions such as azoospermia (Ambulkar et al., 2014). Environmental exposure to toxins (López-Botella and Velasco 2021), unhealthy lifestyles (Balawender and Orkisz 2020), and medical conditions such as diabetes (Ding et al., 2015) also compromise male fertility by affecting sperm quality and testicular function.

Heat stress, one of the core mediators that negatively affects male reproductive function (Hoang-Thi et al., 2022), alters the expression of genes and proteins involved in spermatogenesis, the cornerstone of male reproductive health. It reduces the expression of antioxidant enzymes, such as GPx4, GSTm5, and PRx4, and SOD activity, increasing the vulnerability of the testes to oxidative damage (Bui-Le and Hoang-Tan 2023, Karimi et al., 2019, Kim M. K. et al., 2017, Kumar Roy et al., 2016). Heat stress also affects spermatogenesis proteins, reducing CREB1 and INHA expression (Kim M. K. et al., 2017, Kopalli et al., 2019), which are essential for FSH regulation, while increasing the levels of certain proteins, such as collagen chains and histones (Panpan et al., 2023). Additionally, heat stress reduces the expression of sex hormone receptors, disrupting hormone regulation and testicular function (Bui-Le and Hoang-Tan 2023, Kopalli et al. 2019).

Several parameters can serve as indicators of reproductive health outcomes. Testicular and epididymal weights, for example, are known reflections of reproductive function (Boeri et al. 2021), with reductions in these weights often signifying testicular damage and impaired fertility. In some studies, heat stress has been shown to significantly reduce epididymal weight in mice, and STZ-induced diabetes leads to decreased testicular weight (Shi et al. 2017). Similarly, cisplatin (Aslan et al. 2021) and lead (Pb) (Udefa et al. 2020) are known to reduce testicular weight. In addition to organ weight, other methods, such as semen analysis, histological examination of the testes, and evaluation of reproductive hormone levels, are used to assess reproductive health. Semen analysis provides insights into sperm count, motility, and morphology, whereas histological analysis reveals structural damage to the testes. Hormone levels, such as testosterone, follicle-stimulating hormone (FSH), and luteinizing hormone (LH), are also crucial; alterations in these levels can indicate reproductive dysfunction.

The use of herbal medicines for the treatment of male reproductive disorders has become increasingly popular. This is mainly because conventional treatments for male infertility, including hormonal therapies and assisted reproductive technologies (ART), are not only costly but also fraught with risks and variable success rates (Wal et al. 2022). Moreover, many cases remain idiopathic, increasing the need for therapeutic approaches (Eisenberg and Esteves 2023). The trend of using herbal medicines is driven by factors such as the safety and lower side effects of herbs than conventional methods do, their easy accessibility and low cost, especially in developing countries, and, more importantly, their proven effectiveness through traditional use, as proven by a meta-analysis by Nguyen-Thanh et al. (2024) (Nguyen-Thanh et al. 2024).

Studies on the effectiveness of herbal medicines in the male reproductive system in humans have often investigated sperm quality, hormonal balance, and oxidative stress profiles (Nguyen-Thanh et al. 2024). However, these studies have significant heterogeneity and methodological limitations, such as inconsistent dosing, inadequate control groups, and insufficient reporting of safety profiles, which weakens the strength of the evidence (Barratt et al. 2017). In addition, high-quality, placebo-controlled, randomized trials focusing on clinically meaningful endpoints such as time-to-pregnancy (TTP) and live birth rates, rather than observational studies, are lacking.

Animal studies, particularly those using murine models, have addressed the many limitations inherent to human research. Owing to their genetic similarity to humans and well-characterized reproductive systems, murine family members offer an effective model for studying the impact of various treatments on spermatogenesis (Cunha et al. 2019). These models allow for controlled examination of spermatogenesis at the cellular and molecular levels. They help uncover the mechanisms by which herbal medicines may protect, repair, or enhance testicular function under stress, such as heat, toxins, or oxidative damage. For example, heat stress has been shown to impair key molecular pathways in spermatogenesis, including the reduced expression of antioxidant enzymes and hormone receptors (Hou et al. 2020, Kim M. K. et al. 2017). Animal studies have refined the development of herbal medicines by determining their optimal dosages, routes of administration, formulations, and toxicity profiles. They also provide foundational data for translational research, bridging the gap between preclinical findings and clinical applications.

Therefore, we carried out this systematic review to evaluate the protective effects of herbal medicines on murine spermatogenesis, their mechanistic actions, and their potential to inform future human trials.

2. Materials and Methods

2.1. Information sources and search strategy

Embase, Web of Science, PubMed, Google Scholar, and various other scientific websites were used to identify potentially relevant studies on the effects of herbal medicines on weight parameters, sperm parameters, serum reproductive hormone levels, serum biochemistry, and oxidative stress markers in murine reproductive dysfunction. The search keys used throughout the system were "sexual dysfunction", "fertility impairments", "male reproductive system", "spermatogenesis", "testicular and spermatozoal toxicity", "antioxidants", "reproductive hormones", "herbal medicines", "testicular damage", "oxidative stress", "testosterone", "semen parameters", "sex hormones", "seminal biochemical", “testicular proteomics”, "seminal antioxidant", "semen analysis", "antioxidants", "lipid peroxides", "seminal plasma", and "catalase activity". The search was performed exclusively in American English, following its spelling, terminology, and expressions.

2.2. Inclusion and exclusion criteria

The selection criteria were as follows: (i) studies examining the associations among herbal medicine remedies, weight parameters, sperm parameters, serum biochemical levels, sex hormones, Johnson's score, and the testicular antioxidant profile. (ii) These studies should have presented at least one of the following outcome measures: physical and organ weights (body weight, testis weight, relative testis weight, epididymal weight, relative epididymal weight, seminal vesicle weight, and relative seminal vesicle weight); reproductive parameters (seminiferous tubule diameter, Johnson's score, sperm count, sperm motility, sperm viability, and sperm abnormality); hormonal levels (testosterone, luteinizing hormone (LH), and follicle-stimulating hormone (FSH)); metabolic and biochemical markers (glucose concentration, glutamic oxaloacetic transaminase activity (GOT), glutamic pyruvic transaminase activity (GPT), and creatinine); and oxidative stress and antioxidant markers (malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx)). We excluded

duplicated scientific publications, in vitro studies, nonmurine studies, studies that had insufficient data, unmeasurable data displayed through graphs, or conference abstracts and reviews. Additionally, any studies with no available information on the subjects of murine and herbal medicines were also excluded.

2.3. Data collection for analysis

The data extracted from each study included the first author's name, publication year, and country of origin, as well as the number of subjects. Organ weights, including body weight in grams, testis weight in milligrams, relative testis weight as a percentage, epididymis weight in milligrams, relative epididymal weight as a percentage, seminal vesicle weight in milligrams, and relative seminal vesicle weight as a percentage, were reported. Reproductive parameters such as seminiferous tubule diameter in micrometers, Johnson's score on a scale of 1--10, sperm count expressed as million sperm per milliliter, sperm motility and viability as percentages, and abnormal sperm percentage were also included. Hormonal testosterone levels are reported in nanograms per milliliter, and follicle-stimulating hormone (FSH) and luteinizing hormone (LH) levels are reported in milli-international units per milliliter. Metabolic and biochemical markers such as glucose concentration in milligrams per deciliter, glutamic oxaloacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT) activity levels in international units per liter, creatinine levels in micromoles per liter, and malondialdehyde (MDA) activities in serum and testes in nanomoles per milliliter and nanomoles per milligram, respectively, were also recorded. Additionally, superoxide dismutase (SOD) activity in the serum and testes, catalase activity, and glutathione peroxidase (GPx) activity were measured in units per milliliter and nanomoles per milligram, respectively. The metric values are presented as the means ± standard deviations (SDs).

2.4. Statistical analysis

A meta-analysis was conducted via Stata SE version 15.0 software (Stata Corp., College Station, TX, USA) to investigate the effects of herbal medicines on murine spermatogenesis. A random effects model was used to summarize the relationship between the two variables. The values of the injury and injury treatment groups were compared via the standard mean difference (SMD) method to assess the effects of herbal medicines on body weight, reproductive organ weight, semen parameters, serum biochemical levels, sex hormones, Johnson's score, and testicular antioxidant profiles. This study used 95% confidence intervals (CIs) to estimate the mean effects of herbal medicines on the aforementioned indices. Publication bias was evaluated via Egger's test and funnel plots with a significance level of 0.05. Heterogeneity between the studies was assessed via the Higgins I^2 metric.

3. Results

3.1. Characterization of eligible studies

Studies were searched through Embase, Web of Science, PubMed, Google Scholar, and other scientific websites. The process of searching for and filtering meta-analyses is illustrated in Figure 1. A total of 1413 articles were retrieved through a comprehensive search using designated keywords. After the titles, summaries, and full texts were analyzed, 1137 articles were excluded because they did not meet the inclusion criteria. The remaining 276 full-text articles were evaluated. Consequently, 232 articles were excluded for the following reasons: nonmurine studies, in vitro studies, studies with small sample sizes, duplicate content, review papers, studies with data only in graphic form, studies with data not provided, and studies that did not report the data of interest. After applying all the inclusion criteria, 23 articles were included in the meta-analysis or systematic review. This study analyzed 48 studies from 44 scientific articles over 16 years from 2007--2023 in terms of the effects of herb treatment on the male reproductive system, sperm parameters, sex hormones, blood biochemical levels, and oxidative stress markers in 1597 male mice. The search was completed on October 21, 2024 (Table 1). Among these 44 scientific reports, 30 articles reported on the male reproductive system, 23 articles reported information on semen parameters, and 20 articles related to sex hormones. Blood biochemical levels were reported in seven articles, and details concerning oxidative stress markers were reported in 19 articles.

3.2. Associations between herbal medicines and the male murine reproductive system

The effects of herbal treatments on body weight were analyzed in 35 studies from 19 scientific articles (Figure 2a). The analysis employed a random effects model to evaluate these studies. The difference between the studies was large, with an I-squared heterogeneity index of 87.8%. Each sample was weighed (%weight) from 0.22 to 3.61. The results of the meta-analysis revealed that the standard mean difference (SMD) between the injury model group and the herbal treatment group was 1.83 (95% CI: 1.24, 2.42). These results suggest that herbal treatments increase body weight. The Egger test was used to evaluate publication bias in the studies that examined the impact of herbal treatments on body weight. The results revealed publication bias (P= 0.000) (not presented graphically).

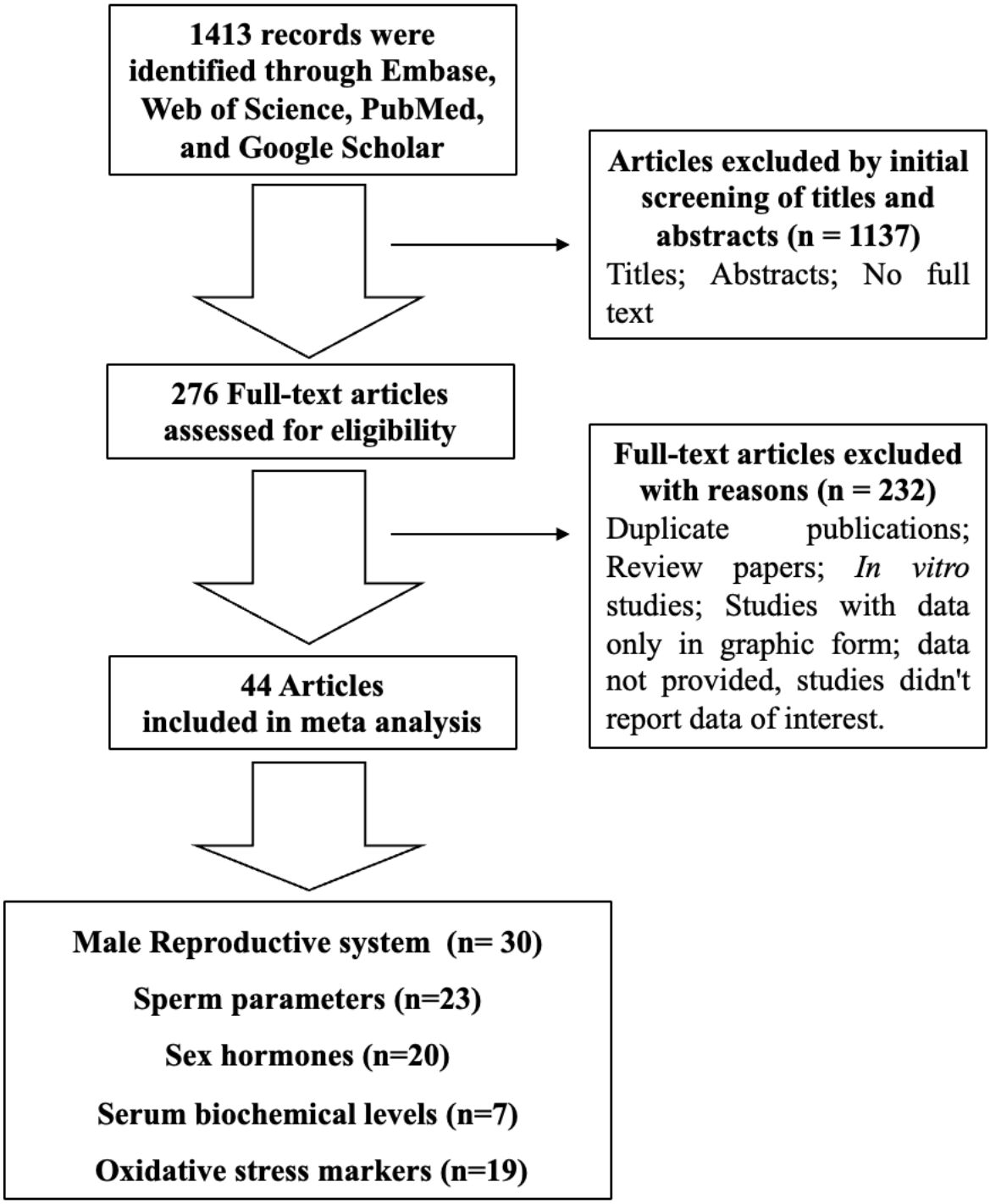

Figure 1 The process of selecting articles for the meta-analysis.

Figure 2b presents an analysis of the effects of herbal treatments on testis weight in 46 studies from 21 scientific articles, which revealed a significant difference between studies (I-squared heterogeneity = 88.6%, P<0.001). The weight allocated to each study ranged from 0.37% to 2.60%. The results of the analysis revealed that the SMD between the injury model group and the herbal treatment group was 1.94 (95% CI: 1.42, 2.46), suggesting that the use of medicinal plants can increase the testis weight. The effects of herbal treatments on the relative testis weight (testis weight/body weight × 100%) were analyzed in 26 studies. The meta-analysis results revealed that the difference in SMD between the injury model group and the herbal treatment group was 2.01 (95% CI: 1.27, 2.75). The use of medicinal plants significantly increased the relative weight of the testes. The Egger test results (P for bias = 0.000) indicated evidence of publication bias in the studies that examined the effects of herbal treatments on testis weight and relative testis weight in male mice.

Table 1 Summary of studies included in the meta-analysis.

No	Study	ID	Murine	Injury factor	Herbal/Formulation	Composition	Dosage	Length of treatment (week)	Sample size			Main outcome				
									Total	Injury model	Herbal treatment	Male Reproductive system	Sperm parameters	Sex hormones	Serum biochemical levels	Oxidative stress markers
1	(Shahraki et al. 2019)	Mohammad 2019 (1)	Rat	Streptozotocin	*A pyrethrum*	Ethanol extract of root	50 mg/kg/day	4	20	10	10	Bw; Tw, Ew				
		Mohammad 2019 (2)	Rat	Streptozotocin	*A pyrethrum*	Ethanol extract of root	100 mg/kg/day	4	20	10	10	Bw; Tw, Ew				
		Mohammad 2019 (3)	Rat	Streptozotocin	*A pyrethrum*	Ethanol extract of root	150 mg/kg/day	4	20	10	10	Bw; Tw, Ew				
2	(Sharma et al. 2021)	Vikas 2021 (1)	Rat	Lead acetate	*A pyrethrum*	Ethanol extract of root	150 mg/kg/day	13	12	6	6	Bw; Rtw; Rew; Rsvw	S; M; V	T		
3	(Bahmanpour et al. 2012)	Soghra 2012 (1)	Rat	Busulfan	*C tinctorius*	Aqueous extract of herbal	10 mg/kg/day	5	24	12	12	Tw, Ew; Svw	S; M;	T		
		Soghra 2012 (2)	Rat	Busulfan	*C tinctorius*	Aqueous extract of herbal	25 mg/kg/day	5	24	12	12	Tw, Ew; Svw	S; M;	T		
		Soghra 2012 (3)	Rat	Busulfan	*C tinctorius*	Aqueous extract of herbal	50 mg/kg/day	5	24	12	12	Tw, Ew; Svw	S; M;	T		
4	(Huang H. et al. 2017)	Hefei 2017	Rat	Streptozotocin	*C officinalis*	Total terpenes	50 mg/kg/day	6	25	12	13			T, FSH, LH	BGL	
5	(Bui-Le and Hoang-Tan 2023)	Thanh 2023 (1)	Mice	Heat stress	*C orchioides*	Aqueous extract of rhizomes	100 mg/kg/day	5	12	6	6	Js		T		
		Thanh 2023 (2)	Mice	Heat stress	*C orchioides*	Aqueous extract of rhizomes	200 mg/kg/day	5	12	6	6	Js		T		
		Thanh 2023 (3)	Mice	Heat stress	*C orchioides*	Aqueous extract of rhizomes	400 mg/kg/day	5	12	6	6	Js		T		
6	(Lin et al. 2015)	Chunmei 2015 (1)	Mice	Heat stress	*C longa*	Curcumin	20 mg/kg/day	2	40	20	20			T		MDAt; SODt
		Chunmei 2015 (2)	Mice	Heat stress	*C longa*	Curcumin	40 mg/kg/day	2	40	20	20			T		MDAt; SODt

		Chunmei 2015 (3)	Mice	Heat stress	*C longa*	Curcumin	80 mg/kg/day	2	40	20	20			T		MDA; SODt
7	(Karimi et al. 2019)	Samaneh 2019	Mice	Titanium dioxide	*C longa*	Curcumin	200 mg/kg/day	7	16	8	8	Bw; Tw; Rtw	S; A			
8	(Sudjarwo et al. 2017)	Sri 2017 (1)	Rat	Lead acetate	*C longa*	Curcumin	100 mg/kg/day	6	16	8	8		S; M; V			MDA; SODt; GPx
		Sri 2017 (2)	Rat	Lead acetate	*C longa*	Curcumin	200 mg/kg/day	6	16	8	8		S; M; V			MDA; SODt; GPx
		Sri 2017 (3)	Rat	Lead acetate	*C longa*	Curcumin	400 mg/kg/day	6	16	8	8		S; M; V			MDA; SODt; GPx
9	(Hady et al. 2023)	Alsayed 2023	Rat	Lead acetate	*C longa*	Curcumin	100 mg/kg/day	4	20	10	10			T; FSH; LH		MDA; SODt; CAT; GPx
10	(Bustani et al. 2022)	Ghadeer 2022	Rat	Nicotine	*C longa*	Curcumin	300 mg/kg/day	4	20	10	10		M; V			
11	(Udefa et al. 2020)	Augustine 2020	Rat	Lead acetate	*C esculentus*	Ethanol extract of herbal	500 mg/kg/day	3	10	5	5	Bw; Tw; Rtw; Ew; Rew	S; M; V; A			MDA; SODt; GPx
		Augustine 2020	Rat	Lead acetate	*C esculentus*	Ethanol extract of herbal	1000 mg/kg/day	3	10	5	5	Bw; Tw; Rtw; Ew; Rew	S; M; V; A			MDA; SODt; GPx
12	(Nouri et al. 2009)	Mohammad 2009	Rat	Gentamicin	*D carota*	Ethanol extract of seeds	400 mg/kg/day	4	18	9	9		M	T; FSH; LH		
13	(Yuan et al. 2014)	Ding 2014 (1)	Mice	Cyclophosphamide	*Epimedium* spp.	Total Flavonoids	200 mg/kg/day	5	20	10	10	Bw; Tw; Rtw; Ew; Rew	S; M			MDA; SODt; GPx
		Ding 2014 (2)	Mice	Cyclophosphamide	*Epimedium* spp.	Total Flavonoids	400 mg/kg/day	5	20	10	10	Bw; Tw; Rtw; Ew; Rew	S; M			MDA; SODt; GPx
14	(Fungfuang et al. 2016)	Wirasak 2016 (1)	Rat	Streptozotocin	*K parviflora*	Ethanol extract of rhizomes	140 mg/kg/day	6	12	6	6	Tw; Ew; Svw; Std; Js			BGL	
		Wirasak 2016 (2)	Rat	Streptozotocin	*K parviflora*	Ethanol extract of rhizomes	280 mg/kg/day	6	12	6	6	Tw; Ew; Svw; Std; Js			BGL	
		Wirasak 2016 (3)	Rat	Streptozotocin	*K parviflora*	Ethanol extract of rhizomes	420 mg/kg/day	6	12	6	6	Tw; Ew; Svw; Std; Js			BGL	

15	(Shi et al. 2017)	Guang 2017 (1)	Mice	Streptozotocin	*L barbarum*	Polysaccharides Lycium barbarum	10 mg/kg/day	10	50	25	25	Bw; Tw; Rtw; Ew; Rew; Svw; Rsvw	S; M; V; A			
		Guang 2017 (2)	Mice	Streptozotocin	*L barbarum*	Polysaccharides Lycium barbarum	20 mg/kg/day	10	50	25	25	Bw; Tw; Rtw; Ew; Rew; Svw; Rsvw	S; M; V; A			
		Guang 2017 (3)	Mice	Streptozotocin	*L barbarum*	Polysaccharides Lycium barbarum	40 mg/kg/day	10	50	25	25	Bw; Tw; Rtw; Ew; Rew; Svw; Rsvw	S; M; V; A			
16	(Kumar Roy et al. 2016)	Vikas 2015	Rat	Heat stress	*M roxbhurghianus*	Methanol extract of leaves	400 mg/kg/day	2	14	7	7			T		MDAt; SODt; CAT; GPx
17	(Cele et al. 2017)	Nkosinathi 2017 (3)	Rat	n-butanol	*M procumbens*	Methanol extract of root	50 mg/kg/day	4	10	5	5	Tw	S	T	SGOT; SGPT; Cr	MDAt; SODs
		Nkosinathi 2017 (4)	Rat	n-butanol	*M procumbens*	Methanol extract of root	250 mg/kg/day	4	10	5	5	Tw	S	T	SGOT; SGPT; Cr	MDAt; SODs
18	(Lamondo et al. 2014)	Djuna 2014 (1)	Rat	Lead acetate	*M pendans*	Ethanol extract of herbal	135 mg/kg/day	16	14	7	7					MDAs; SODs
		Djuna 2014 (2)	Rat	Lead acetate	*M pendans*	Ethanol extract of herbal	270 mg/kg/day	16	14	7	7					MDAs; SODs
		Djuna 2014 (3)	Rat	Lead acetate	*M pendans*	Ethyl acetate fraction of herbal	20 mg/kg/day	16	14	7	7					MDAs; SODs
		Djuna 2014 (4)	Rat	Lead acetate	*M pendans*	Ethyl acetate fraction of herbal	40 mg/kg/day	16	14	7	7					MDAs; SODs
19	(Mohajeri and Kaffashi Elahi 2015)	Mohajeri 2015 (1)	Mice	Heat stress	*N sativa*	Seeds	10% basal diet	10	20	10	10					MDAt; SODt; CAT; GPx
		Mohajeri 2015 (2)	Mice	Heat stress	*N sativa*	Seeds	20% basal diet	10	20	10	10					MDAt; SODt; CAT; GPx
20	(El-Ashmawy et al. 2007)	Ibrahim 2007 (1)	Rat	Ethanol	*O majorana*	Marjoram volatile oil	0,16 ml/kg/day	10	20	10	10	Tw, Ew	S; M; A	T		MDAt; GPx

21	(Cele et al. 2017)	Nkosinathi 2017 (1)	Rat	n-butanol	*O paniculosa*	Methanol extract of root	50 mg/kg/day	4	10	5	5	Tw	S	T	SGOT; SGPT; Cr	SODs; MDAt
		Nkosinathi 2017 (2)	Rat	n-butanol	*O paniculosa*	Methanol extract of root	250 mg/kg/day	4	10	5	5	Tw	S	T	SGOT; SGPT; Cr	SODs; MDAt
22	(Jang et al. 2011)	Mi 2014 (1)	Mice	Ethanol	*P ginseng*	Ethanol extract of root	2,0 g/kg/day	5	20	10	10	Bw; Tw; Rtw; Ew; Rew; Svw; Rsvw				
		Mi 2014 (2)	Mice	Ethanol	*P ginseng*	Red Ginseng Wine	6,0 g/kg/day	5	20	10	10	Bw; Tw; Rtw; Ew; Rew; Svw; Rsvw				
23	(Kopalli et al. 2019)	Spandana 2019 (1)	Rat	Heat stress	*P ginseng*	Ethanol extract of root	100 mg/kg/day	24	20	10	10	Tw; Ew	M		BGL	
		Spandana 2019 (2)	Rat	Heat stress	*P ginseng*	Ethanol extract of root	200 mg/kg/day	24	20	10	10	Tw; Ew	M		BGL	
24	(Kim Y. H. et al. 2010)	Young 2010	Rat	Testicular Torsion/Detorsion	*P ginseng*	Steamed ginseng	100 mg/kg/day	4	20	10	10			T; FSH; LH		
25	(Park J. S. et al. 2006)	Jeong 2006	Rat	TCDD	*P ginseng*	Ethanol extract of tissue cultured root	30 mg/kg/day	4	18	9	9	Bw; Tw; Ew				
26	(Kim M. K. et al. 2017)	Min 2017 (1)	Rat	Heat stress	*P ginseng*	Equeous extract of root	100 mg/kg/day	8	20	10	10	Tw; Ew	M		BGL; SGOT; SGPT; Cr	
		Min 2017 (2)	Rat	Heat stress	*P ginseng*	Equeous extract of root	200 mg/kg/day	8	20	10	10	Tw; Ew	M		BGL; SGOT; SGPT; Cr	
27	(Aslan et al. 2021)	Esra 2021	Rat	Cisplatin	*P ginseng*	Ginseng powder	200 mg/kg/day	4	14	7	7	Bw; Tw; Rtw; Js				
28	(Sharma et al. 2021)	Vikas 2021 (3)	Rat	Lead acetate	*P murex*	Ethanol extract of fruits	150 mg/kg/day	13	12	6	6	Bw; Rtw; Rew; Rsvw	S; M; V	T		
29	(Besong and Ateufack 2018)	Egbe 2018 (1)	Rat	Amitriptyline	*P arboreus*	Methanol extract of leaf	46.5 mg/kg/day	8	16	8	8	Tw; Ew; Svw		T; FSH; LH		
		Egbe 2018 (2)	Rat	Amitriptyline	*P arboreus*	Methanol extract of leaf	93 mg/kg/day	8	16	8	8	Tw; Ew; Svw		T; FSH; LH		
30	(Yang et al. 2016)	Woong 2016 (1)	Rat	Bisphenol A	*R sativus*	Ethanol extract of herbal	25 mg/kg/days	2	10	5	5	Bw; Rtw				
		Woong 2016 (2)	Rat	Bisphenol A	*R sativus*	Ethanol extract of herbal	50 mg/kg/days	2	10	5	5	Bw; Rtw				

		Woong 2016 (3)	Rat	Bisphenol A	*R sativus*	Ethanol extract of herbal	100 mg/kg/days	2	10	5	5	Bw; Rtw				
31	(Offor et al. 2019)	Samuel 2019 (1)	Rat	Lead Acetate	*S Anomalum*	Ethanol extract of fruit	452 mg/kg/day	4	12	6	6	Bw; Rtw	S; M; V			MDAs; SODs
		Samuel 2019 (2)	Rat	Lead Acetate	*S Anomalum*	Ethanol extract of fruit	678 mg/kg/day	4	12	6	6	Bw; Rtw	S; M; V			MDAs; SODs
32	(Sharma et al. 2021)	Vikas 2021 (2)	Rat	Lead acetate	*S acmella*	Ethanol extract of flowers	150 mg/kg/day	13	12	6	6	Bw; Rtw; Rew; Rsvw	S; M; V	T		
33	(Panpan et al. 2023)	Zhang 2022	Rat	Cold stress	*S oleracea*	Phytoestrogens	30% basal diet	24	24	12	12	Bw; Tw; Rtw; Ew; Rew	M; V	T; FSH; LH		
34	(Boudou et al. 2013)	Farouk 2013	Rat	Manganese (II) Chloride tetrahydrate	*S aromaticum*	Essential oil from flower buds	0.1 ml/kg/day	3	12	6	6	Bw; Tw	A			
35	(Pomjunya et al. 2017)	Atchariya 2017 (1)	Rat	Streptozotocin	*V cinerea*	Ethanol extract of herbal	10 mg/kg/day	5	12	6	6	Bw; Tw; Ew	S; M; V; A	T	BGL	
		Atchariya 2017 (2)	Rat	Streptozotocin	*V cinerea*	Ethanol extract of herbal	40 mg/kg/day	5	12	6	6	Bw; Tw; Ew	S; M; V; A	T	BGL	
36	(El-Ashmawy et al. 2007)	Ibrahim 2007 (2)	Rat	Ethanol	*V vinifera*	Ethanol extract of seeds	75 mg/kg/day	10	20	10	10	Tw, Ew	S; M; A	T		MDAt; GPx
37	(Hala et al. 2010)	Hala 2010	Rat	Aluminum chloride	*V vinifera*	Ethanol extract of seeds	75 mg/kg/day	10	10	5	5	Bw; Rtw; Rew	S; M; V; A	T		SODt
38	(Alkhedaide et al. 2016)	Adel 2016	Rat	Cadmium	*V vinifera*	Ethanol extract of seeds	400 mg/kg/day	12	20	10	10					MDAt; CAT; GPx
39	(Bayatli et al. 2013)	Firuze 2013	Rat	Testicular Torsion/Detorsion	*V vinifera*	Seed pronthocyanidin extract	100 mg/kg/day	1	18	9	9	Std; Js				MDAt
40	(Hozayen 2013)	Walaa 2013 (1)	Rat	Ethanol	*Z Officinale*	Aqueous extract of root	500 mg/kg/day	4	12	6	6			T; FSH; LH		MDAt; GPx
		Walaa 2013 (2)	Rat	Ethanol	*Z Officinale*	Methanol extract of root	200 mg/kg/day	4	12	6	6			T; FSH; LH		MDAt; GPx
41	(Mt et al. 2018)	Mohammad 2018	Rat	Streptozotocin	*Z Officinale*	Ethanol extract of rhizome	200 mg/kg/day	6	24	12	12	Bw; Tw; Rtw				
42	(Mallick et al. 2007)	Chhanda 2007	Rat	Streptozotocin	MTEC	Methanol extract of herbal	1200 mg/kg/day	2	16	8	8	Bw; Rtw; Rsvw; Std	S;V			
43	(Hou et al. 2020)	Ying 2020 (1)	Rat	Heat stress	*DZBTS*	Ethanol extract of herbal	0.4853 g/kg/day	2	22	11	11			T; FSH; LH		MDAs; SODs

		Ying 2020 (2)	Rat	Heat stress	*DZBTS*	Ethanol extract of herbal	0.9707 g/kg/day	2	22	11	11			T; FSH; LH		MDAs; SODs
44	(Park H. J. and Koo 2017)	Hyun 2017 (1)	Rat	LH	*KH-465*	Ethanol extract of herbal	200 mg/kg/day	4	30	15	15	Bw; Tw; Ew	M	T; FSH; LH		SODt
		Hyun 2017 (2)	Rat	LH	*KH-465*	Ethanol extract of herbal	400 mg/kg/day	4	30	15	15	Bw; Tw; Ew	M	T; FSH; LH		SODt
45	(Behdarvand-Magha and Ahangarpour 2021)	Zeinab 2020	Mice	Methylglyoxa	*Gallnuts; Sumac; Tea leaves; Oak bark*	Gallic acid	50 mg/kg/day	7	20	10	10	Tw; Std	S;		BGL	MDAs; SODs
46	(Hwang et al. 2015)	Deok 2015 (1)	Mice	Heat stress	*KOK*	Aqueous extract	0.25 g/kg/day	5	16	8	8	Bw, Tw; Rtw	S;M			
		Deok 2015 (2)	Mice	Heat stress	*KOK*	Aqueous extract	0.50 g/kg/day	5	16	8	8	Bw, Tw; Rtw	S;M			
		Deok 2015 (3)	Mice	Heat stress	*KOK*	Aqueous extract	2.00 g/kg/day	5	16	8	8	Bw, Tw; Rtw	S;M			
47	(Zhang et al. 2020)	Kaishu 2020 (1)	Rat	Tripterygium glycosides	*QLPs*	QLPs powder	1.62 g/kg/day	9	20	10	10			T; FSH; LH		MDAt; SODt
		Kaishu 2020 (2)	Rat	Tripterygium glycosides	*QLPs*	QLPs powder	3.24 g/kg/day	9	20	10	10			T; FSH; LH		MDAt; SODt
48	(Ji et al. 2016)	Hai 2016 (1)	Mice	X-rays	*WZYZP*	WZYZP powder	0.25 g/kg/day	3	24	12	12	Tw; Ew;	S, M; A	T; FSH; LH		
		Hai 2016 (2)	Mice	X-rays	*WZYZP*	WZYZP powder	1.0 g/kg/day	3	24	12	12	Tw; Ew;	S, M; A	T; FSH; LH		

Note: Bw: Body weight (g); Tw: Testis weight (mg); Rtw: Relative testicular weights (%); Ew: Epididymis weight (mg); Rew: Relative epididymis weight (%); Svw: Seminal vesicle weight (mg); Rsvw: Relative seminal vesicle weight (%); Std: Seminiferous tubule diameter (μm); Js: Johnson's score; Sperm count (million/ml); M: Motility (%); V: Viabillity (%); A: Abnormality (%); T: Testosterone (ng/ml); FSH: Follicle stimulating hormone (mIU/ml); LH: Luteinizing hormone (mIU/ml): BGL: Blood glucose level (mg/dL); SGOT: Serum glutamic-oxaloacetic transaminase; SGPT: Serum glutamic pyruvic transaminase; Cr: Creatinine (μmol/L); MDAs: Serum malondialdehyde (nmol/mL); SODs: Serum superoxide dismutase (U/ml); MDAt: Testicular malondialdehyde (nmol/mg); SODt: Testicular superoxide dismutase (U/mg protein); CAT: Testicular catalase (μmol/mL); TCDD: 2,3,7,8-Tetrachlorodibenzo-p- dioxin.

The components of MTEC include the roots of *Musa paradisiaca*, seeds of *Tamarindus indica*, seeds of *Eugenia jambolana*, and leaves of *Coccinia indica (ratios of 2:2:1:1*, respectively*)*.

The components of Duzhong Butiansu (DZBTS) include *Eucommia ulmoides* Oliv. (3.69%), *Cuscuta australis* R. Br. (3.69%)*; Cistanche deserticola* Y. C. (3.69%)*; Polygala tenuifolia* Willd. (3.69%); *Angelica sinensis* (Oliv.) Diels. (3.69%), *Nelumbo nucifera* Gaertn. (3.69%); *Alisma orientale* (Sam.) Juzep. (3.69%); *Paeonia suffruticosa* Andr. (3.69%); *Paeonia suffruticosa* Andr. (3.69%); *Epimedium brevicomu* Maxim. (3.32%); *Astragalus membranaceus* (Fisch.) Bge. (7.38%), *and Rehmannia glutinosa* Libosch. (7.38%)*; Dioscorea opposita* Thunb. (7.38%)*; Poria cocos* (Schw.) Wolf (7.38%); *Atractylodes macrocephala* Koidz. (7.38%), *and Citrus reticulata* Blanco. (1.85%), *Amomum villosum* Lour. (1.85%), *Ligustrum lucidum* Ait. (1.66%), *and Rosa laevigata* Michx. (1.66%), *Cornus officinalis Sieb. et Zucc.* (0.37%), *Morinda officinalis* How (0.37%), *Platycladus orientalis* (L.) Franco (0.37%), *Codonopsis pilosula* (Franch.) Nannf. (7.38%), *Lycium barbarum* L. (7.38%), *Glycyrrhiza uralensis* Fisch. (3.69%).

The components of the Wuzi Yanzong pill (WZYZP) include *Lycium barbarum* L. (35%) and *Cuscuta chinensis* Lam. (35%); *Rubus chingii* Hu. (17%); *Schizandra chinensis* (Turcz.) Baill. (5%), and *Plantago asiatica* L. (8%).

The components of Qilin pills (QLPs) include *Polygonum multijiorum* Thunb., *Ecliptae Eclipta prostrala* L., *Epimedium brevicornu* Maxim., *Cuscuta chinensis* Lam. ; *Cynomorium songaricum* Rupr.; *Codonopsis pilosula* (Franch.) Nannf. ; *Curcuma aromatica Salisb*. ; *Lycium chinense* Mill. ; *Rubus idaeus* Linn.; *Dioscorea oppositifolia* L.; *Salvia miltiorrhiza* Bunge. ; *Astragalus membranaceus* (Fisch.) Bge. ; *Paeonia lactiflora* Pall. ; *Citrus reticulata* Blanco. ; *Morus alba* L.

The components of Kyung-Ok-Ko (KOK) include *Rehmannia glutinosa var. purpurea*, *Panax ginseng*, *Poriacocos*, *Lycium chinense*, *Aquilaria agallocha*, and honey.

The components of KH-465 include *Epimedium koreanum* Nakai and *Angelica gigas* Nakai.

Figure 2c shows the analysis of the effects of herbal treatments on epididymal weight across thirty-four studies, which revealed significant variation in their results. The heterogeneity index was 83.8% (P<0.001) and ranged from 0.06 to 3.51. The results of the analysis indicated that treatment with herbal medicines increased the epididymal weight. The mean SMD calibration difference between the injury model group and the herbal treatment group was 1.22 (95% CI: 0.79, 1.65). The

findings of the relative epididymal weight survey involving 13 studies revealed substantial variations among the studies. The meta-analysis results revealed that the difference in SMD between the injury model group and the herbal treatment group was 1.93 (95% CI: 0.93, 2.93). The use of medicinal plants significantly increased the relative epididymal weight. Additionally, the Egger test demonstrated no publication bias among the studies related to epididymal weight and relative epididymal weight after male mice were treated with herbal medicines (P= 0.057 and 0.077, respectively).

The results presented in Figure 2d show that treatment with herbal medicines increased the seminal vesicle weight. The mean SMD calibration difference between the injury model group and the herbal treatment group was 1.25 (95% CI: 0.75, 1.75). Each study contributed an average of 4.02–9.99% of the total weight (I2 = 73.8%, P<0.001). Compared with the injury model group, the herbal treatment group presented an increase in the relative seminal vesicle weight, with an SMD of 1.67 (95% CI: 0.81, 2.54). The results revealed publication bias for both seminal vesicle weight and relative seminal vesicle weight, with p values for bias of 0.031 and 0.000, respectively.

Six studies from four scientific articles were conducted to investigate the effects of herbal treatment on seminiferous tubule diameter. The level of heterogeneity among the studies was considerable (I-squared heterogeneity=93.0%, P<0.001), ranging from 7.34--24.42. The overall meta-analysis revealed that treatment with herbal products significantly increased the seminiferous tubule diameter (SMD=9.10; 95% CI: 4.93, 13.28). The results revealed that the publication bias (P for bias) was 0.000.

The findings of this study examining the relationship between herbal medicine treatment and Johnson's score are presented in Figure 3b. The studies included in the analysis displayed significant variation in their results, with an I-squared value of 89.2%, indicating high heterogeneity. The percentage weight of each study ranged from 4.61% to 16.88%. The results revealed that herbal medicines were effective at increasing Johnson's score (SMD 6.40, 95% CI: 3.73, 9.06). The results revealed that the publication bias (P for bias) was 0.000.

3.3. Associations between herbal medicines and murine sperm parameters

Thirty-five studies investigated the effects of herbal medicine treatment on the sperm count (Figure 4a). The difference between the studies was large (I-squared heterogeneity=84.9%, P<0.001), ranging from 0.47--3.77. The mean SMD calibrated mean dissimilarity between the injury model group and herbal treatment group was 3.47 (95% CI: 2.72, 4.21). The results of the analysis revealed that treatment with herbal medicines increased sperm counts. There was publication bias, with a p value of 0.000 (not illustrated).

The outcomes of a meta-analysis, which included 37 studies, on the impact of herbal medicine treatment on sperm motility were analyzed via a random effects model. The analysis revealed significant variability among the studies, with each study contributing an average of 0.02–3.60% weight (I2 = 92.8%, P<0.001). The analysis depicted in Figure 4b suggests that treatment with herbal medicines can significantly increase sperm motility (SMD=3.57, 95% CI: 2.70, 4.44). The results revealed no publication bias (P= 0.879).

The results of the analysis, which involved 19 studies that investigated the effects of medicinal plant treatments on sperm viability, are shown in Figure4C. Research indicates that treatment with medicinal plants can increase sperm viability in semen. There was a substantial disparity between the studies (I-squared heterogeneity index=88.3%, P<0.001). The proportion of weight allocated to each study ranged from 0.54% to 6.71%. A comprehensive evaluation of the effects of these herbs on the sperm viability index revealed that the standard mean difference (SMD) between the injury model group and the herbal treatment group was 2.04 (95% CI: 1.21, 2.87), suggesting that treatment with medicinal plants can increase sperm viability. The analysis also revealed evidence of publication bias, with a p value of 0.000.

Figure 4d displays the results of the analysis of 14 studies that examined the effects of herbal medicine treatment on abnormal sperm morphology. The results revealed a significant difference between the studies (I-squared heterogeneity=89.3%, P<0.001). The weight allocated to each study ranged from 1.69% to 9.53%. The meta-analysis results revealed that the difference in SMD between the injury model group and the herbal treatment group was -2.28 (95% CI: -3.65, -1.72). This study suggests that treatment with medicinal herbs can reduce abnormal sperm morphology. Egger test results (P for bias = 0.000) indicated evidence of publication bias in studies examining the effects of plant medicine treatment on abnormal sperm morphology in males (not illustrated).

3.4. Associations between herbal medicines and male murine sex hormones

Data from thirty-nine studies from twenty-one scientific articles were analyzed to investigate the effects of herbal treatment on testosterone levels. The level of heterogeneity among the studies was considerable (I-squared heterogeneity=90.8%, P<0.001), ranging from 0.80--3.02. A comprehensive meta-analysis revealed that herbal product treatments significantly increased testosterone levels in the serum (SMD=1.98; 95% CI: 1.33, 2.64). Evidence of publication bias was observed, with a p value of 0.000.

The findings of the FSH survey involving 17 studies from 11 scientific articles were analyzed, revealing a significant difference between the studies (I-squared heterogeneity=87.8%, P<0.001). The weights allocated to each study ranged from

0.09% to 6.99%. The comprehensive meta-analysis results depicted in Figure 5b indicate that the difference in the SMD between the injury model group and the herbal treatment group was 0.08 (95% CI: -0.64, 0.80). Notably, the FSH levels in the herbal treatment group did not differ from those in the injury model group. A publication bias was observed for FSH levels, with a p value of 0.018 (data not shown).

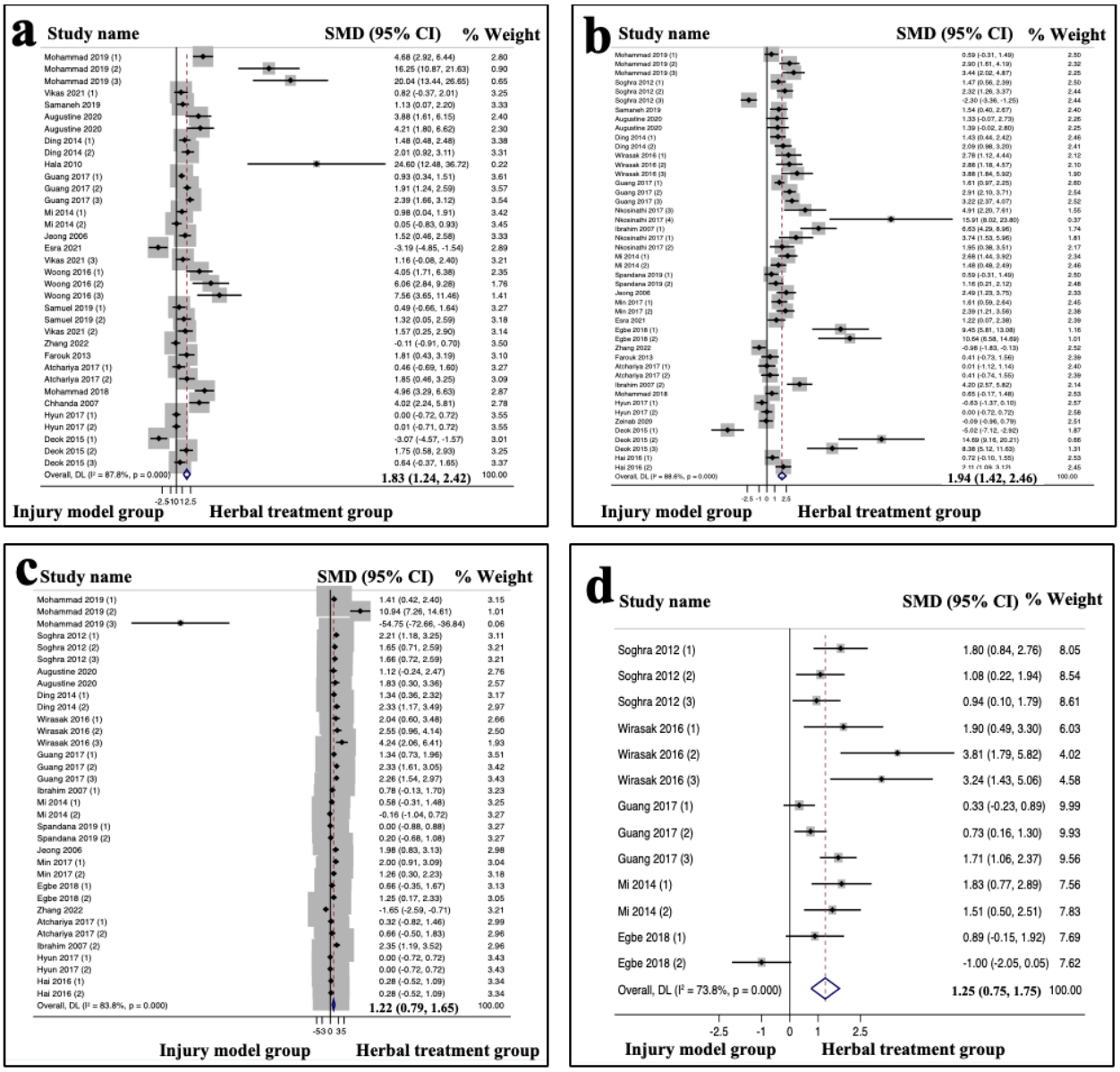

Figure 2 The effects of herbal medicine on male murine reproductive organs a. Body weight; b. testis weight; c. epidermis weight; d. seminal vesicle weight.

Figure 5c shows the analysis of the effects of treatment with herbal products on LH levels in serum across 17 studies, which revealed significant variation in their results. The heterogeneity index (I-squared) was 90.3% (P<0.001), indicating a high level of heterogeneity among the studies, with a weighted range of 3.57 6.55. The mean difference in SMD calibration between the injury model group and the herbal treatment group was 0.41 (95% CI: − 0.40, 1.23). The meta-analysis results revealed that the herbal products did not have constant serum LH levels. Additionally, the Egger test demonstrated no publication bias among studies related to serum LH levels after male mice were treated with herbal medicine (p for bias = 0.921).

3.5. Associations between herbal medicines and male murine blood serum biochemical parameters

Figure 6a shows the effects of herbal medicine treatment on the blood glucose concentration, which consisted of 11 studies from 6 research papers. The research revealed significant variability among the studies, with each contributing an average of 2.57–10.73% of the total weight (I2 = 90.3%, P<0.001). Overall, the standardized mean difference (SMD) between the injury model group and the herbal treatment group was -1.49 (95% CI: -2.71, -0.26). Treatment with herbal medicines

reduces the blood glucose concentration. The results of the publication bias assessment of these articles on the impact of herbal medicine on blood glucose concentration via Egger's test revealed publication bias (P= 0.001).

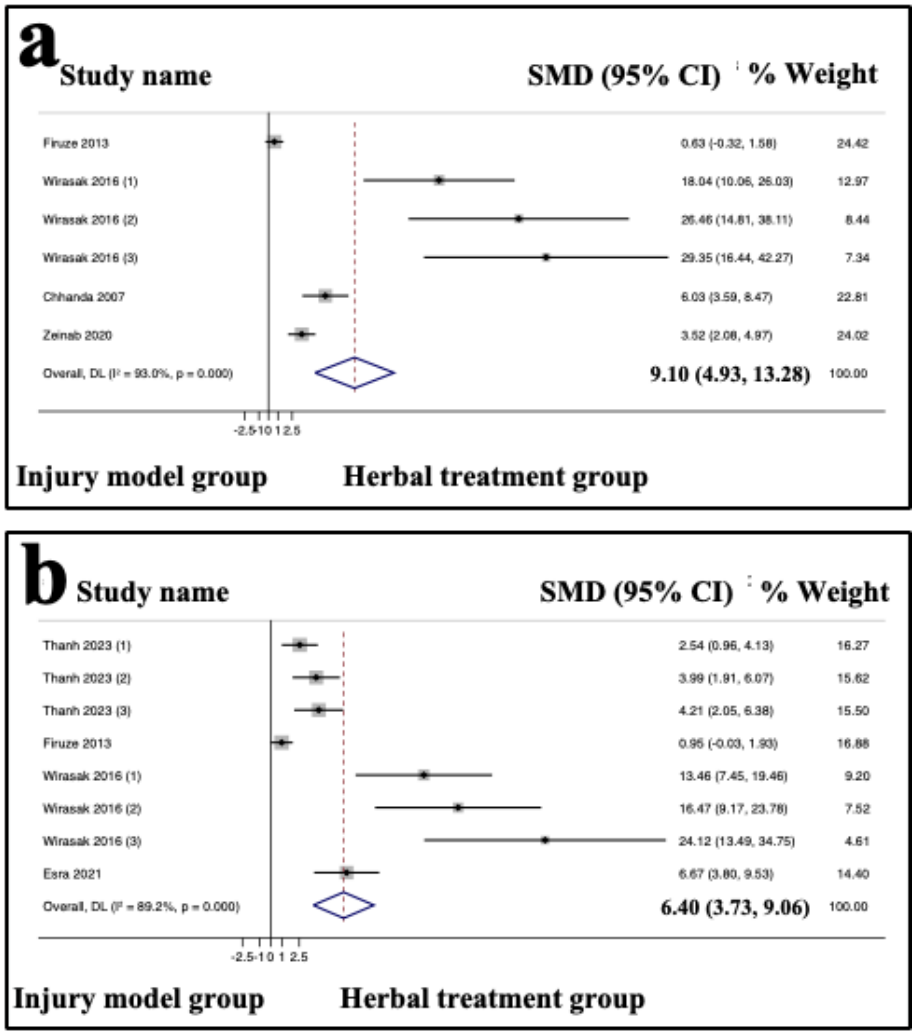

Figure 3 The effects of herbal medicine on murine seminiferous tubules and Johnson's score. a. Seminiferous tubule diameter; b. Johnson's score.

Figure 6b shows the results of the examination of the effects of herb treatment on the activity of serum glutamic oxaloacetic transaminase (GOT). A random effects model was used to evaluate the collected data. The difference between the studies was large (I-squared heterogeneity index=69.7%, P>0.001). Each study weighed 9.21% to 21.56% weight. The comparison between the injury model and herbal treatment groups revealed a standard mean difference (SMD) of -1.55 (95% CI: -2.56, -0.54). These findings indicated that herb treatment resulted in a decrease in serum glutamic oxaloacetic transaminase (GOT) activity. Egger's test was used to assess publication bias among the selected studies that examined the effect of herbal treatment on serum glutamic oxaloacetic transaminase activity. Evidence of publication bias was found, with a p value of 0.016.

Six studies indicated that phytomedicine treatment led to a decrease in serum glutamate pyruvate transaminase (GOT) activity (Figure 6c). Significant variation was observed among the studies (I-squared heterogeneity=90.4%, P>0.001), with weights ranging from 8.31--22.60. The mean standardized difference between the injury model and herbal treatment groups was -4.45 (95% CI: -7.01, -1.90). Evidence of publication bias was detected in the analytical outcomes, with a p value of 0.000.

Six studies were conducted to investigate the effects of herbal product treatment on blood creatinine levels. The investigations revealed significant heterogeneity (I-squared heterogeneity=87.0%, P<0.001), with the percentage weight of each study ranging from 11.02% to 20.11%. A comprehensive meta-analysis revealed that herbal treatment led to increased creatinine levels in the blood (SMD=2.11; 95% CI: 0.41, 3.81). The analysis of publication bias produced a p value of 0.000, indicating evidence of bias.

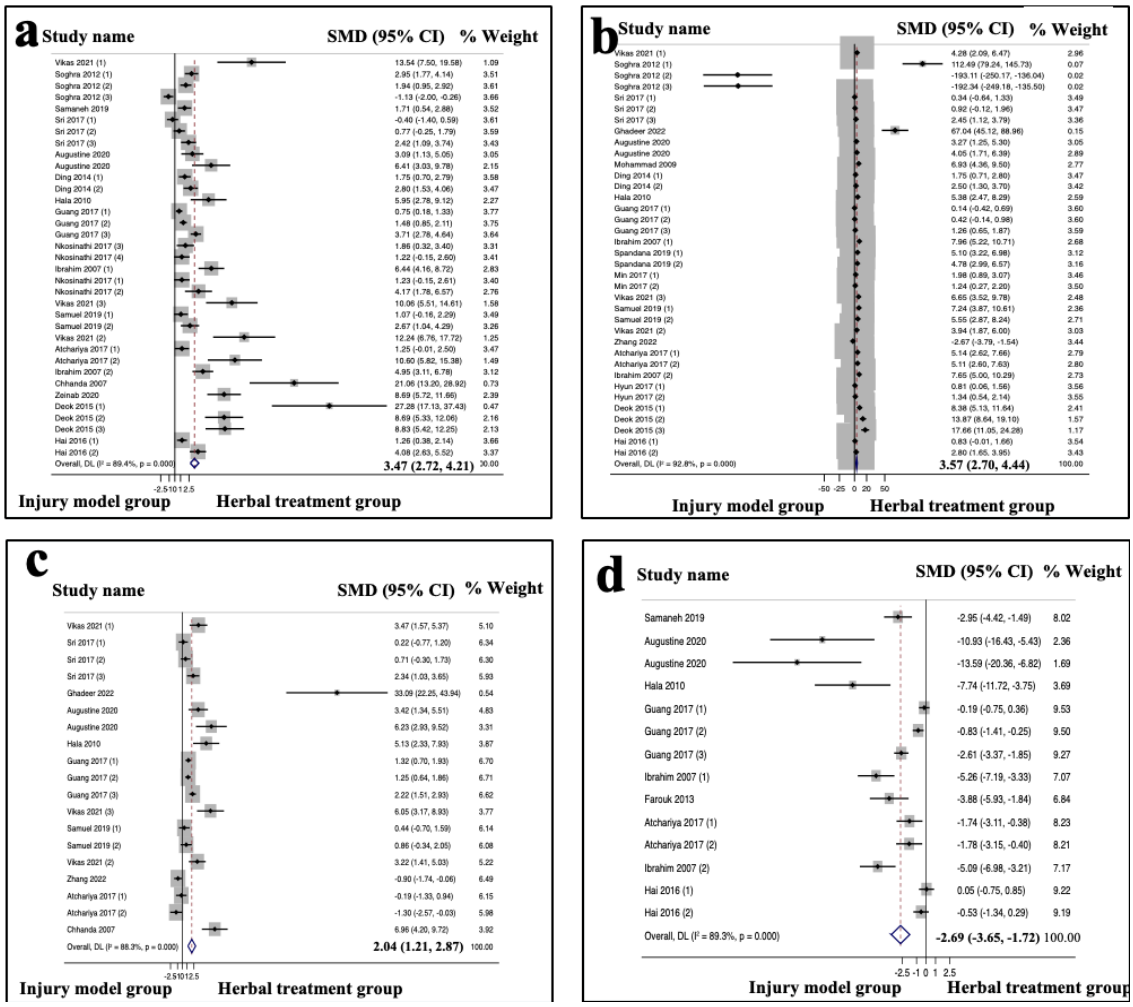

Figure 4 The effects of herbal medicine on murine sperm parameters. a. Sperm count; b. motility; c. viability; d. abnormality.

3.6. Associations between herbal medicines and male murine oxidative stress markers

A comprehensive evaluation of the effects of herbal treatment on the serum concentration of malondialdehyde (MDA) in nine studies from four scientific publications was conducted via a random effects model. The findings revealed heterogeneity among the studies, with each contributing an average weight of 8.22–13.92% (I2 = 47.8%, P>0.001). These results suggest that botanical medicine treatment reduces the serum concentration of MDA in the blood. The mean SMD calibration difference between the injury model and herbal treatment groups was -1.86 (95% CI: -2.43, -1.28). Egger's test revealed evidence of publication bias among the studies examining the blood serum concentration of MDA following phytomedicine treatment in males (p for bias = 0.049).

Figure 7b illustrates the meta-analysis of 24 studies that examined the effects of herbal therapeutics on testicular malondialdehyde levels. The level of heterogeneity among the studies was considerable (I-squared heterogeneity=89.8%, P<0.001), with weights ranging from 0.37--5.30. The overall findings indicated a standard mean difference in the SMD of -3.60 (95% CI: −4.57, − 2.63). These results suggest that herbal therapy effectively decreases testicular MDA levels. Egger's test revealed publication bias among studies investigating testicular MDA levels following the use of herbal medicines in male murines (P for bias = 0.000).

The meta-analysis examined the relationship between herbal therapy and serum superoxide dismutase (SOD) levels, and the findings are displayed in Figure 7c. Considerable variation was observed among the studies (I-squared heterogeneity=63.2%, P>0.001), with weights ranging from 0.03 to 12.67. The mean SMD calibration difference between the injury model and herbal treatment groups was 2.39 (95% CI: 1.62, 3.15). These findings indicate that botanical therapy increases the serum SOD level. Egger's test (P for bias =0.000) revealed publication bias among studies investigating serum SOD levels following male murine botanical treatment.

The analytical results of the 19 studies on the effects of botanical therapy on testicular SOD levels are shown in Figure 7d. There was considerable variation among related studies (I-squared heterogeneity=81.8%, P<0.001), with weights ranging from 0.90--7.09. The standardized mean difference (SMD) between the injury model and herbal treatment groups was 2.30 (95% CI: 1.64, 2.95), as shown in Figure 7d. These findings indicated that phytomedicine treatment led to increased levels of

SOD in the testes. The results of the publication bias assessment. Statistically significant publication bias was detected among the selected articles that examined the effects of phytomedicine treatment on testicular SOD levels (P for bias = 0.000).

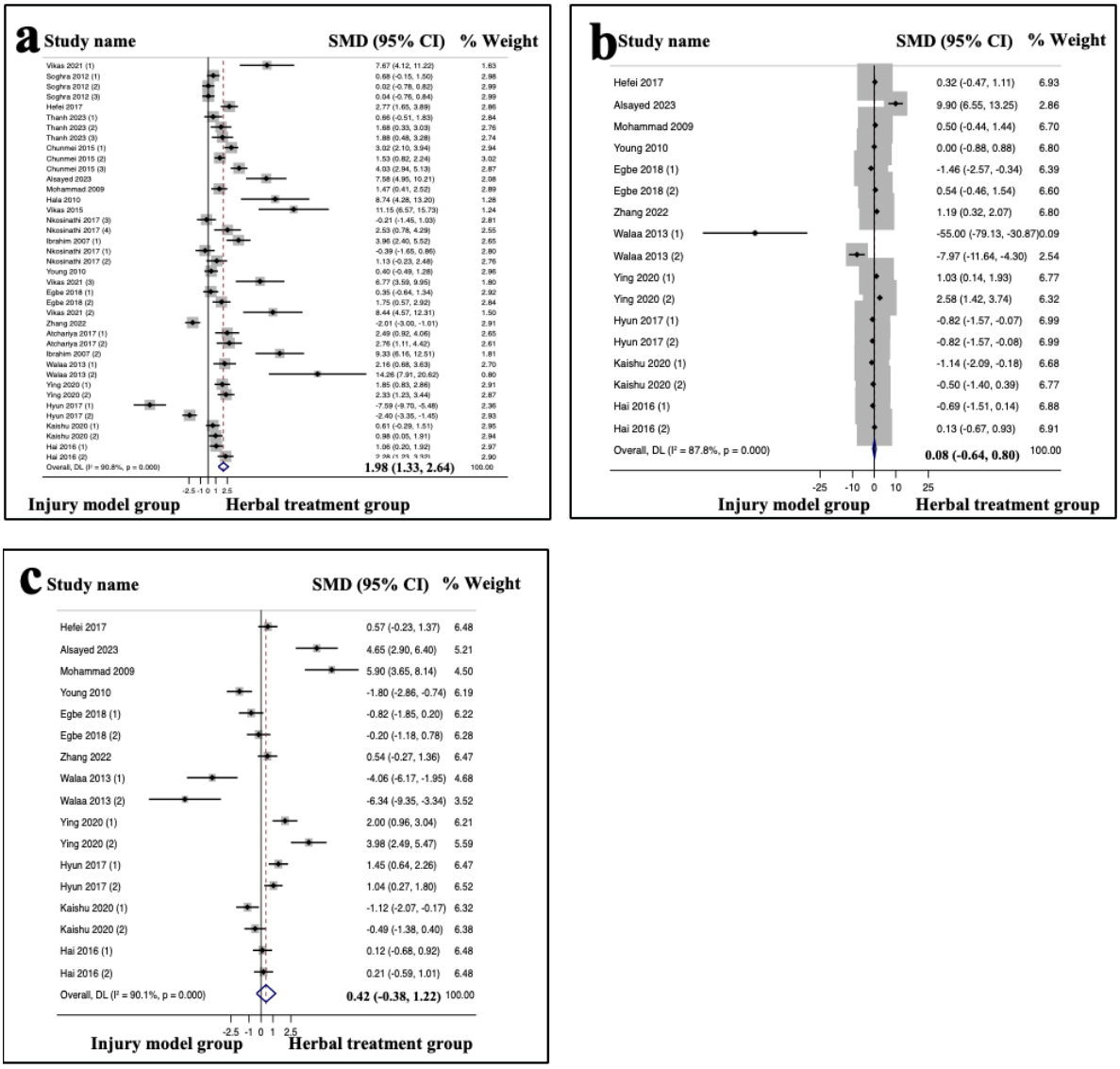

Figure 5 The effects of herbal medicine on male murine hormone levels. a. Testosterone; b. FSH; c. LH

The results of the analysis of five studies investigating the effects of botanical therapy on the levels of testis catalase (CAT) are illustrated in Figure 7e. Previous studies have indicated that treatment with medicinal plants can increase testicular CAT levels. There were no significant differences between the studies (I-squared heterogeneity index= 0.00%, P>0.001). The proportion of the weight allocated to each study ranged from 11.42% to 26.36%. A comprehensive evaluation of the effects of phytomedicine therapy on the levels of testis CAT revealed that the standard mean difference (SMD) between the injury model and herbal treatment groups was 4.16 (95% CI: 3.41, 4.91), suggesting that medicinal plant therapy can increase testicular CAT levels. The analysis also revealed evidence of publication bias, with a p value of 0.032.

The results of a meta-analysis of 15 studies from 9 scientific articles were examined to assess the effects of herbal treatments on the activity of glutathione peroxidase (GPx) in testicular tissues. A random-effects model was used to analyze the data, revealing substantial variability among the studies, with each study contributing an average weight of 1.00–8.90% (I-squared heterogeneity = 88.4%, P<0.001). A comprehensive meta-analysis demonstrated that treatment with herbal medicines increased GPx activity in testicular tissues. The mean SMD calibration difference between the injury model and herbal treatment groups was 3.48 (95% CI: 2.32, 4.63). No publication bias was observed (P= 0.000).

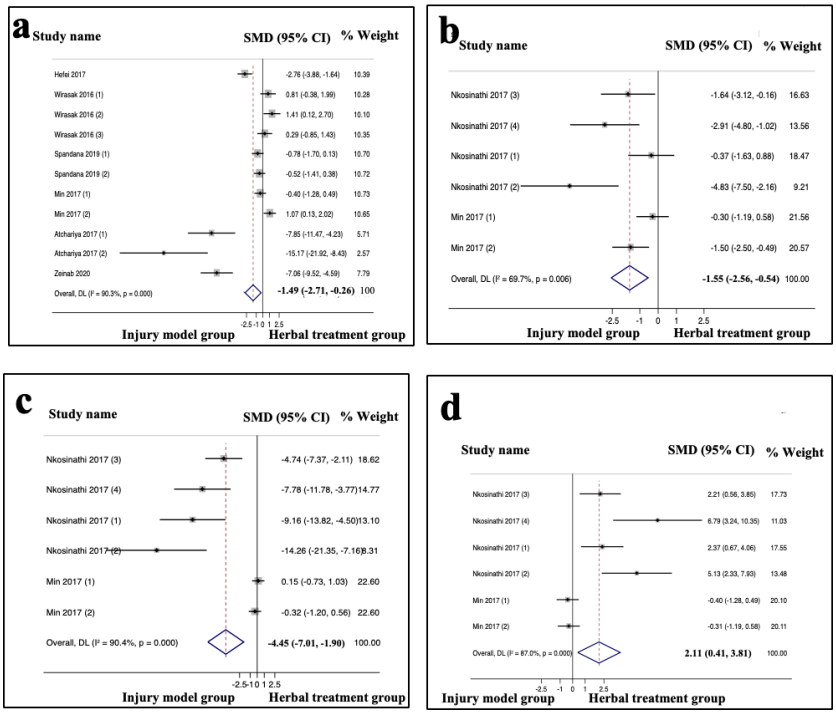

Figure 6 The effects of herbal medicine on male murine blood serum biochemical parameters. a. GLU; b. GOT (AST); c. GPT (ALT); d. Cre.

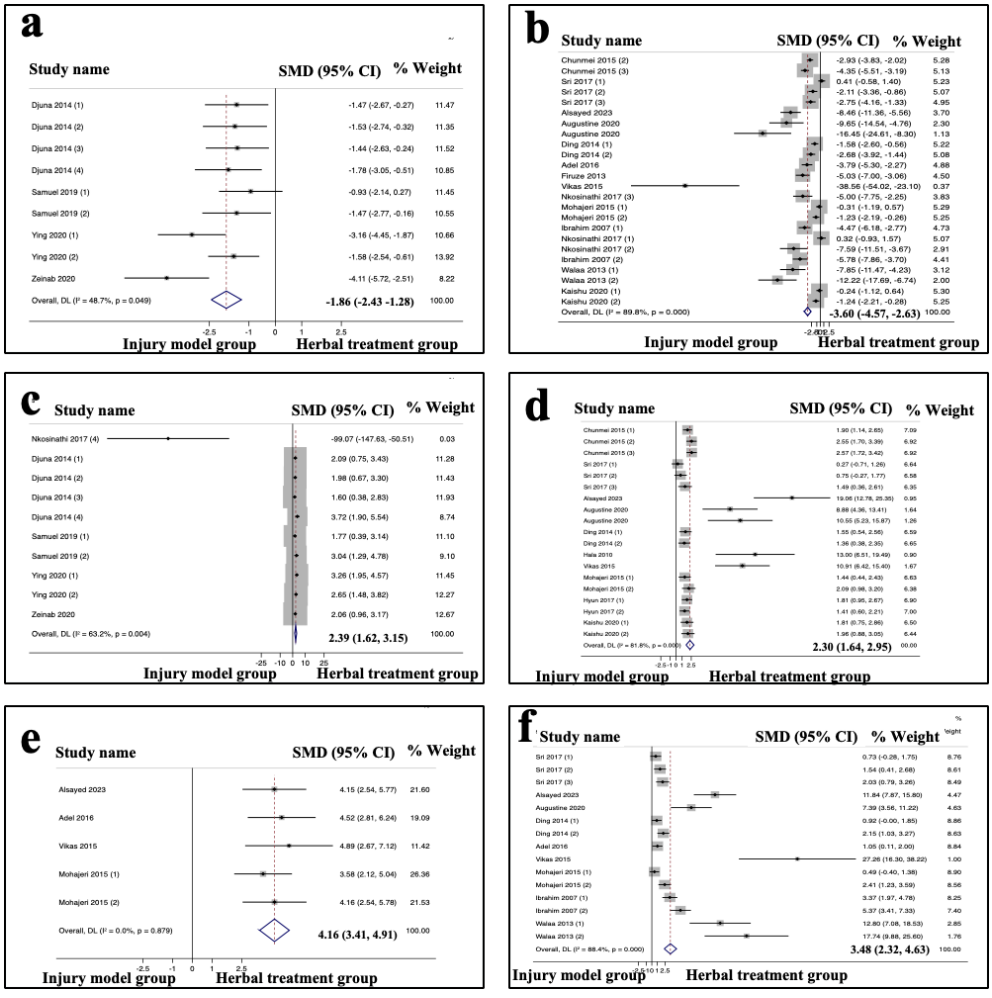

Figure 7 The effects of herbal medicine on male murine oxidative stress markers. a. Blood serum concentration of malondialdehyde (MDA); b. Testicular malondialdehyde level; c. Serum SOD; d. Testicular SOD; e. Testicular CAT; f. Testicular GPx a. GLU; b. GOT (AST); c. GPT (ALT); d. Cre.

4. Discussion

The present study investigated the effects of herbal medicine treatment on male murine spermatogenesis by analyzing 48 studies from 44 articles involving 1597 males and 27 plant species, and seven formulations. The results of the meta-analysis revealed that treatment with herbal plants significantly increased male murine reproductive organs, including body weight (SMD =1.83; 95% CI: 1.24, 2.42), testis weight (SMD =1.94; 95% CI: 1.42, 2.46), relative testis weight SMD = 2.01; 95% CI: 1.27, 2.75), epididymis weight (SMD= 1.22; 95% CI: 0.79, 1.65), relative epididymal weight (SMD=1.93; 95% CI: 0.93, 2.93), seminal vesicle weight (SMD=1.25; 95% CI: 0.75, 1.75), and relative seminal vesicle weight (SMD=1.67; 95% CI: 0.81, 2.54). Moreover, it also increased the seminiferous tubule diameter (SMD=9.10; 95% CI: 4.93, 13.28) and Johnson's score (SMD 6.40, 95% CI: 3.73, 9.06). After treatment, males showed improvements in semen parameters, including sperm count (SMD = 3.47; 95% CI: 2.72, 4.21), sperm motility (SMD=3.57, 95% CI: 2.70, 4.44), sperm viability (SMD=2.04; 95% CI: 1.21, 2.87), and abnormal sperm morphology (SMD=-2.28; 95% CI: -3.65, -1.72). While significant changes in FSH and LH levels were not observed, they favorably increased the serum testosterone levels. In addition, blood serum biochemical parameters and oxidative stress markers improved after treatment with herbal medicine in male mice in the injury model.

In studies using murine models, herbal medicine has been shown to improve sperm quality parameters, such as count, motility, and morphology, and to increase testosterone levels (Hou et al. 2020, Hwang et al. 2015, Nouri et al. 2009, Shi et al. 2017). For example, Daucus carota L. seed extract (CSE) has been shown to increase cauda epididymis sperm reserves (CESRs), significantly increase testosterone levels, and improve sperm quality parameters, including sperm count and motility, by protecting testes from gentamicin-induced necrosis (Nouri et al. 2009). Cornus officinalis (Kumar Roy et al. 2016) improved testosterone levels in diabetic mice (Huang B. et al. 2023), whereas Mallotus roxburghianus (Kumar Roy et al. 2016) and Curculigo orchioides (Bui-Le and Hoang-Tan 2023) mitigated testicular dysfunction. Additionally, grape seed extract (El-Ashmawy et al. 2007) and curcumin (Lin et al. 2015) restored testosterone and antioxidant enzyme activity in toxin-exposed mice, whereas Cyperus esculentus L. (Udefa et al. 2020) and Anacyclus pyrethrum (Sharma et al. 2021) counteracted lead-induced testicular damage. These improvements may also be linked to other mechanisms such as oxidative stress reduction and gene expression regulation.

Oxidative processes in murines can be countered by numerous herbs. These herbs originate from diverse regions and plant families, and many have shown efficacy in reducing oxidative stress in testes (Hou et al. 2020, Hwang et al. 2015, Kumar Roy et al. 2016, Shi et al. 2017). Oxidative stress, which involves an imbalance between the production of reactive oxygen species (ROS) and antioxidant defenses, can impair sperm function and lead to cellular damage. Commonly analyzed markers include malondialdehyde (MDA) for oxidative damage and antioxidant enzymes, such as superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px). The role of oxidative stress has been further clarified in rat studies of marjoram volatile oil and grape seed extract, which neutralize free radicals and protect cell membranes from oxidative damage (El-Ashmawy et al. 2007).

Herbal medicines effectively inhibit apoptosis in the testes and protect against reproductive damage. By neutralizing harmful free radicals, natural antioxidants reduce apoptosis, a process that is essential for the removal of damaged cells, and impairs testicular function when overactive. For example, the traditional extract Kyeong-Ok-Ko has been shown to decrease the expression of Bax, a pro-apoptotic protein, while modulating key apoptosis-related factors such as cytochrome c and caspase-3, in heat-stressed mouse testes (Hwang et al. 2015).

Herbal medicines play a key role in the regulation of reproductive functions by modulating gene and protein expression. Natural antioxidants found in herbs can influence gene and protein expression, thereby improving reproductive health. For example, Duzhong Butiansu (DZBTS) has been shown to increase spermatogenesis in mice with heat stress-induced dysfunction by regulating proteins involved in spermatogenesis and the HSF/Hsp70 signaling pathway (Hou et al. 2020). This pathway is critical for protecting cells from heat stress, as HSF1 activates the expression of heat shock proteins such as Hsp70, which stabilize proteins and prevent denaturation. DZBTS helps restore heat-induced changes by modulating this pathway and reducing oxidative stress, ultimately protecting testicular function (Hou et al. 2020).

The ability of herbal medicines to protect spermatogenesis from different inducers such as heat stress, radiation, and chemical toxins should be investigated. Red ginseng (KRG) and pectinase-treated Panax ginseng (GINST), for example, have shown promise in alleviating heat stress-induced damage by increasing sperm motility and regulating spermatogenesis-related molecules (Kim M. K. et al. 2017, Kopalli et al. 2019). In diabetic mice, Lycium barbarum polysaccharide (LBP) restored sexual function and fertility by regulating the hypothalamic-pituitary-gonadal axis activity (Shi et al. 2017). Similarly, in heat-stressed rats, traditional Chinese medicines, such as Duzhong Butiansu (DZBTS), enhance spermatogenesis by modulating the androgen receptor (AR), CREB1, and HSF1/Hsp70 signaling pathways (Hou et al. 2020). Heavy metal-induced testicular damage can be partially reversed with the use of herbal medicines. For example, radish sprout ethanolic extract mitigates bisphenol A (BPA)-induced dysfunction (Yang et al. 2016), whereas Cyperus esculentus extract improves testicular and epididymal weights, sperm parameters, and testosterone levels in lead-exposed rats (Udefa et al. 2020).

A review of animal studies is required to refine these herbal medicines by optimizing their doses, routes, formulations, toxicity profiles, pharmacokinetics, and drug interactions, which are challenging to conduct in humans. This ensures safer and

more precise development for rigorous human clinical trials, which offer stronger evidence than observational studies do. The optimal dosage that maximizes therapeutic benefits while minimizing potential adverse effects is highly important. For example, in a study on partially sterile male rats, Carthamus tinctorius extract improved sperm parameters at a dose of 10 mg/kg, although higher doses reduced the efficacy (Bahmanpour et al. 2012). This finding suggests that there may be a negative feedback mechanism at higher doses, potentially due to the effects on the hypothalamic–hypophyseal axis (Zirkin et al. 1989). Polyherbal formulations represent a promising avenue for the development of targeted therapies for male infertility. The Wuzi Yanzong pill has demonstrated efficacy in alleviating testicular damage by increasing the sperm count, reducing oxidative stress, and promoting the expression of spermatogenesis-related proteins (Hou et al. 2020). Another study has explored the effects of a traditional herbal prescription, Kyung-Ok-Ko, on heat-induced male infertility in mice (Hwang et al. 2015). In addition, several animal studies have evaluated the cytotoxic effects of herbal extracts on various cell lines. One study reported that root extracts of Maytenus procumbens and Ozoroa paniculosa showed moderate to weak toxicity in both cancer and normal cells (Cele et al. 2017). The authors highlighted the need to further assess the potential kidney toxicity of these extracts because of the observed increase in serum creatinine levels in extract-treated groups. Another study determined the acute toxicity of an aqueous extract of Cyperus esculentus using the Lorke method (Udefa et al. 2020).

Our study has several strengths. First, 27 different herbs and seven formulations have been identified in the literature, offering promising alternatives or complements to conventional treatments. Second, studies delving into the potential mechanisms of action of antioxidant enzymes (GSTM5 and GPX4), spermatogenesis-related proteins (CREB1 and INHA), and sex hormone receptors (androgen, luteinizing hormone, and follicle-stimulating hormone receptors) in protecting the testes and investigating these pathways could open new avenues for deeper understanding. Despite these promising results, this study had several limitations. There is a lack of consistency in the designs of these studies, particularly in terms of animal models, dosages, treatment durations, and outcome measures. This variability makes it challenging to compare results across different studies and draw definitive conclusions. Many studies suffer from sample size bias, with small sample sizes (<10) that reduce statistical power and limit the generalizability of the results. Additionally, detailed information regarding the quality control of herbal preparations, including their origin, extraction methods, and standardization, is lacking, which undermines the reliability of the findings. Furthermore, publication bias may exist, with studies showing positive results, such as those in which herbs have protective effects on the testes, published more frequently than those with negative or inconclusive results. Finally, further human studies are vital to confirm the preliminary results of animal studies.

5. Conclusion

In conclusion, herbal medicines have been demonstrated to exert protective effects against murine spermatogenesis. The provided better understanding of their mechanistic actions has the potential to produce future human trials soon.

Acknowledgments

The authors express gratitude for the partial support by Hue University in Vietnam through the Core Research Program, specifically for the Research Group on Regenerative Medicine (NCTB. DHH.2024.02).

Ethical Considerations

Ethical approval is not required.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Funding

Financial support for this research was provided by the Research Projects in Science and Technology program of the Vietnamese Ministry of Education and Training under Grant number B2023-DHH-11.

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