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Review Article

The Profertility and Aphrodisiac Activities of *Tribulus terrestris* L.: Evidence from Meta-Analyses

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Tribulus terrestris L. (TT) is a traditional medicinal plant, which belongs to the Zygophyllaceae family. TT extracts have been widely used for diuretic, analeptic, aphrodisiac, and profertility properties. To quantitatively evaluate the profertility and aphrodisiac effects of *Tribulus terrestris* L., we undertook the present meta-analyses on published data. A thorough literature screening was performed to identify articles evaluating the effect of TT on spermatogenesis, male fertility, reproductive, and aphrodisiac parameters. We shortlisted 30 relevant studies conducted on humans and rodents. Meta-analyses were conducted to evaluate the quantitative impact of TT on various fertility parameters. In case of humans, the pooled analysis on 133 subjects showed significant improvements in sperm concentration (SDM = 0.624, 95% CI = 0.13 to 1.117, $p = 0.013$) and sperm motility (SDM = 0.742, 95% CI = 0.331 to 1.152, $p = 0.001$). TT resulted in nonsignificant increases in testosterone and LH and a nonsignificant decrease in FSH. Similar to the above, TT improved sperm count, sperm motility, and sperm viability in rodents with normal or compromised fertility. The effect on hormone levels was less credible with frequent variations across studies and animal models. The aphrodisiac activity was studied in castrated animal models or normal rodents, both of which showed significant improvements in mounting frequency and intromission frequency and significant declines in mounting latency and intromission latency. These meta-analyses suggested that TT possesses profertility and aphrodisiac activities.

1. Introduction

Infertility is defined as the failure to achieve a clinical pregnancy after 12 months or more of unprotected regular sexual intercourse [1]. Approximately 40% of infertility cases worldwide are due to the male factor infertility [2, 3]. A large number of the infertile cases are still classified as idiopathic, and diagnosis tends to be descriptive, often leading to ineffective medical approaches for treatment [2]. Semen quality is the main predictor of male fertility and is used as the primary checkpoint to assess the causes of infertility. As per the WHO 2010 guidelines, sperm count and motility are important criteria to establish infertility in males [4]. However, not all cases of infertility are explained by a decline in semen parameters. This leads to the failure of establishment of an appropriate cause of infertility in a large number of patients. Plant products offer an attractive alternative for the treatment of such cases. Plants products are often a complex mixture of natural products, exerting multipronged effects required for improvement in fertility.

Till date, a variety of traditional medicinal plants from India, China, Africa, and other countries have been claimed to have aphrodisiac and profertility effects [5]. *Tribulus terrestris* L. (TT), known as Gokshura in Ayurveda, has been claimed to be effective in treating urogenital diseases including the loss of libido [6, 7]. In addition to the profertility effects in males, it is often prescribed for the treatment of infertility, impotence, erectile dysfunction, and low libido [8–11]. *Tribulus terrestris* L., also known as Gokshura, caltrop, Maxican sandbur, and goathead, is a small leafy plant that belongs to the family Zygophyllaceae, largely inhabitant to the tropical and temperate

regions of Asia, Africa, Australia, and Europe [12, 13]. Root, fruit, or leaf extracts of TT are commonly employed for various male ailments, including the loss of virility and fertility. TT contains the steroidal saponins, mainly protodioscin, prototribestin and other important ingredients such as alkaloids, flavonoids, terpenoids, and phenol carboxylic acid. TT extracts have been found to improve sperm parameters, sex hormonal profile, and libido by some studies [11, 14–18], while others have reported its adverse effects [19–21]. Meanwhile, in some human clinical trials, TT showed no impact on the seminal parameters ([22–24]. Similarly, other studies showed no improvement in sex hormone levels [25–28].

On taking these contradictory reports into account, the impact of TT on semen parameters, hormones, and libido remains unclear. Therefore, we have performed this systematic review and meta-analyses to undertake qualitative and quantitative analysis of the available data to draw conclusions regarding the potency of TT as a profertility and aphrodisiac plant. We found that TT has profertility and aphrodisiac properties.

2. Material and Methods

This study was excluded from authorization by the Institutional Review Board since it was a review and meta-analysis. We used the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) chart for presenting the results of this systematic review and meta-analysis [29].

2.1. Search Strategy. We performed a broad electronic search to identify the articles evaluating the effect of *Tribulus terrestris* L. (TT) on sperm parameters, sex hormone levels, and aphrodisiac activity. We searched the major public databases such as PubMed (MEDLINE), Web of Science, Google Scholar, and EMBASE for the identification of relevant studies. The electronic search keywords were "*Tribulus terrestris* L. and male infertility," "male infertility and protodioscin," "*Tribulus terrestris* L. and sperm parameters,", "*Tribulus terrestris* L. and Testosterone," "*Tribulus terrestris* L. and Luteinizing Hormone (LH) or Follicle Stimulating Hormone (FSH)," and "*Tribulus terrestris* and aphrodisiac activity." We also looked for the major clinical trials registry databases like WHO, ISRTCN, and US-based clinical trial registry for the identification of more studies (https://www.isrctn.com; https://www.clinicaltrials.gov).

2.2. Inclusion and Exclusion Criteria. All the articles obtained through the electronic search were subjected to a set of inclusion/exclusion criteria. Inclusion criteria were as follows: (a) pre/post and control/treated studies on human, rat, and mouse to evaluate the effect of TT administration on semen parameters, sex hormones, and aphrodisiac activity; (b) patients in the studies were diagnosed using the standard diagnostic parameters; (c) studies used the standard methods and sufficient data were provided. The exclusion criteria consisted of the following: (a) the studies that failed to provide a detailed description of the subjects, raw data, and other information required to specifically understand the study design and the data therein; (b) review articles, meta-analyses, case reports, and research on males with disorders such as varicocele and cryptorchidism; (c) the studies that administered TT along with other dietary nutraceuticals which would mix up the effect of TT.

2.3. Data Extraction. Using a spreadsheet, data were collected to document research design, number of participants, dose and duration, quantitative outcomes, and the primary findings. Whenever the data were available in the form of graphs, the values were extracted using web plot digitizer (https://apps.automeris.io/wpd//). The data were divided in four major groups: (i) pre- or postdata of human infertile males treated with TT, (ii) control vs. TT-treated animals, (iii) chemically induced/castrated infertile rat/mouse models vs. TT treatment (therapeutic effect), (iv) coadministration of *Tribulus terrestris* and infertility-inducing agents in rat/mouse (prophylactic effect). Quantitative evidence, therapies given, and other information were collected by two authors independently (AA and RV). The inconsistencies were overcome through discussion with the senior author, leading to a consensus.

2.4. Quantitative Data Analysis. Meta-analysis was performed as detailed in our previous study [30]. Standard difference in mean (SDM) was used as the 'effect size' statistic in human studies and Hedges 'g' was used as the 'effect size' statistic in case of animal studies. Q and I^2 represent the heterogeneity taking into account that I^2 value < 25% means low heterogeneity, 50 percent means moderate heterogeneity and 75 percent suggests considerable heterogeneity [31, 32]. Both the fixed effect and the random effects models were used to measure the pooled effect size value. The fixed effect model was used for drawing inference where the heterogeneity was not significant but in the case of significant heterogeneity, the random effects model was used. Treatment protocols for the dose and length of TT therapy were significantly heterogeneous across studies (Table 1). The methodological information and other details for the studies included in this analysis are given in Table 2. For human studies, pre- and postdata were taken, while in rat/mouse studies, control/TT-treated (therapeutic), control/TT-treated (preventive), and infertile model/TT-treated were considered for qualitative analysis, irrespective of the dose and duration of treatment.

2.5. Sensitivity Analysis. Sensitivity analysis was performed by exclusion of one study at a time, followed by reestimation of the effect size after exclusion of each study. A significant change in the overall conclusion was used to identify a sensitive study.

2.6. Publication Bias. Publication bias analysis was performed qualitatively on the basis of asymmetry in the funnel plot and quantitatively using Egger's intercept test value. In case of significant bias, Duval and Tweedie's trim and fill method was used to compute unbiased estimates.

3. Results

3.1. Literature Screening. Literature search was performed using the keywords, such as *Tribulus terrestris* L., reproduction, male infertility, and aphrodisiac property in various

Table 1: Methodological details of the studies included in the meta-analysis.

Study	Model development	TT (post-/pre-/ coadministered)	TT (dosage)	Dosage administration
Animal studies				
[38]	Sodium Valproate (500 mg/kg-last week) (testicular toxicity)	TT (preadministered)	2.5,5,10 mg/kg-60 days	Orally
[39]	Cypermethrin (3.38 mg/kg-28days) (reproductive toxicity)	TT (coadministered)	100 mg/kg-28 days	Orally by gavage
[35]	Copper overloaded (200 mg/kg-90days) (testicular dysfunction)	TT (coadministered)	10 mg/kg-90 days	Orally
[36]	Bisphenol A (25 mg/kg-4weeks) (spermatotoxicity)	TT (coadministered)	20 mg/kg-4 weeks	Orally
[37]	Cyclophosphamide (100 mg/kg-14th day) (reproductive toxicity)	TT (preadministered)	11 mg/kg-14 days	Orally by gavage
[9]	Malathion (250 mg/kg) (free radical development)	TT(postadministered)	2.5,5,10 mg/kg-8 weeks	Orally by gavage
[43]	Morphine (60-80 mg/ml-21days)	TT(postadministered)	6.25% of food-4 weeks	Orally
[42]	2,3,7,8 tetrachlorodibenzo-p dioxin (TCDD) (40ug/kg-1 week) (oligospermia)	TT(postadministered)	200,400 mg/kg-28 days	Injected
[41]	Streptozotocin (50 mg/kg-2weeks) (diabetes)	TT(postadministered)	100,250,500 mg/kg-1 week	IP
[45]	Streptozotocin (55 mg/kg-1day) (diabetes)	TT(postadministered)	10 mg/kg-60 days	Orally by gavage
[44]	Streptozotocin (1dose) (diabetes)	TT(postadministered)	<0.5,162.24 ug/kg-12 weeks	NA
[18]	Castration	TT(postadministered)	5,10,25 mg/kg-14 days	NA
[40]	Castration	TT(postadministered)	5 mg/kg-8 weeks	NA
[11]	Sulphasalazine (100 mg/kg/day) (decrease fertility)	TT(postadministered)	50 mg/kg-60 days	Orally
[15]	Castration	TT(postadministered)	5 mg/kg-8 weeks	Orally
[48]	Control	TT (aqueous extract)	6 mg/kg-8 weeks	Orally
[50]	Control	TT (ethanolic extract)	7.5 mg/kg-4 weeks	Orally
[17]	Control	TT (lypholized aqueous extract)	100,50 mg/kg-28 days	Orally
[51]	Control	TT	5%W/V-21 days	—
[20]	Control	TT (ethanol extract)	42 mg/kg-70 days	Gavage
[47]	Control	TT (ethanol extract)	10 mg/kg-25 days	Orally
[52]	Control	TT	100 mg/kg	Orally by oral catheter
[16]	Control	TT (methanol extract)	300 mg/kg-8 weeks	Orally by intragastric tube
[46]	Control	TT (aqueous extract)	2.5,5,10 mg/kg-8 weeks	Orally
[49]	Control	TT (ethanolic extract)	11,42,110 mg/kg-28 days	Gavage

Table 1: Continued.

Study	Model development	TT (post-/pre-/ coadministered)	TT (dosage)	Dosage administration
Infertile human studies				
[33]	Human infertile (oligospermia)	TT (libilov tablets)	3*2 tablets (250 mg)-60 days	Orally
[25]	Human infertile (oligospermia)	TT (tribestan tablet)	750 mg-12 weeks	Orally
[34]	Human infertile (partial androgen deficiency in aging males)	TT	750 mg-3 months	Orally
[27]	Human infertile (unexplained infertility)	TT	750 mg-3 months	Orally
[10]	Human infertile (abnormal semen)	TT (dried extract)	250 mg-84 days	Orally

combinations. A total of 717 hits were obtained, of which 147 relevant studies were further shortlisted on the basis of titles and abstracts. By strictly following the predefined inclusion and exclusion criteria, we were left with 30 relevant studies. The detailed scheme adopted for literature screening and selection is presented in the PRISMA plot (Figure 1).

The studies included in this meta-analysis had evaluated the effect of TT on semen parameters and sex hormones or its aphrodisiac property (Table 1). The shortlisted studies included five human clinical trials evaluating the therapeutic effect [10, 25, 27, 33, 34], of which two were randomized trials [25, 27] and three were nonrandomized trials [10, 33, 34]. Five animal studies evaluated the preventive effect of TT against various fertility-compromising agents [35–39], ten evaluated the therapeutic effect of TT in model organisms [9, 11, 15, 18, 40–45], and eighteen studies administered TT in normal animals to evaluate improvements in semen parameters, hormone levels, and its aphrodisiac effect [9, 16, 17, 20, 36, 37, 39, 40, 42, 43, 45–52] (Table 2).

3.2. *Tribulus terrestris* L. Positively Impacted Sperm Parameters

3.2.1. Tribulus terrestris L. Improves Sperm Concentration. The effect of TT on sperm concentration was analysed by conducting meta-analyses on human and animal studies. In case of humans, four studies administered TT in a total of 133 infertile oligozoospermic/idiopathic infertile/erectile dysfunction/androgen deficient individuals [10, 25, 27, 33]. These studies used TT dose from 250 mg to 750 mg (tablet, dried extract or ethanolic/methanolic extract). The studies showed significant heterogeneity ($Q = 10.655$, $I^2 = 71.843$, $p = 0.014$), suggesting the use of the random effects model for drawing inference. The pooled effect size estimate showed a significant improvement in sperm concentration upon TT administration (SDM = 0.624, 95% CI = 0.13 to 1.117, $p = 0.013$; Figure 2). The comparison of quantitative data showed an average of 66.36% improvement in sperm concentration in infertile subjects.

Another set of animal studies was conducted on rodent models of infertility created by the administration of sodium valproate, cypermethrin, copper overload, bisphenol A, cyclophosphamide, malathion, morphine, strepozotocin, TCDD, diabetes, castration, or sulphasalazine. TT in these studies was administered either subsequent to the creation of model to score its therapeutic impact or along with the method of model creation to investigate the prophylactic effect of TT against a decrease in sperm count. The therapeutic effect of TT on sperm concentration was determined through pooled analysis of data from four studies with nine datasets [9, 41, 42, 45]. Meta-analysis showed significant heterogeneity ($Q = 27.305$, $I^2 = 70.701$, $p = 0.001$), suggesting the use of the random effects model for drawing inference. The pooled effect size estimate showed significant improvement in sperm concentration upon TT administration (Hedges' $g = 15.003$, 95% CI = 11.198 to 18.808, $p = 0.001$; Figure 2), suggesting a significant therapeutic effect.

The prophylactic effect of TT was studied using data pooled from two studies with four datasets [38, 39]. Pooled analysis showed significant heterogeneity ($Q = 26.976$, $I^2 = 88.879$, $p = 0.001$), suggesting the use of the random effects model for drawing the inference. The pooled effect size estimate showed a significant impact of TT in preventing a drop in sperm count; in fact, TT was able to not only prevent the decline but also improve sperm concentration (Hedges' $g = 3.938$, 95% CI = 1.500 to 6.376, $p = 0.002$; Figure 2).

In another set of seven studies presenting a total of 11 datasets, TT was administered in normal rodents (mouse and rat) [9, 17, 20, 39, 42, 45, 47]. Meta-analysis of these data sets showed significant heterogeneity ($Q = 63.019$, $I^2 = 84.132$, $p = 0.001$), suggesting the use of the random effects model. The pooled effect size analysis showed a significant improvement in sperm concentration upon TT administration (Hedge's $g = 2.149$, 95% CI = 1.035 to 3.263, $p = 0.001$; Figure 2).

3.2.2. Tribulus terrestris L. Improves Sperm Motility. The effect of TT on sperm motility was analysed by conducting meta-analyses on human and animal studies. In case of humans, four studies administered TT (tablet, dried powder, or ethanolic/methanolic extract) in a total of 130 oligozoospermic/idiopathic infertile/erectile dysfunction/androgen-

Table 2: Summary of the meta-analyses performed on various parameters.

Parameters	Subjects	Group	Test of association				Test of heterogeneity			Significance
			Hedges' g or SDM	95% CI		p	Q	p	I^2	
				Lower limit	Higher limit					
Sperm concentration	Human	Infertile	0.624	0.130	1.117	0.013	10.655	0.014	71.843	Significant using random model
	Animal model	Therapeutic	15.003	11.198	18.808	0.001	27.305	0.001	70.701	Significant using random model
		Prophylactic	3.938	1.500	6.376	0.002	26.976	0.001	88.879	Significant using random model
		Control	2.149	1.035	3.263	0.001	63.019	0.001	84.132	Significant using random model
Sperm motility	Human	Infertile	0.742	0.331	1.152	0.001	7.187	0.066	58.26	Significant using random model
	Animal model	Therapeutic	10.29	6.502	14.091	0.001	75.129	0.001	89.352	Significant using random model
		Prophylactic	3.316	1.684	4.947	0.001	25.655	0.001	84.409	Significant using random model
		Control	0.805	-0.434	2.044	0.203	13.651	0.003	78.024	Nonsignificant using random model
Sperm viability	Human	Infertile	—	—	—	—	—	—	—	—
	Animal model	Therapeutic	7.304	3.525	11.083	0.001	19.041	0.001	84.245	Significant using random model
		Prophylactic	5.581	2.882	8.280	0.001	21.371	0.001	85.962	Significant using random model
		Control	0.622	0.184	1.060	0.005	3.229	0.665	0.000	Significant using fixed model
Testosterone	Human	Infertile	0.438	-0.290	1.173	0.243	11.288	0.004	82.282	Nonsignificant using random model
	Animal model	Therapeutic	3.523	2.164	4.822	0.001	183.855	0.001	91.298	Significant using random model
		Prophylactic	2.524	0.963	4.065	0.001	63.105.	0.001	90.492	Significant using random model
		Control	0.736	-0.134	1.606	0.097	188.345	0.001	89.912	Nonsignificant using random model
FSH	Human	Infertile	-0.852	−2.049	0.345	0.163	28.270	0.001	92.925	Nonsignificant using random model
	Animal model	Therapeutic	-3.430	-6.263	-0.598	0.018	77.988	0.001	93.589	Significant using random model
		Prophylactic	3.979	1.516	6.441	0.002	29.559	0.001	89.851	Significant using random model
		Control	-2.096	-4.879	0.688	0.140	50.109	0.001	92.017	Nonsignificant using random model
LH	Human	Infertile	0.096	−0.119	0.311	0.383	1.421	0.841	0.000	Nonsignificant using fixed model
	Animal model	Therapeutic	-1.535	-4.437	1.367	0.300	64.599	0.001	93.808	Nonsignificant using random model
		Prophylactic	5.190	2.356	8.025	0.001	53.600	0.001	92.537	Significant using random model
		Control	-0.970	-2.466	0.527	0.204	34.239	0.001	88.317	Nonsignificant using random model
Mounting latency	Human	Infertile	—	—	—	—	—	—	—	—
	Animal model	Therapeutic	-18.106	-29.702	-6.509	0.002	41.959	0.001	92.850	Significant using random model

Table 2: Continued.

Parameters	Subjects	Group	Test of association 95% CI Hedges' g or SDM	Lower limit	Higher limit	p	Test of heterogeneity Q	p	I^2	Significance
Mounting frequency	Human	Prophylactic	—	—	—	—	—	—	—	—
		Control	-6.085	-6.988	-5.182	0.001	11.903	0.064	49.594	Significant using fixed model
		Infertile	—	—	—	—	—	—	—	—
	Animal model	Therapeutic	7.355	2.456	12.255	0.003	39.418	0.001	92.389	Significant using random model
		Prophylactic	—	—	—	—	—	—	—	—
		Control	8.678	5.638	11.718	0.001	47.915	0.001	87.478	Significant using random model
Intromission latency	Human	Infertile	—	—	—	—	—	—	—	—
	Animal model	Therapeutic	-12.477	-17.663	-7.292	0.001	13.128	0.004	77.148	Significant using random model
		Prophylactic	—	—	—	—	—	—	—	—
		Control	-4.480	-6.522	-2.439	0.001	74.514	0.001	91.948	Significant using random model
Intromission frequency	Human	Infertile	—	—	—	—	—	—	—	—
	Animal model	Therapeutic	11.365	3.093	19.638	0.007	42.431	0.001	92.930	Significant using random model
		Prophylactic	—	—	—	—	—	—	—	—
		Control	7.580	5.329	9.832	0.001	29.640	0.001	79.759	Significant using random model

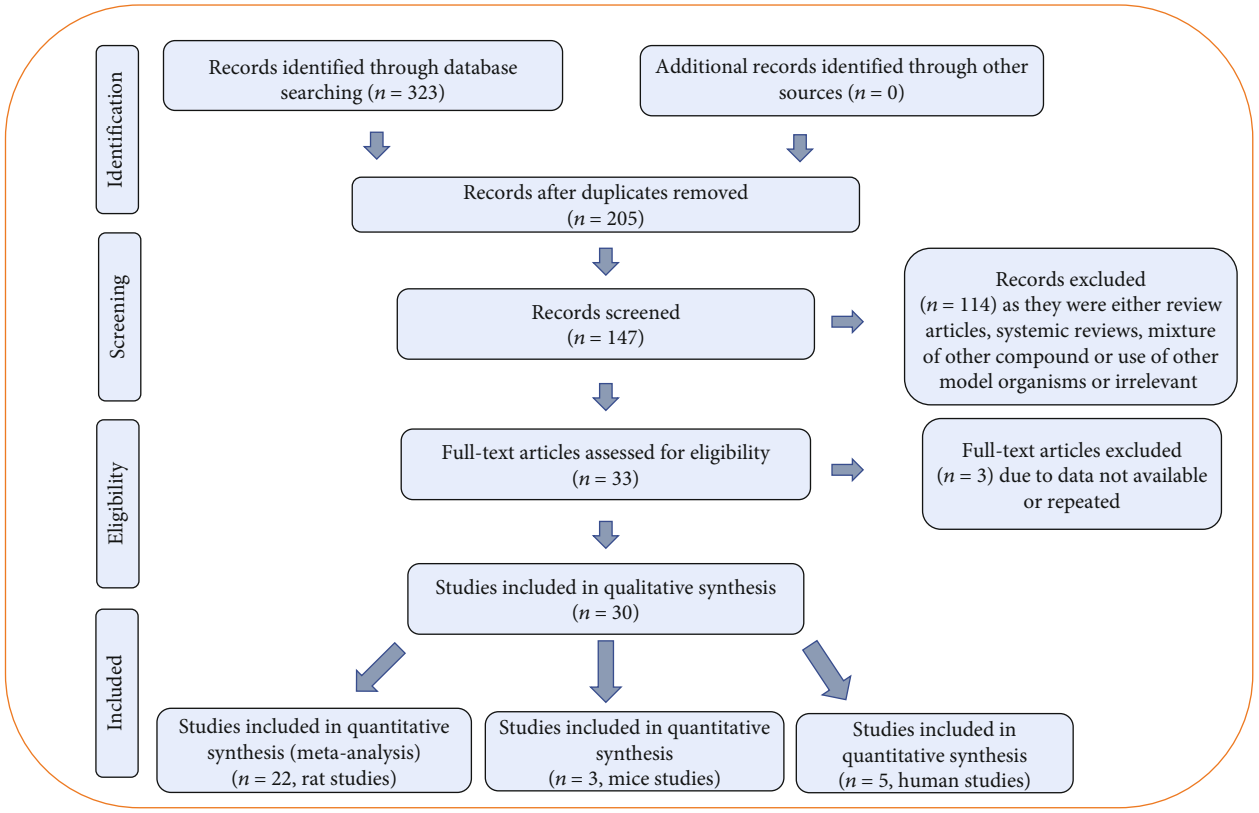

Figure 1: The PRISMA flow diagram showing the process of literature screening and study selection.

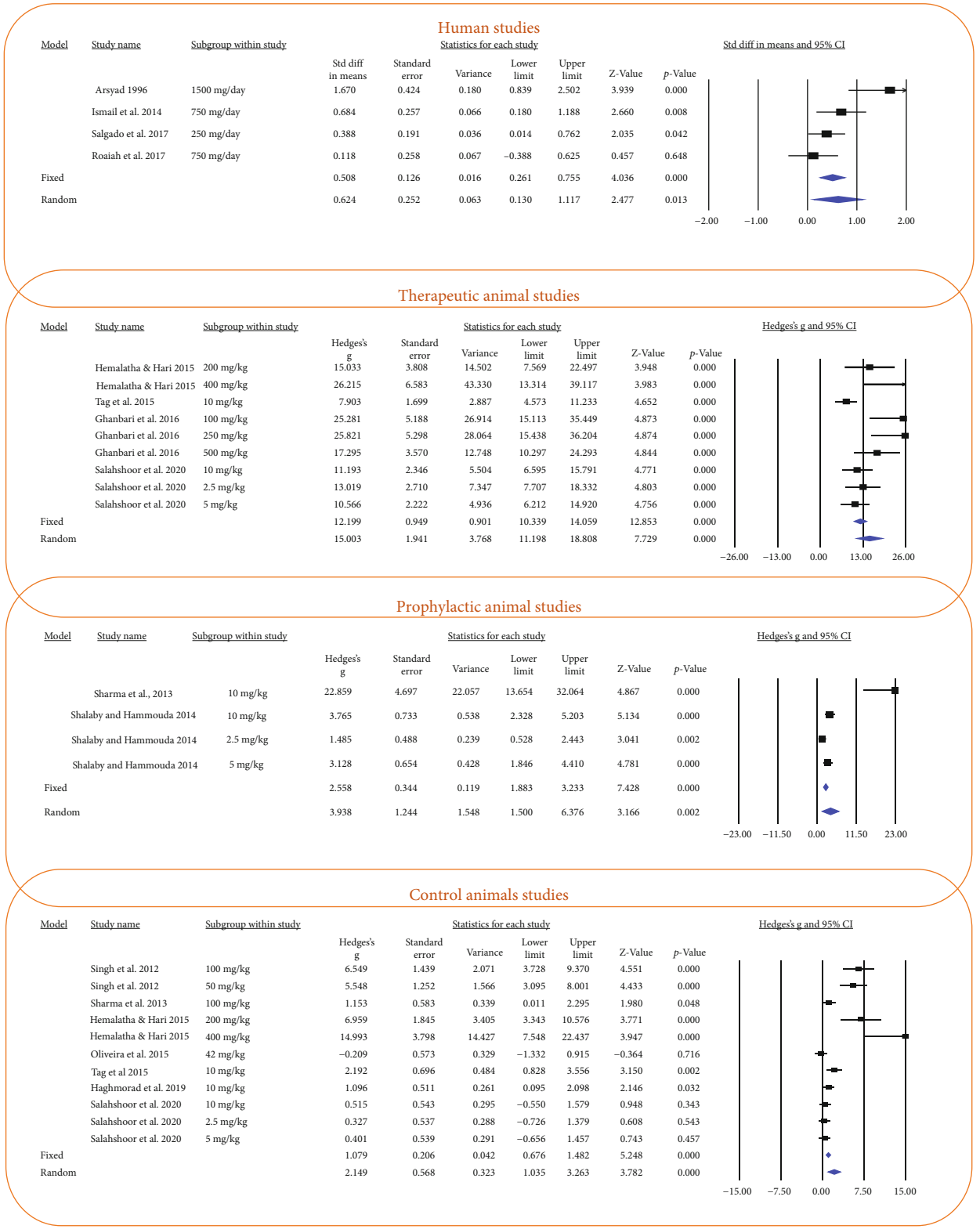

FIGURE 2: The forest plot showing the effect of TT on sperm concentration. The p value represents the significance, the horizontal black bar represents 95% CI with Hedges' g/SDM in the centre. The overall association is shown by a diamond-shaped box (blue).

deficient infertile patients [10, 25, 27, 33]. The studies showed significant heterogeneity ($Q = 7.187$, $I^2 = 58.26$, $p = 0.066$), suggesting the use of the random effects model for drawing inference. The pooled effect size estimate showed a significant improvement in sperm motility (SDM = 0.742, 95% CI = 0.331 to 1.152, $p = 0.001$; Figure 3). The

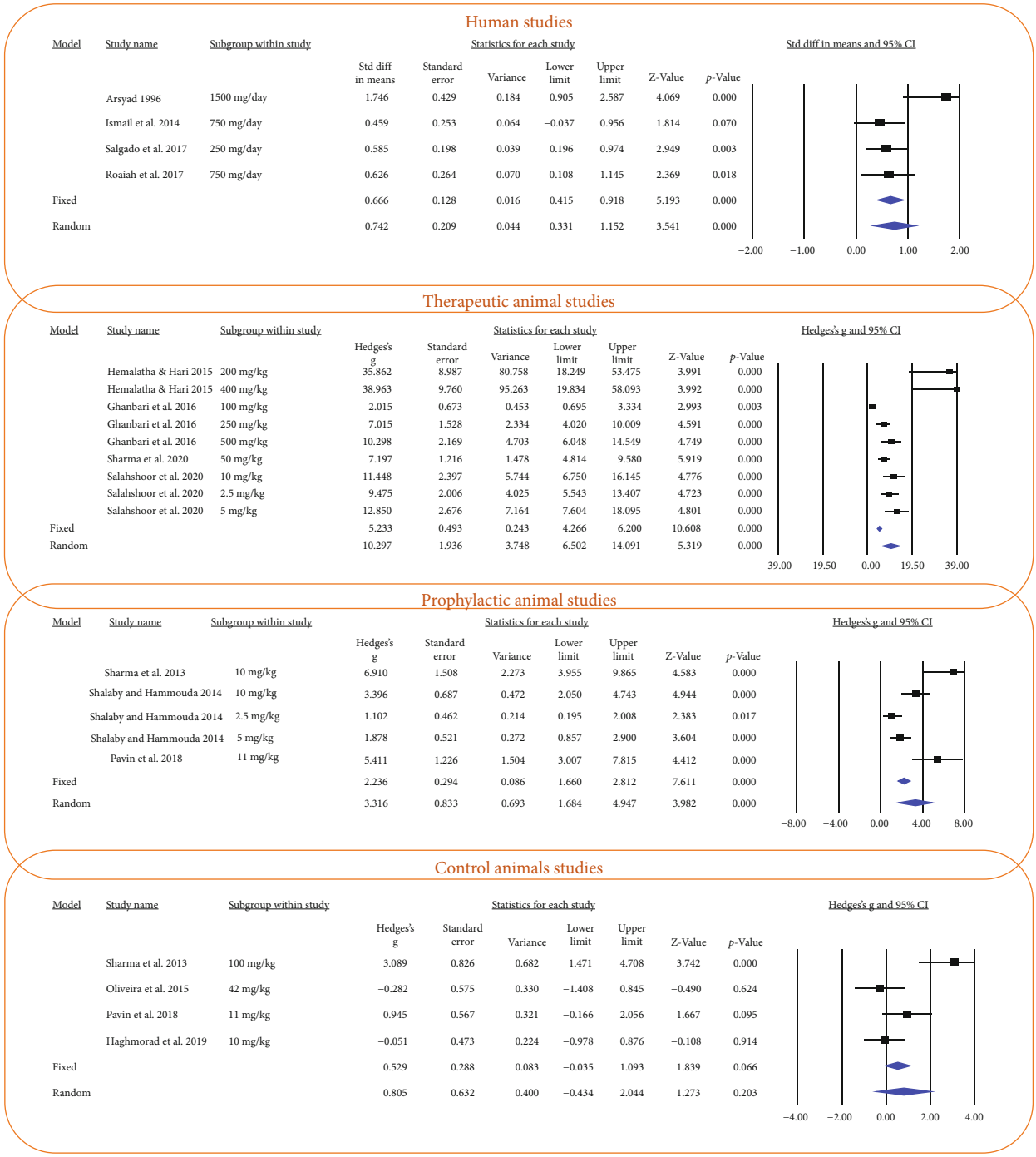

Figure 3: The forest plot showing the effect of TT on sperm motility. The p value represents the significance, the horizontal black bar represents 95% CI with Hedges' g/SDM in the centre. The overall association is shown by a diamond-shaped box (blue).

comparison of quantitative data showed an average of 41.23% improvement in sperm motility in infertile subjects.

Animal studies were conducted on rodent models of infertility generated by various means. The therapeutic effect of TT was determined through a pooled analysis on four studies [9, 11, 41, 42] with nine datasets. The test of heterogeneity showed significant heterogeneity ($Q = 75.129$, $I^2 = 89.352$, $p = 0.001$), suggesting the use of the random effects model for drawing inference. The pooled effect size estimate showed a significant improvement in sperm motility upon TT administration (Hedges' $g = 10.29$, 95% CI = 6.502 to 14.091, $p = 0.001$; Figure 3).

Three studies with five datasets [37–39] had studied the prophylactic effect of TT. The test of heterogeneity showed significant heterogeneity ($Q = 25.655$, $I^2 = 84.409$, $p = 0.001$), suggesting the use of the random effects model for

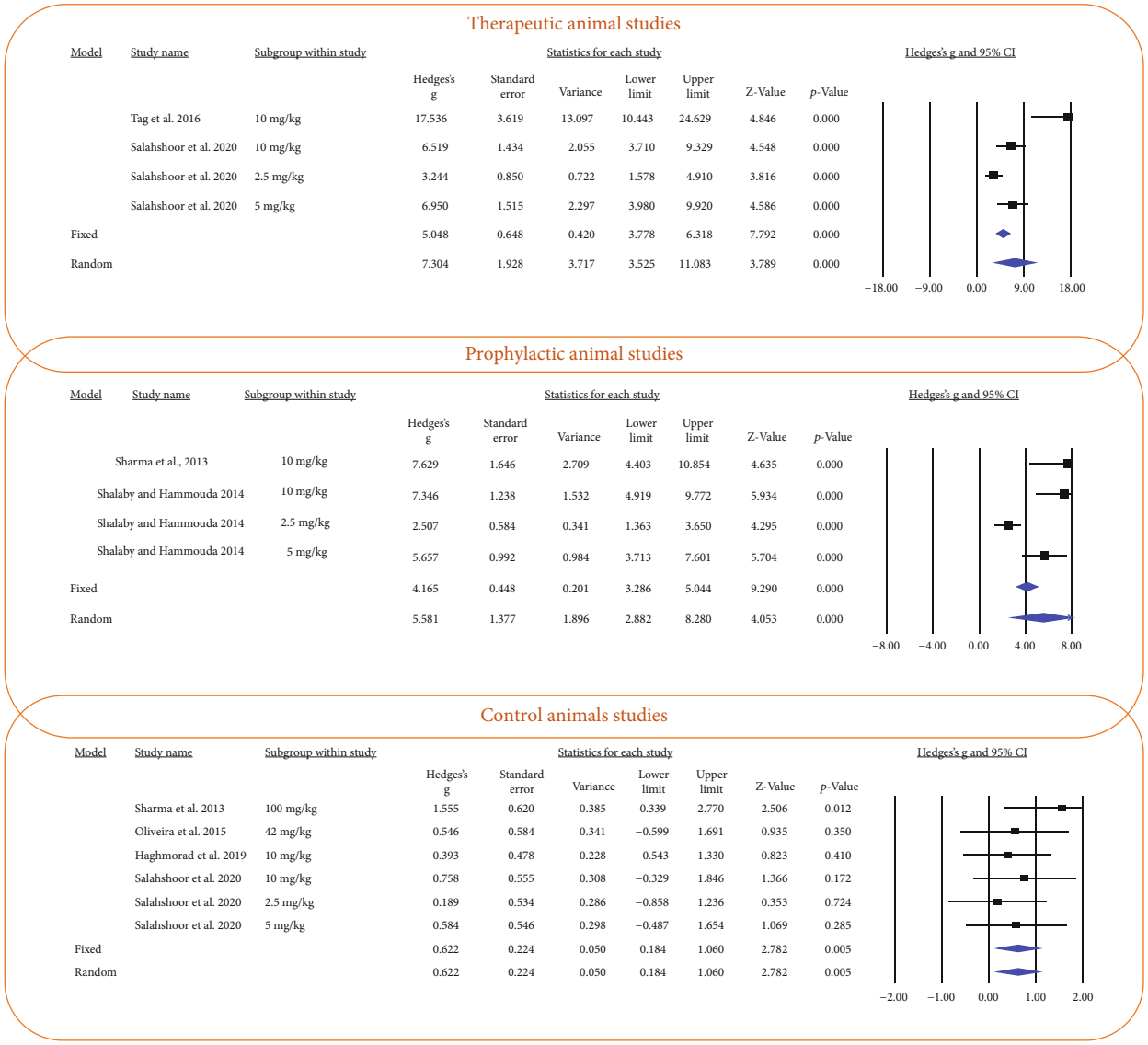

FIGURE 4: The forest plot showing the effect of TT on sperm viability. The p value represents the significance, the horizontal black bar represents 95% CI with Hedges' g/SDM in the centre. The overall association is shown by a diamond-shaped box (blue).

inference. The pooled effect size estimate showed the efficacy of TT not only in preventing a decline in sperm motility but also in improving sperm motility (Hedges' $g = 3.316$, 95% CI = 1.684 to 4.947, $p = 0.001$; Figure 3).

Apart from studies on animal models, TT was also administered to normal rodents (rat and mouse) in four studies [20, 37, 39, 47] with four datasets. Meta-analysis showed a significant heterogeneity ($Q = 13.651$, $I^2 = 78.024$, $p = 0.003$), suggesting the use of the random effects model for drawing inference. The pooled effect size estimate showed no significant improvement in sperm motility upon TT administration (Hedges' $g = 0.805$, 95% CI = −0.434 to 2.044, $p = 0.203$; Figure 3).

3.2.3. Tribulus terrestris L. Improves Sperm Viability. The effect of TT administration on sperm viability was studied in animals only. In these studies, TT was given to rodents with or without fertility compromise.

The therapeutic effect of TT on sperm viability was studied using various models of infertility [9, 45]. Significant heterogeneity ($Q = 19.041$, $I^2 = 84.245$, $p = 0.001$) existed in these studies. Upon TT administration, a significant improvement in sperm viability was observed in the random effects model (Hedges' $g = 7.304$, 95% CI = 3.525 to 11.083, $p = 0.001$; Figure 3). Similarly, the prophylactic effect of TT was studied in combination with fertility deteriorating agents [38, 39]. Four data sets from these two studies were pooled for meta-analysis. Significant heterogeneity was seen ($Q = 21.371$, $I^2 = 85.962$, $p = 0.001$) and the pooled effect size estimate showed significant prevention of sperm viability deterioration in challenged animals (Hedges' $g = 5.581$, 95% CI = 2.882 to 8.280, $p = 0.001$; Figure 4).

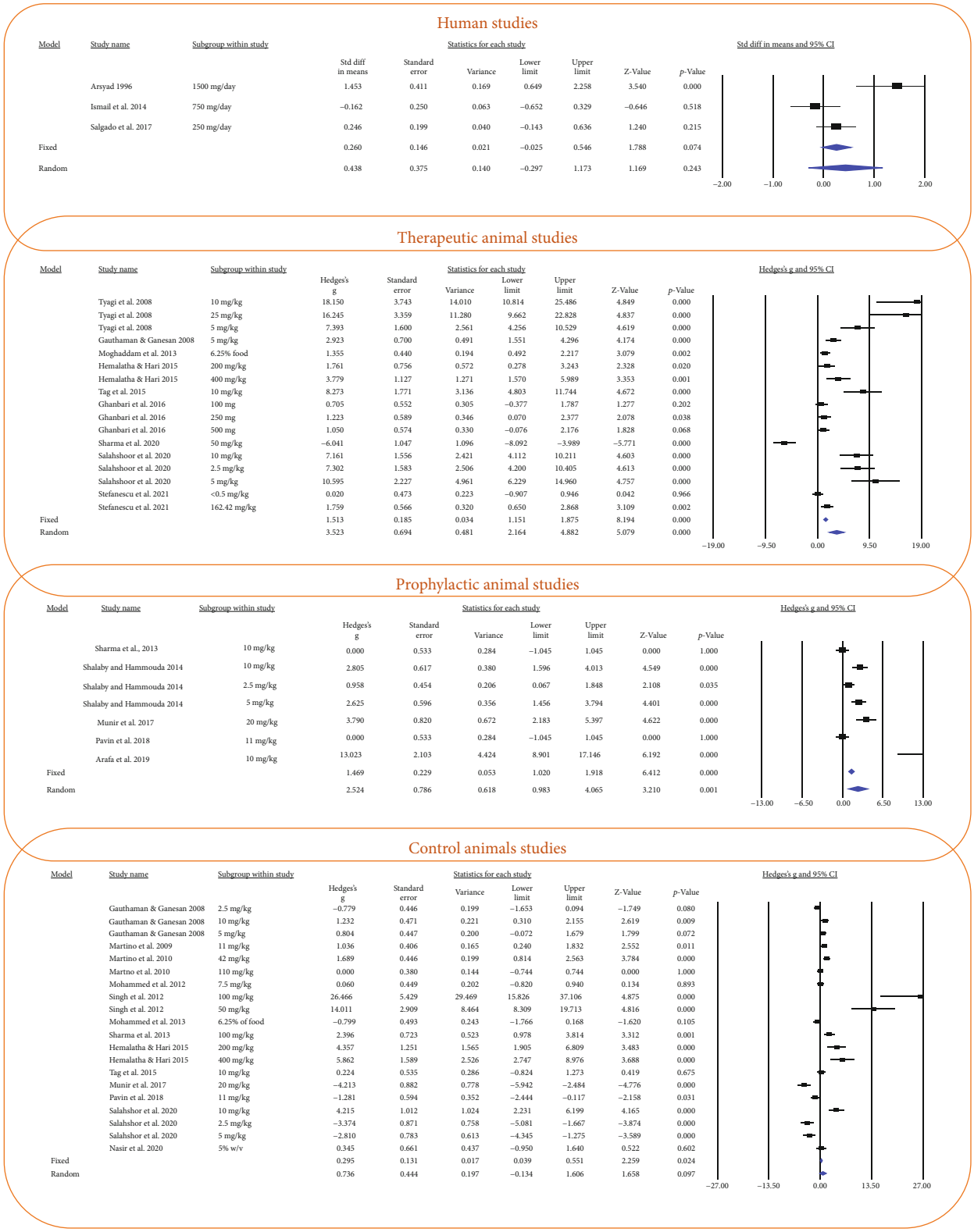

Figure 5: The forest plot showing the effect of TT on Testosterone. The p value represents the significance, the horizontal black bar represents 95% CI with Hedges' g/SDM in the centre. The overall association is shown by a diamond-shaped box (blue).

In case of normal rodents, four studies with six datasets [9, 20, 39, 47] were pooled to evaluate the effect of TT on sperm viability. The test of heterogeneity showed no significant heterogeneity ($Q = 3.229$, $I^2 = 0.000$, $p = 0.665$); therefore, the fixed effect model estimates were taken for drawing inference. The pooled effect size analysis showed significant improvement in sperm viability upon TT administration (Hedges' $g = 0.622$, 95% CI = 0.184 to 1.060, $p = 0.005$; Figure 4).

3.3. Tribulus terrestris L. Regulates Hormone Levels

3.3.1. Tribulus terrestris L. Improves Testosterone Level Marginally. The effect of TT on testosterone was analysed by conducting meta-analyses on human and animal studies.

In case of infertile human patients, three studies [10, 25, 33] with significant heterogeneity ($Q = 11.288$, $I^2 = 82.282$, $p = 0.004$) were pooled to draw inferences using the random effects model. The pooled effect size estimate showed no significant improvement in testosterone upon TT administration in infertile patients (SDM = 0.438, 95% CI = −0.29 to 1.173, $p = 0.243$; Figure 5).

Animal studies were conducted on rodent models of infertility generated by various means. TT in these studies was administered either afterwards to evaluate the therapeutic effect or along with the method of model creation to investigate the prophylactic effect. Nine studies with seventeen datasets had administered TT in therapeutic mode in animal models of infertility generated by various means [9, 11, 18, 40–45]. The data across these studies were heterogenous ($Q = 183.855$, $I^2 = 91.298$, $p = 0.001$), favoring the random effects model. The pooled effect size estimate showed significant improvement in testosterone upon TT administration (Hedges' $g = 3.523$, 95% CI = 2.164 to 4.822, $p = 0.001$; Figure 5).

Five studies with seven datasets having significant heterogeneity ($Q = 63.105$, $I^2 = 90.492$, $p = 0.001$) evaluated the prophylactic effect of TT against deterioration in testosterone levels as a result of various exposures [35–39]. The pooled effect size estimate showed that TT not only offered effective prevention but also resulted in a significant improvement in testosterone (Hedges' $g = 2.524$, 95% CI = 0.983 to 4.065, $p = 0.001$; Figure 5).

In case of control animal studies, twelve studies with 20 datasets administered TT in control animals [9, 17, 36, 37, 39, 40, 42, 43, 45, 49–51]. Meta-analysis showed significant heterogeneity ($Q = 188.345$, $I^2 = 89.912$, $p = 0.001$), favoring the use of the random effects model. The pooled effect size estimate showed no significant improvement in testosterone upon TT administration (Hedges' $g = 0.736$, 95% CI = −0.134 to 1.606, $p = 0.097$; Figure 5).

3.3.2. Tribulus terrestris L. Affects the FSH Level. Three studies with three data sets had analysed the effect of TT administration on FSH level in infertile human patients [10, 25, 33]. The data across these studies were heterogenous ($Q = 28.27$, $I^2 = 92.925$, $p = 0.001$), suggesting the use of the random effects model for interpretation. TT administration decreased the FSH level, but the effect was not statistically significant (SDM = −0.852, 95% CI = −2.049 to 0.345, $p = 0.163$; Figure 6).

In animal models of infertility, the therapeutic effect of TT administration on FSH level was analysed by pooling data from four studies [42–45]. Pooled analysis identified significant heterogeneity across these studies ($Q = 77.988$, $I^2 = 93.589$, $p = 0.001$), suggesting the use of the random effects model for inference. The pooled effect size using the random effects model showed a significant reduction in the level of FSH upon TT administration (Hedges' $g = -3.430$, 95% CI = −6.263 to -0.598, $p = 0.018$; Figure 6).

Two studies with four data sets [38, 39] evaluated the prophylactic impact of TT in resisting a change in the FSH level. In the presence of significant heterogeneity ($Q = 29.559$, $I^2 = 89.851$, $p = 0.001$), the random effects model was preferred. The pooled effect size estimate showed significant improvement in FSH upon TT administration (Hedges' $g = 3.979$, 95% CI = 1.516 to 6.441, $p = 0.002$; Figure 6).

In another set of four studies [39, 42, 43, 45], TT was administrated in control animals. Meta-analysis showed significant heterogeneity ($Q = 50.109$, $I^2 = 92.017$, $p = 0.001$), suggesting the use of the random effects model for drawing inference. The pooled effect size estimate showed a decline in the FSH level, but the effect was not statistically significant (Hedges' g = −2.096, 95% CI = −4.879 to 0.688, $p = 0.140$; Figure 6).

3.3.3. Tribulus terrestris L. Affects the LH Level. In case of human infertile patients, five studies with five datasets [10, 25, 27, 33, 34] showed no significant heterogeneity ($Q = 1.421$, $I^2 = 0$, $p = 0.841$). Pooled effect size estimation using the random effects model showed no significant improvement in LH upon TT administration in infertile patients (SDM = 0.096, 95% CI = −0.119 to 0.311, $p = 0.383$; Figure 7).

In case of animal model studies, the therapeutic effect of TT on LH was determined through pooled analysis on three studies with five datasets [42–44]. Significant heterogeneity ($Q = 64.599$, $I^2 = 93.808$, $p = 0.001$) across these studies advocated the use of the random effects model. The pooled effect size estimate showed no significant improvement in LH upon TT administration (Hedges' $g = -1.535$, 95% CI = −4.437 to 1.367, $p = .300$; Figure 7).

Three studies [35, 38, 39] evaluated the prophylactic effect of TT. Pooled analysis revealed significant heterogeneity ($Q = 53.600$, $I^2 = 92.537$, $p = 0.001$), suggesting the use of the random effects model for inference. The pooled effect size estimate showed significant improvement in LH upon TT administration (Hedges' $g = 5.190$, 95% CI = 2.356 to 8.025, $p = 0.001$; Figure 7).

Four studies ([39, 42, 43, 48]) administered TT in normal animals to assess its impact on LH. Meta-analysis showed significant heterogeneity ($Q = 34.239$, $I^2 = 88.317$, $p = 0.001$), suggesting the use of the random effects model for drawing inference. The pooled effect size estimate showed no significant change in LH upon TT administration (Hedges' $g = -0.970$, 95% CI = −2.466 to 0.527, $p = 0.204$; Figure 7).

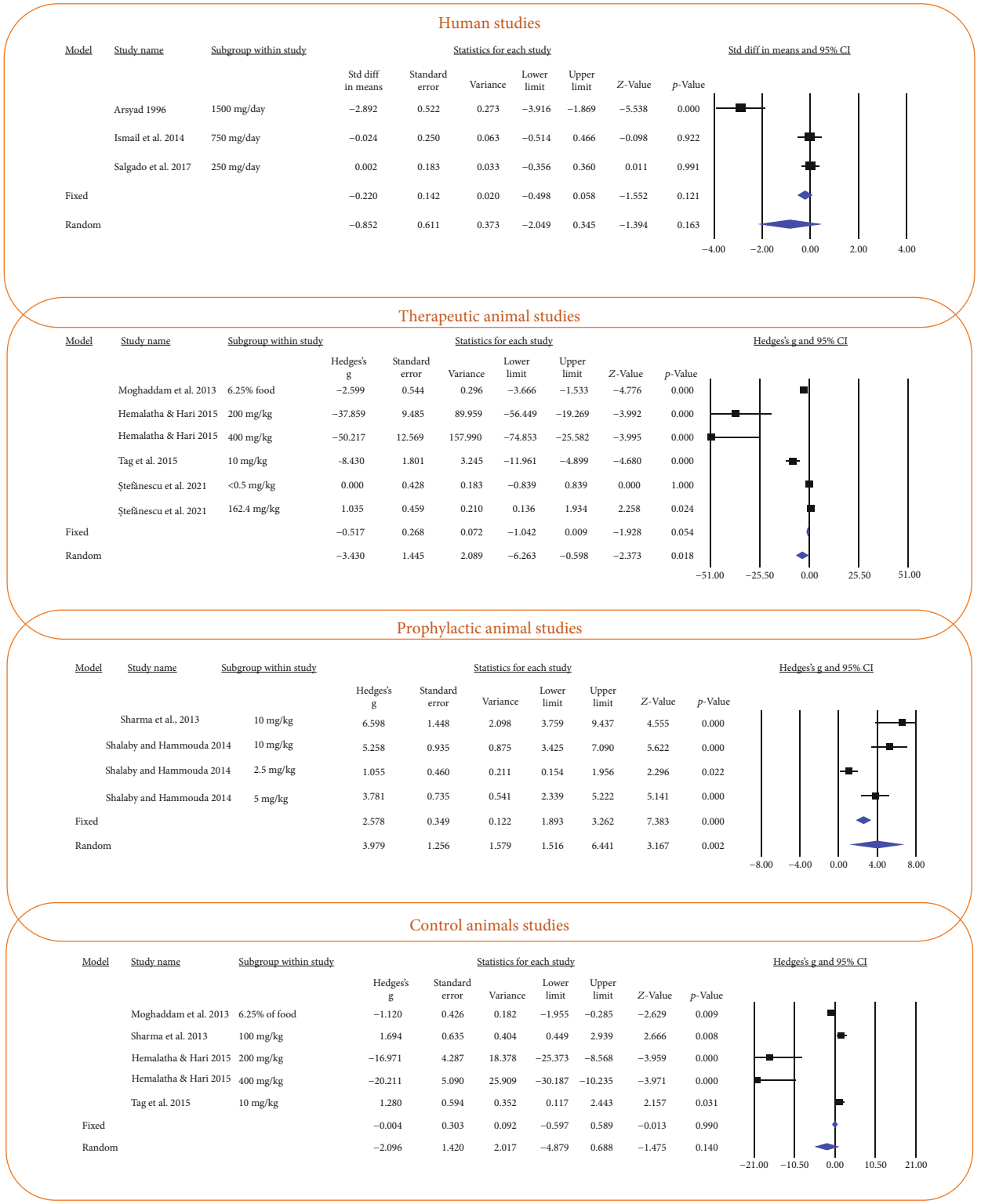

Figure 6: The forest plot showing the effect of TT on FSH. The *p* value represents the significance, the horizontal black bar represents 95% CI with Hedges' *g*/SDM in the centre. The overall association is shown by a diamond-shaped box (blue).

3.4. Tribulus terrestris L. Possesses Aphrodisiac Activity. Tribulus terrestris L. has also been claimed to possess aphrodisiac activity. Aphrodisiac activity across most of the studies was assessed in animals by quantitative analysis of the mounting latency (ML), mounting frequency (MF), intromission latency (IL), and intromission frequency (IF).

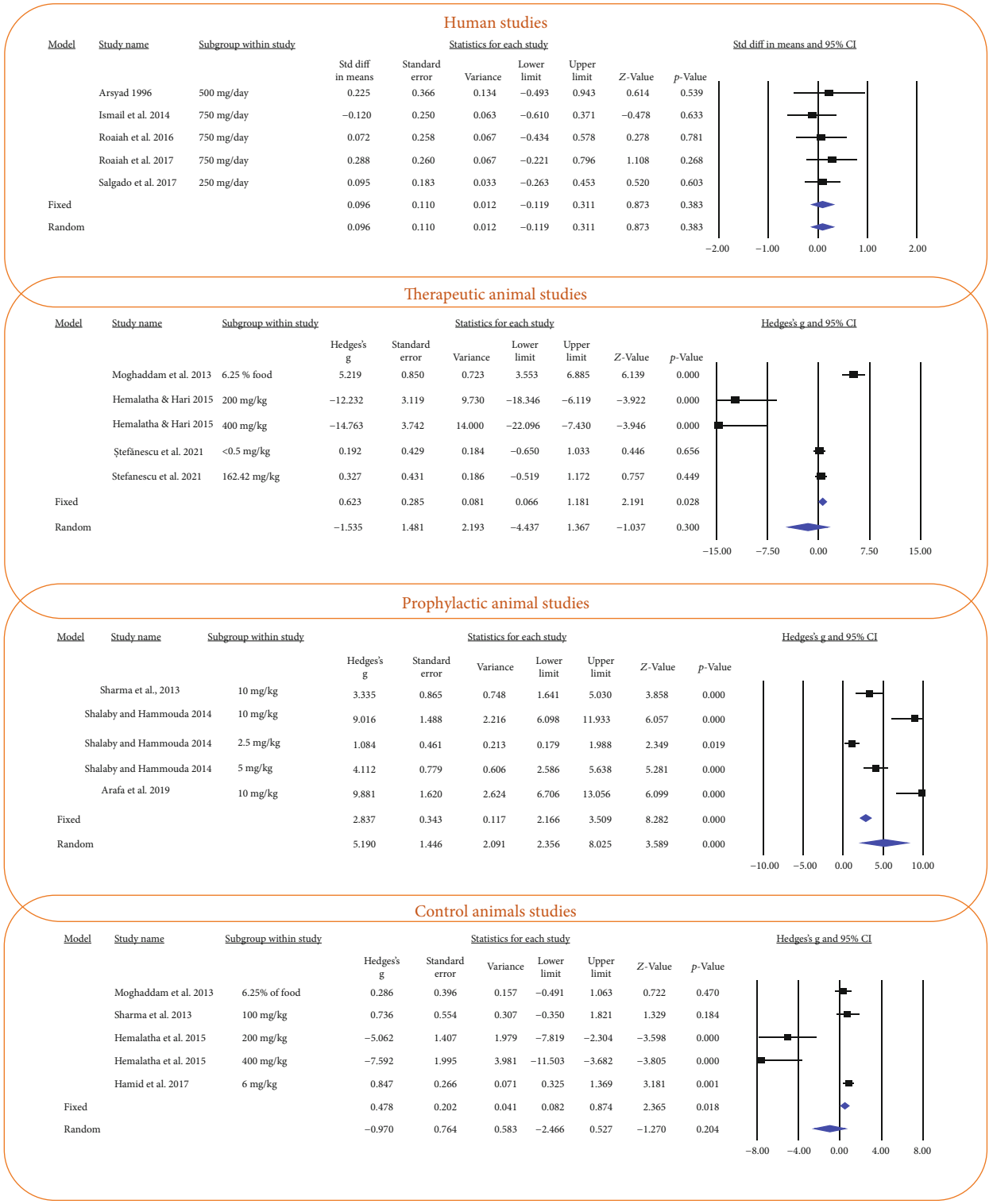

FIGURE 7: The forest plot showing the effect of TT on LH. The p value represents the significance, the horizontal black bar represents 95% CI with Hedges' g/SDM in the centre. The overall association is shown by a diamond-shaped box (blue).

3.4.1. Tribulus terrestris L. Reduces the Mounting Latency (ML). Two studies [15, 18] had evaluated the effect of TT administration on ML in castrated rat models. Pooled analysis on these studies with four datasets showed significant heterogeneity ($Q = 41.959$, $I^2 = 92.850$, $p = 0.001$), suggesting the use of the random effects model for inference. The pooled effect size estimate showed significant improvement in ML upon TT administration (Hedges' g = −18.106, 95% CI = −29.702 to -6.509, $p = 0.002$; Figure 8.

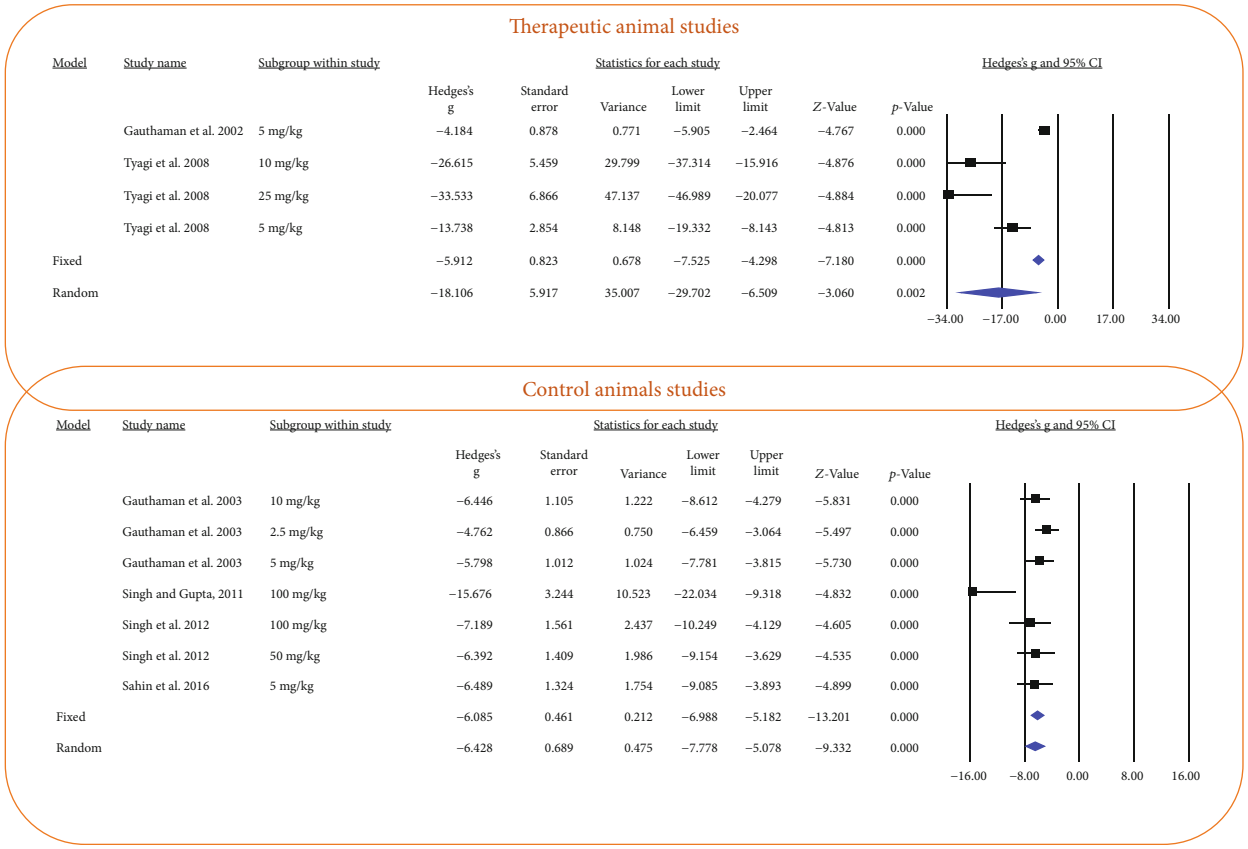

Figure 8: The forest plot showing the effect of TT on the mounting latency. The p value represents the significance, the horizontal black bar represents 95% CI with Hedges' g/SDM in the centre. The overall association is shown by a diamond-shaped box (blue).

TT was administered in control animals in four studies with seven datasets [16, 17, 46, 52]. Meta-analysis on the pooled data showed no significant heterogeneity ($Q = 11.903$, $I^2 = 49.594$, $p = 0.064$), suggesting the use of the fixed effect model for drawing inference. The pooled effect size estimate showed significant reduction in ML upon TT administration (Hedges' $g = -6.085$, 95% CI = −6.988 to -5.182, $p = 0.001$; Figure 8).

3.4.2. Tribulus terrestris L. Improves the Mounting Frequency (MF). Pooled analysis including four datasets from two therapeutic studies [15, 18] showed significant heterogeneity ($Q = 39.418$, $I^2 = 92.389$, $p = 0.001$), suggesting the use of the random effects model for inference. The pooled effect size estimate showed significant improvement in MF upon TT administration (Hedges' $g = 7.355$, 95% CI = 2.456 to 12.255, $p = 0.003$; Figure 9).

Four studies with seven datasets presented the effect of TT in normal (control) animals [16, 17, 46, 52]. Meta-analysis showed significant heterogeneity ($Q = 47.915$, $I^2 = 87.478$, $p = 0.001$), suggesting the use of the random effects model for drawing inference. The pooled effect size estimate showed significant improvement in MF upon TT administration (Hedges' $g = 8.678$, 95% CI = 5.638 to 11.718, $p = 0.001$; Figure 9).

3.4.3. Tribulus terrestris L. Reduces the Intromission Latency (IL). The effect of TT on intromission latency was evaluated on castrated rat models in two studies with four datasets [15, 18]. Pooled analysis on these studies showed significant heterogeneity ($Q = 13.128$, $I^2 = 77.148$, $p = 0.004$), suggesting the use of the random effects model for inference. The pooled effect size estimate showed significant reduction in IL upon TT administration (Hedges' $g = -12.477$, 95% CI = −17.663 to -7.292 $p = 0.001$; Figure 10).

Four studies including seven datasets had evaluated the effect of TT on IL in control animals [16, 17, 46, 52]. Meta-analysis showed significant heterogeneity ($Q = 74.514$, $I^2 = 91.948$, $p = 0.001$), suggesting the use of the random effects model for drawing inference. The pooled effect size estimate showed significant reduction in IL upon TT administration (Hedges' $g = -4.480$, 95% CI = −6.522 to -2.439 $p = 0.001$; Figure 10).

3.4.4. Tribulus terrestris L. Augments the Intromission Frequency (IF). The therapeutic effect of TT on IF was also evaluated in castrated animal models [15, 18]. Pooled analysis on four datasets from two studies showed significant heterogeneity ($Q = 42.431$, $I^2 = 92.930$, $p = 0.001$), suggesting the use of the random effects model for inference. The pooled effect size estimate showed significant improvement in IF upon TT administration (Hedges' $g = 11.365$, 95% CI = 3.093 to 19.638, $p = 0.007$; Figure 11).

Four studies with seven data sets evaluated the effect of TT on intromission frequency in control animals [16, 17, 46, 52]. Meta-analysis showed significant heterogeneity ($Q = 29.64$, $I^2 = 79.759$, $p = 0.001$), suggesting the use of the

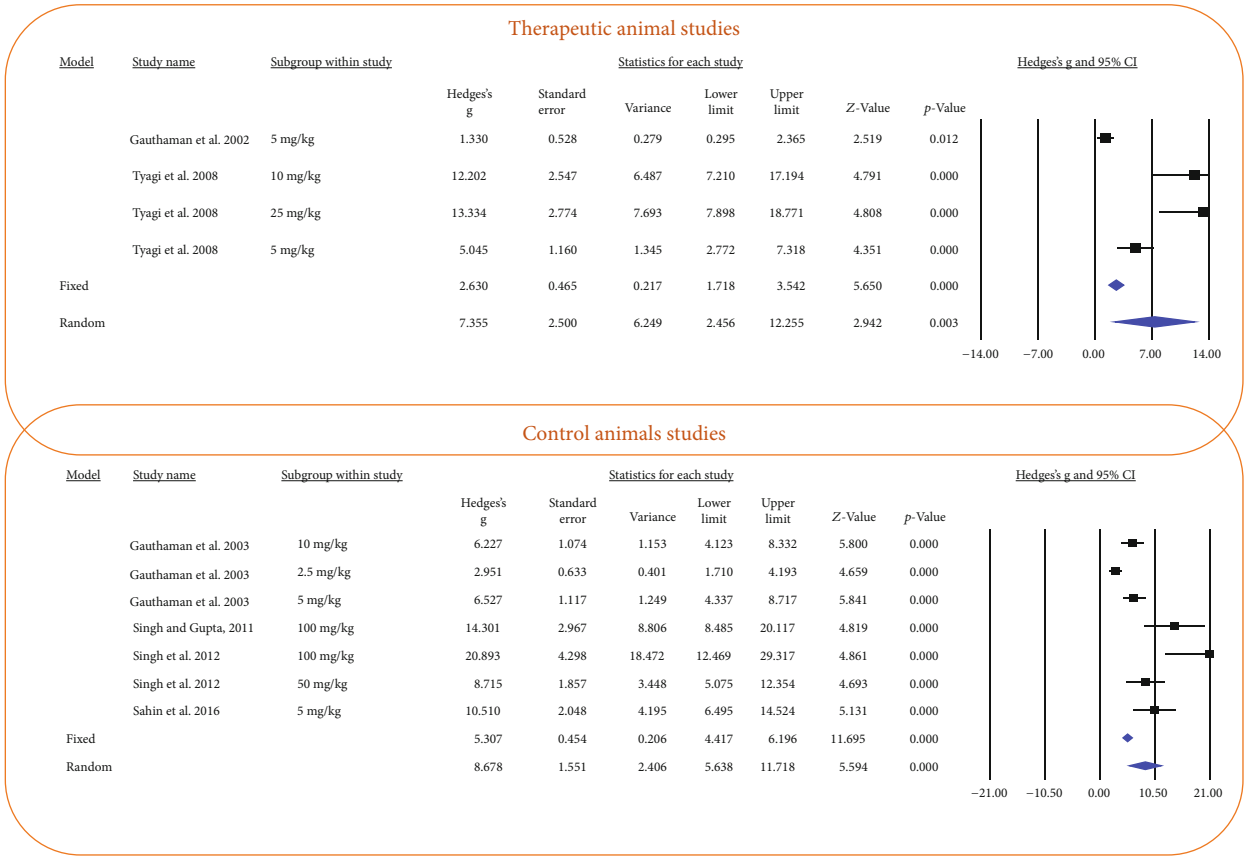

FIGURE 9: The forest plot showing the effect of TT on the mounting frequency. The p value represents the significance, the horizontal black bar represents 95% CI with Hedges' g/SDM in centre. The overall association is shown by a diamond-shaped box (blue).

random effects model for drawing inference. The pooled effect size estimate showed significant improvement in IF upon TT administration (Hedges' $g = 7.580$, 95% CI = 5.329 to 9.832, $p = 0.001$; Figure 11).

3.5. Publication Bias. The funnel plot and Egger's intercept tests were used to assess publication bias. In case of human studies, the pooled effect size calculations for sperm parameters, such as sperm concentration (Egger's intercept = 4.8876; p value (2tailed) = 0.23053), and sperm motility (Egger's intercept = 4.644; p value (2tailed) = 0.1425), and hormones, such as testosterone (Egger's intercept = 5.30902; p value (2 tailed) = 0.47351), FSH (Egger's intercept = −8.05273; p value (2tailed) = 0.21751), and LH (Egger's intercept = 0.5948; p value (2tailed) = 0.69837), were bias free.

In case of control animal studies, sperm motility (Egger's intercept = 8.67334; p value (2tailed) = 0.128), sperm viability (Egger's intercept = 6.586; p value (2tailed) = 0.0992), testosterone (Egger's intercept = 2.68113; p value (2tailed) = 0.1167), and FSH (Egger's intercept = −3.433; p value (2 tailed) = 0.259) were bias free.

Sperm concentration, LH, ML, MF, IL, and IF in control animal groups; sperm concentration, sperm motility, sperm viability, T, FSH, ML, MF, IL, and IF in therapeutic animal groups; sperm concentration, sperm motility, sperm viability, T, FSH, and LH in prophylactic groups showed publication bias. For these parameters, the trim and fill method was used to generate bias-free estimates (Figure 12). After adjustment, the conclusions for different parameters in normal animal study (sperm concentration, LH, ML, IL, and IF), therapeutic study (sperm concentration, motility, testosterone, FSH, ML, and IF), and prophylactic study (motility and viability) were changed. Therefore, this data must be taken with caution.

3.6. Sensitivity Analysis. Sensitivity analysis was undertaken with the exclusion of one study at a time to identify sensitive studies. The effect size estimates along with 95% CI after omission of each study are presented in Figure 13 for the parameters where a sensitive study was identified. We found Ismail et al. [25] on sperm concentration in humans, Salahshoor et al. and Munir et al. [9, 36] on testosterone estimation in control rats, Sharma et al. and Tag et al. [39, 45] in FSH estimation in control rats, to be sensitive. In therapeutic studies, Hemalatha and Hari and Tag et al. [42, 45] in FSH, Moghaddam et al. [43] in LH and Tyagi et al. [18] sub-group with 5 mg/kg dosage in MF estimation, were sensitive.

4. Discussion

Tribulus terrestris L. is a traditional medicinal plant that has been claimed to have aphrodisiac and profertility effects in the Ayurvedic and Chinese medicinal systems [6, 7]. Apart from its curative effects in infertility, the impact on sperm parameters in normal individuals and its effect on libido and

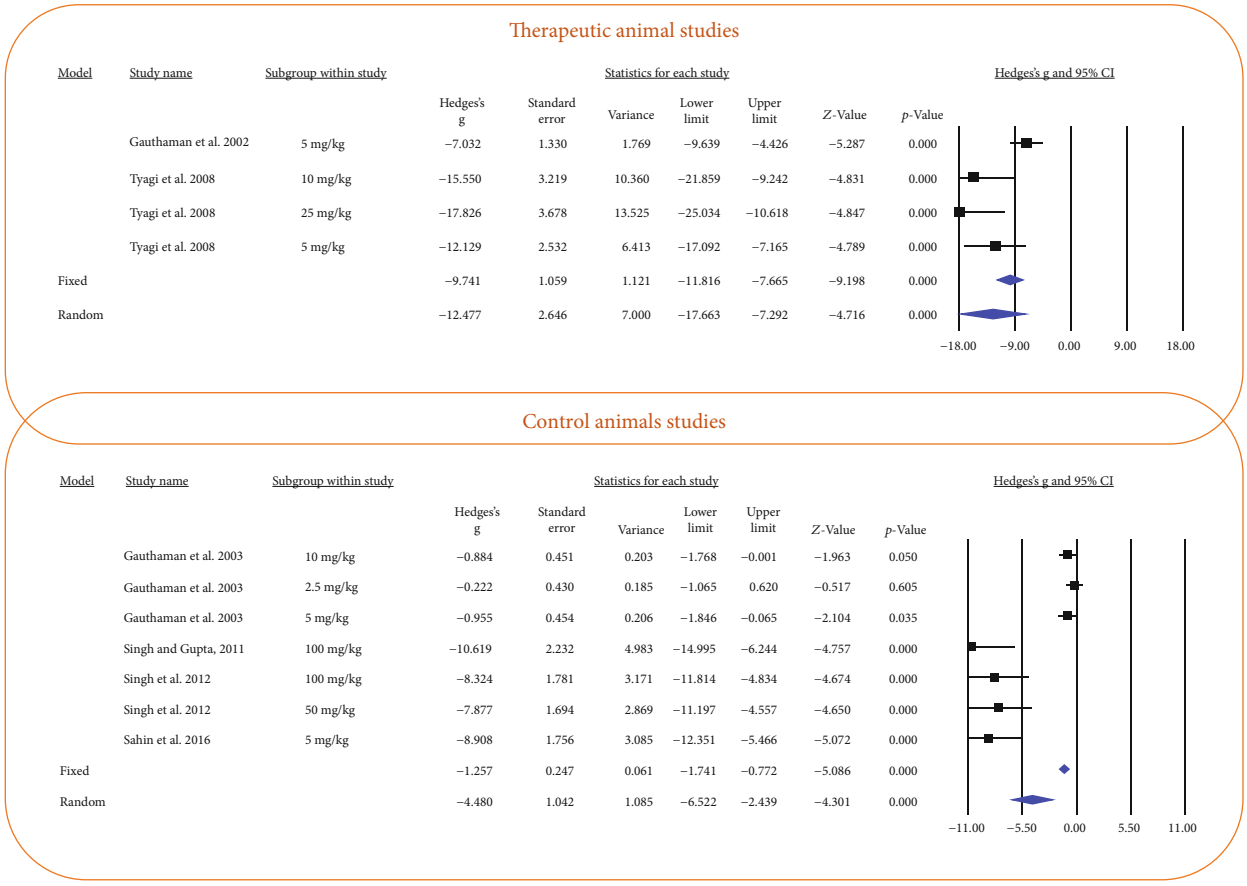

Figure 10: The forest plot showing the effect of TT on the intromission latency. The *p* value represents the significance, the horizontal black bar represents 95% CI with Hedges' *g*/SDM in the centre. The overall association is shown by a diamond-shaped box (blue).

hormones are equivocal across studies. Therefore, we undertook the present meta-analyses to evaluate the effects of TT supplementation on sperm parameters, libido, and sex hormones by pooling data from animal and human studies.

4.1. Tribulus terrestris L. Improves Sperm Parameters Irrespective of Fertility Status. The findings of this meta-analysis suggested that TT administration improves sperm parameters (sperm count, motility, and viability) significantly, irrespective of the status of fertility. TT administration resulted in a significant improvement in sperm concentration, sperm motility, sperm viability in infertile human patients, infertile animal models, and normal rodents. It also provided a prophylactic effect in animals exposed to various fertility compromising agents, further endorsing its profertility effect. Pooled analysis on animal experimentation suggested that TT may also improve sperm parameters under normal conditions, but the effects were more pronounced under stress conditions or infertility. This is supported by the studies on humans as well as infertile animal models. This meta-analysis supports the profertility effects of TT in more than one ways.

4.2. Tribulus terrestris L. Exerts Aphrodisiac Effect. TT showed capability to improve the male sexual functions, including erectile function and libido. The most interesting data in this regard was generated on the castrated rats, in which TT showed significant aphrodisiac activity [18, 40]. Since castration leads to low androgen status, affecting structural, biochemical, pharmacological, and erectile function [53, 54], improvement in libido parameters in these animals provides the strongest evidence in support of its aphrodisiac activity. Physiologically, low level of androgen, as seen in hypogonadism, is associated with decreased sexual desire and activity [55, 56], which was counteracted by TT. We found that TT administration led to improvement in mounting frequency, intromission frequency with a reduction in mounting latency and intromission latency, suggesting a strong aphrodisiac effect of TT.

4.3. Mechanism of Action of Tribulus terrestris L. It has been suggested that protodioscin works by increasing the conversion of testosterone into the more potent androgen, i.e., dihydrotestosterone [33]; however, this meta-analysis suggested that the effect on testosterone may account for its biological effects only to a little extent. TT is believed to be a scavenger of free radicals due to its active constituents (saponins, alkaloids, flavonoids), which might improve sperm parameters, particularly under stress conditions [57]. The effect of TT on the Ca^{2+} channel could be a possible reason for its positive impact on sperm motility [58]. TT may improve semen parameters because of its strong antioxidant

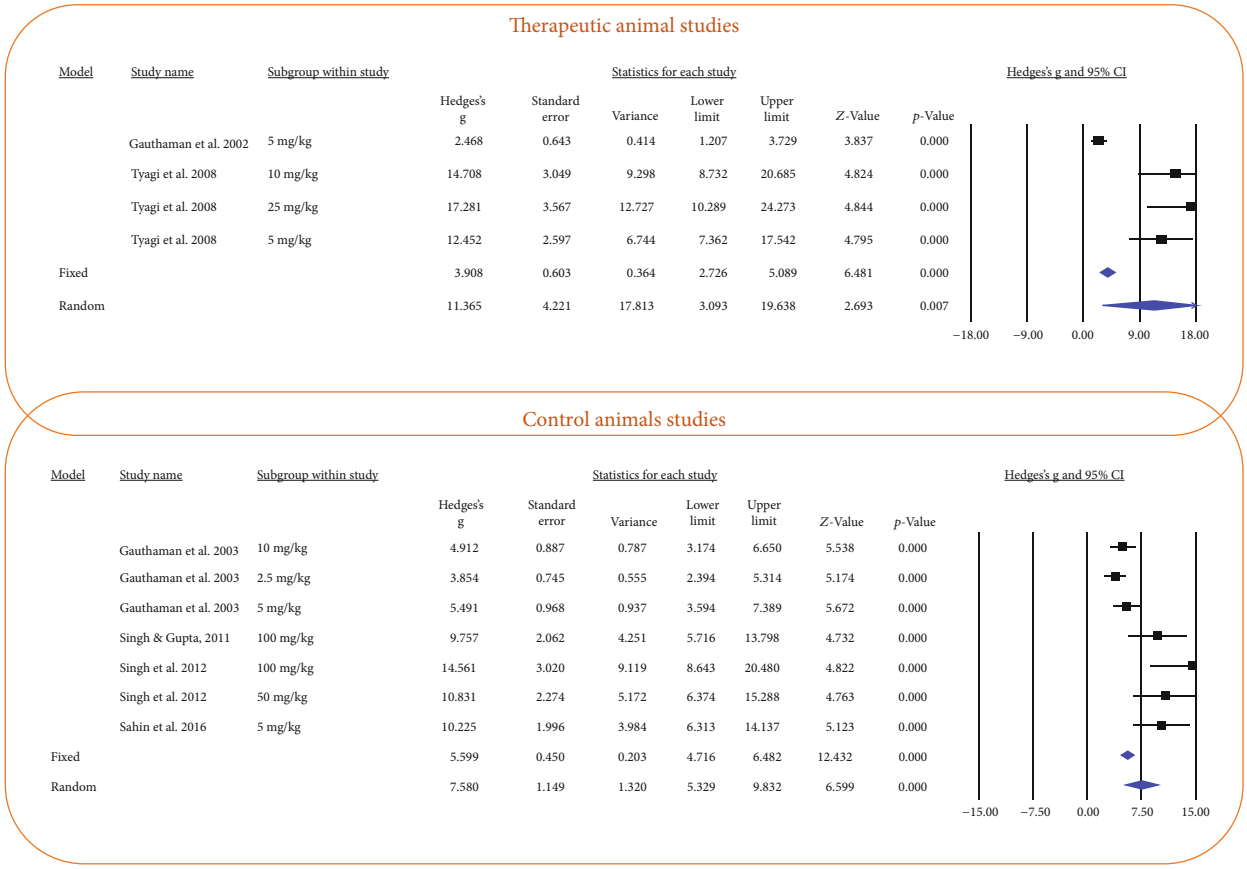

Figure 11: The forest plot showing the effect of TT on the intromission frequency. The *p* value represents the significance, the horizontal black bar represents 95% CI with Hedges' *g*/SDM in centre. The overall association is shown by a diamond-shaped box (blue).

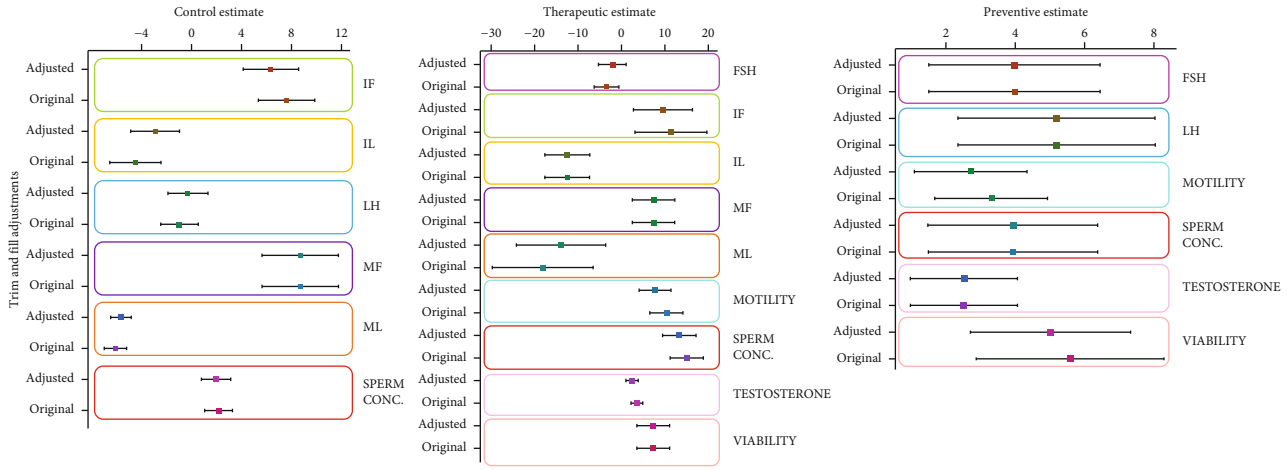

Figure 12: The original and adjusted bias-free estimates computed using Duval and Tweedie's trim and fill method for various parameters presented in human and animal studies.

activity by regulating Nuclear factor erythroid 2–related factor (Nrf-2) and Heme-oxygenase-1 (HO-1) signalling. TT increases Nrf-2 and HO-1 in reproductive tissue that restore antioxidant enzyme activity, which might account for its aphrodisiac activity. Nrf2, a transcriptional factor for the expression of various antioxidant genes, may explain its key role in oxidative stress response. TT increases HO-1, which is induced by an increase in factors like oxidative stress and reactive oxygen species in order to exert its antioxidant property [16].

Among possible reasons for its aphrodisiac effect may be the enhanced conversion of protodioscin to dehydropiandrosterone (DHEA), a neurosteroid. DHEA is an antagonist to Gama-amino butyric acid (GABA), which has an

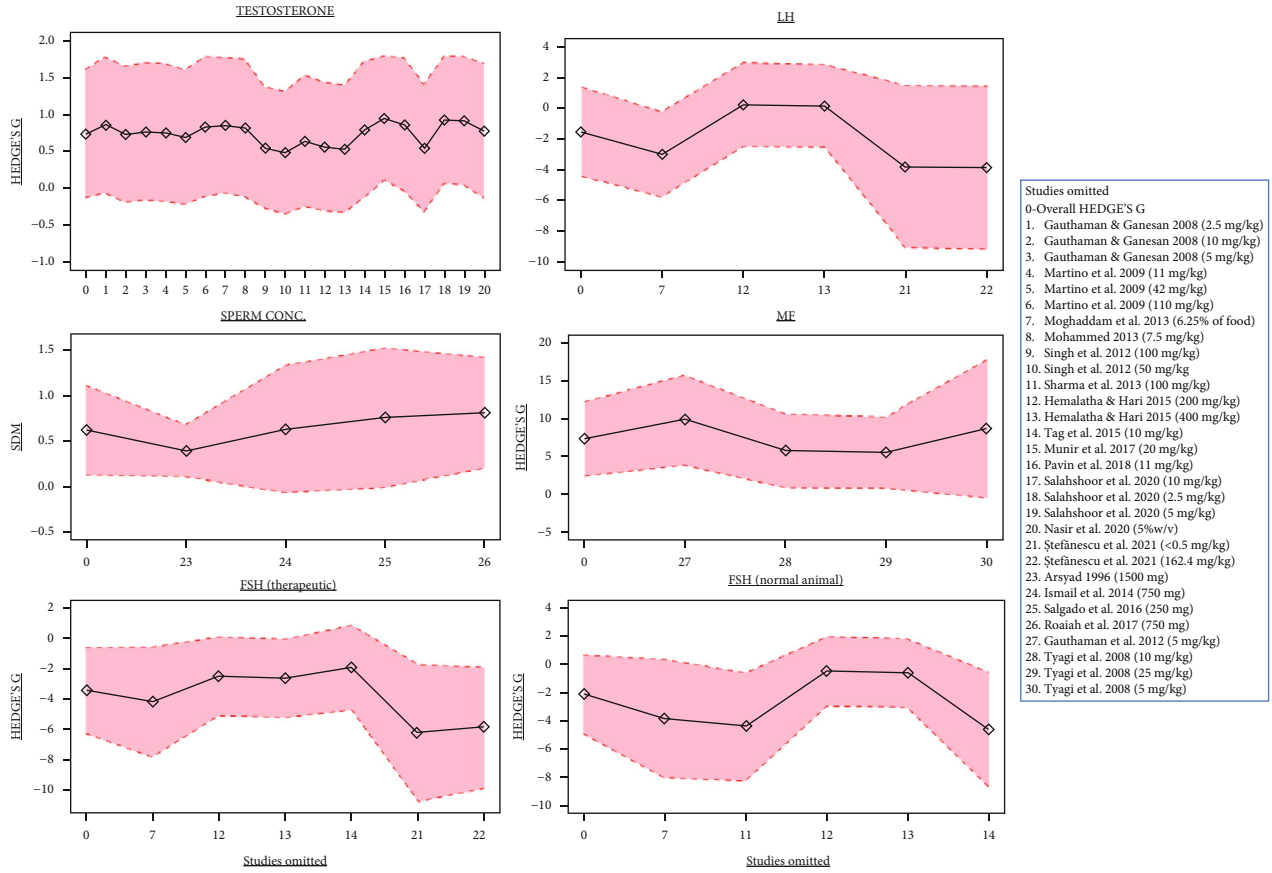

Figure 13: Representation of sensitivity analysis of parameters where sensitivity was found. For visual comparison, the overall effect size and the computed effect size after exclusion of one study at a time are presented. The diamonds represent the SDM (Hedge's g) value and the pink zone represents 95% CI.

inhibitory effect on intromission activity, thus exerting an aphrodisiac effect [15, 46]. However, the aphrodisiac effect of TT does not appear to be because of its effect on testosterone levels, as it resulted in only a marginal improvement in testosterone level. While protodioscin has been suggested to act on hypothalamus to stimulate LH and FSH secretion that further improves testosterone production by the Leydig cells [10, 33, 40], we found a mild effect of TT on T, FSH, and LH levels, suggesting that TT may provide such benefits in infertility or in adverse exposures but not under normal conditions. Ca^{2+} inhibits enzyme phosphodiesterase that may explain its impact on sperm motility by preventing the degradation of cAMP and its aphrodisiac activity by preventing the degradation of cGMP [59]. cGMP regulates contractile state of corporal smooth muscles through its cGMP-dependent protein kinase activity [60]. Oxidative stress might also result in increased cytotoxic effect of nitric oxide, a crucial player in controlling sperm viability and aphrodisiac effect. Apart from the above, there may be other mechanisms by which TT exerts aphrodisiac effect, such as its effect on cavernous smooth muscles, NO release, and cGMP degradation.

4.4. Protodioscin May Account for the Profertility and Aphrodisiac Activity of Tribulus terrestris L. *Tribulus terrestris* L. is rich in a number of chemical constituents such as steroids, saponins, flavonoids, alkaloids, unsaturated fats, vitamins, and tannins [57], of which protodioscin has been investigated in particular. Interestingly, protodioscin has also been clinically tested for its usefulness or benefits in treating male infertility, especially oligozoospermic infertility [22, 33]). Adimoelja et al. debated over TT for improving fertility in idiopathic oligoasthenoteratozoospermia (OTA) patients by conversion of its phytochemical derivative, protodioscin to dehydroepiandrosterone (DHEA) [61, 62]. However, protodioscin has also been shown to exert aphrodisiac activity by acting on neurotransmitters like nitric oxide that help in the relaxation of corpus cavernosum smooth muscles [63]. Although we cannot rule out the fact that other phytochemicals present in the TT extract could contribute to the observed effects, the results obtained in these studies suggest that protodioscin present in this extract could account for the beneficial effects of TT.

4.5. Other Evidence That Supports the Profertility Effect of Tribulus terrestris L. In an in vitro study, treatment with TT improved the Leydig and spermatogonia cell numbers significantly [47]. TT saponin root extract also showed favorable effect in illnesses like edema, leucorrhea, ascites, inflammations, and urinary tract infections. On the other hand, some studies showed the beneficial effect of TT in male infertility, whereas Adimoelja et al. concluded that *Tribulus terrestris* L. improves acrosome morphology of spermatozoal cells and enhanced the acrosome reaction, which contributes to improved fertility [22].

5. Conclusions

In conclusion, these meta-analyses showed a significant impact of TT on sperm parameters (sperm concentration, sperm motility, and sperm viability) in humans and animal models. The profertility effect was seen not only in infertility but also in normal animals. Significant improvements in sperm parameters under stress and normal conditions suggests that TT may be an excellent profertility medicine. The most commonly used dosage was 750 mg/day, split in one or two doses, though rigorous studies on the variation of dosage are required. Similarly, these meta-analyses support that TT has significant aphrodisiac activity, characterized by significant increases in the MF and IF and significant decreases in the ML and IL. However, no consistent pattern of change (improvement) was seen in hormones (LH, FSH), except for testosterone where nonsignificant improvement upon TT administration was seen. Therefore, the aphrodisiac activity of TT may be because of its impact on other parameters, such as cGMP, protein kinases, and contractile state of corporal smooth muscles through as yet unknown mechanisms. With regard to its mechanism of action, further studies are required. While protodioscin appears to be one of the active constituents of TT, other compounds have been seldom experimented with. Therefore, we encourage more number of studies on TT in order to get a better picture of its mechanism of action. The small number of studies pooled in these meta-analyses was a limitation of this study. Subgroup analysis with regard to the type of extract could not be conducted due to the availability of a limited number of studies.

Data Availability

All representative data are provided within the manuscript and also in the supporting information.

Ethical Approval

This study did not need an ethical approval because all the work was developed using published data.

Conflicts of Interest

The authors have declared no conflict of interest with the content of this article.

Authors' Contributions

AA, RV, and PM planned this study and collected data. The experiment was supervised by SR and performed by AA and PM. All authors contributed in writing the manuscript which was corrected by SR. All authors agree to be accountable for all aspects of work ensuring integrity and accuracy. Anam Ara and Rahul Vishvkarma contributed equally to this work.

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