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REVIEW ARTICLE

Treatment with Rapamycin in Animal Models of Traumatic Brain Injuries; a Systematic Review and Meta-Analysis

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Abstract: **Introduction:** In light of the potential of enhanced functional and neurological recovery in traumatic brain injury (TBI) with the administration of rapamycin, this systematic review and meta-analysis aimed to investigate the efficacy of rapamycin treatment in animal models of TBI. **Methods:** An extensive search was conducted in the electronic databases of Medline, Embase, Scopus, and Web of Science by July 1st, 2023. Two independent researchers performed the screening process by reviewing the titles and abstracts and the full texts of the relevant articles, including those meeting the inclusion criteria. Apoptosis rate, inflammation, locomotion, and neurological status were assessed as outcomes. A standardized mean difference (SMD) with a 95% confidence interval (95%CI) was calculated for each experiment, and a pooled effect size was reported. Statistical analyses were performed using STATA 17.0 software. **Results:** Twelve articles were deemed eligible for inclusion in this meta-analysis. Pooled data analysis indicated notable reductions in the number of apoptotic cells (SMD for Tunnel-positive cells = -1.60; 95%CI: -2.21, -0.99, p<0.001), p-mTOR (SMD=-1.41; 95%CI: -2.03, -0.80, p<0.001), and p-S6 (SMD=-2.27; 95%CI: -3.03, -1.50, p<0.001) in TBI post-treatment. Our analysis also indicated substantial IL-1 reductions after rapamycin administration (SMD= -1.91; 95%CI: -2.61, -1.21, p<0.001). Moreover, pooled data analysis found significant neurological severity score (NSS) improvements at 24 hours (SMD= -1.16; 95%CI: -1.69, -0.62, p<0.001; I^2=0.00%), 72 hours (SMD= -1.44; 95%CI: -2.00, -0.88, p<0.001; I^2=0.00%), and 168 hours post-TBI (SMD= -1.56; 95%CI: -2.44, -0.68, p<0.001; I^2=63.37%). No such improvement was observed in the grip test. **Conclusion:** Low to moderate-level evidence demonstrated a significant decrease in apoptotic and inflammatory markers and improved neurological status in rodents with TBI. However, no such improvements were observed in locomotion recovery.

Keywords: Brain Injuries, traumatic; Systematic Review; Meta-analysis; Models, animal

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1. Introduction

Traumatic brain injury (TBI) is the leading cause of death in the younger population, disproportionately affecting low- to middle-income countries (1). The prevalence of TBI has risen since the 1990s and is estimated to be approximately 8.4% (2). The numerous long-term consequences of TBI pose a huge burden on those affected and the society; there were 247.6 million global Disability-Adjusted Life Years (DALY) between 1990 and 2013, as well as 374,636 Years of Life Lost (YLL) in Europe in 2013, and more than 69,000 deaths in the United States in 2021 attributed to TBI and its complications (3-5).

The primary injury following the direct impact of trauma comprises extracellular imbalances, vascular disruption, edema, and hematoma. The subsequent inflammation, blood-brain barrier disruption, oxidative stress, and apoptosis lead to further damage at the cellular level (6, 7). The majority of post-TBI neurological deficits are attributable to neuronal loss (8); therefore, prevention, treatment, and research meticulously focus on different pathways involved in these processes to promote tissue regeneration.

Targeting apoptosis – to mediate cell loss – has gained much attention in recent years for neuro-degenerative conditions (9). The mammalian target of rapamycin (mTOR), as a powerful modulator of cell stability, plays an important role in the regulation of cell growth, energy expenditure, and survival (10). Previous studies have reported controversial findings regarding the fluctuations in mTOR activation after TBI (11); nevertheless, the inhibition of mTOR, both in-vitro and in-vivo, demonstrated promising results in neuronal differentiation and migration, dendritic outgrowth and survival of freshly formed neurons (12, 13).

Rapamycin is a macrolide antibiotic that is known to inhibit mTOR and subsequently antagonize anti-apoptotic proteins, reduce protein synthesis, and induce autophagy (14, 15). In

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light of the potential of enhanced functional and neurological recovery in TBI with the administration of rapamycin, this systematic review and meta-analysis aimed at gathering current literature regarding the efficacy of treatment with rapamycin in TBI for the first time.

2. Methods

2.1. Study design and setting

The current systematic review and meta-analysis was designed to investigate the therapeutic effects of rapamycin in TBI. An extensive search was conducted in the electronic databases of Medline, Embase, Scopus, and Web of Science by July 1st, 2023. Search strategies were based on keywords related to rapamycin and TBI. Search syntax for each database is provided in Appendix 1. In addition, a search in the grey literature (Google and Google Scholar) as well as in the references section of the related articles was conducted to avoid missing any articles.

PICO was defined as follows: population (P): animal models of TBI, intervention (I): rapamycin administration, comparison (C): a non-treated TBI group, and outcomes (O): apoptosis rate, inflammation, functional recovery, and neurological status.

2.2. Selection criteria

All original papers published on the therapeutic effects of rapamycin in TBI were included. We excluded letters to the editors and reviews, as well as studies that did not administer rapamycin, did not evaluate our outcomes of interest, did not execute a traumatic model of injury, included transgenic animals or animals other than rats and mice, or administered combination therapies.

2.3. Data synthesis

The results of the systematic search were entered into the 20th version of EndNote software. After removing duplicates, two independent researchers performed the initial screening process by reviewing the titles and abstracts of the obtained records. Then, the full text of the relevant articles was reviewed in detail, and those meeting the eligibility criteria were included. Data were extracted into a checklist designed based on the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines (16).

The extracted data included the name of the author, year of publication, the country in which the study was conducted, study design, sample characteristics, sample size, the dose of administered rapamycin, the method of rapamycin administration, the time interval between the injury and rapamycin administration, the last follow-up, and the final outcomes. The data reported in the figures are extracted using the Plot Digitizer software.

2.4. Risk of bias assessment and certainty of evidence

Systematic Review Centre for Laboratory Animal Experimentation (SYRCLE) risk of bias assessment tool was used to evaluate the quality of the included articles (17). The certainty of evidence was assessed using the Grading of Recommendations, Assessment, Development, and Evaluations (GRADE) framework (18).

2.5. Statistical analysis

The statistical analyses were conducted using the STATA 17.0 software. Data were stratified based on the evaluated outcome. A standardized mean difference (SMD) with a 95% confidence interval (95%CI) was calculated for each experiment, and a pooled effect size was reported. Meta-analyses were performed only if an outcome was reported in at least three distinct sets of experiments. We employed a Galbraith plot to detect outlier studies. In instances where we identified an outlier in a reported outcome, we chose not to include that data in the pooled analysis. A random effect model was chosen since we expected possible methodological heterogeneity.

Heterogeneity between studies was checked based on the I2 value. Funnel plot was used to identify publication bias using Egger's test.

3. Results

3.1. Characteristics of the included studies

Figure 1 illustrates the study selection process. A total of 526 records were initially identified through a systematic search, and 339 non-duplicate records were screened. At last, 12 articles were deemed eligible for inclusion in this meta-analysis (11, 19-29) (Figure 1). Out of the 12 studies, ten used mice, and two used Sprague-Dawley (SD) rats. TBI models were the controlled cortical injury (CCI) in 6 studies, weight drop in 4, and closed head injury (CHI) in 2. TBI severity ranged from mild in 2 studies to moderate in 5 and severe in 4. Rapamycin administration was a single dose in 9 studies and involved two doses in 3. Rapamycin was given at doses of 0.5-6 mg/kg via intra-peritoneal (IP) and per-oral (PO) routes and 0.0016-0.003 mg/kg via intra-cerebroventricular (ICV) route. IP route of administration was the most common route (7 out of 12 studies) (Table 1).

3.2. Meta-analysis

3.2.1 Apoptosis and autophagy

Evaluating rapamycin's effect on apoptotic and autophagic indices, our pooled analysis indicated notable reductions in Tunnel-positive cells (SMD= -1.60; 95%CI: -2.21, -0.99, p<0.001), p-mTOR (SMD=-1.41; 95%CI: -2.03, -0.80, p<0.001), and p-S6 (SMD=-2.27; 95%CI: -3.03, -1.50, p<0.001) in TBI post-treatment. However, Beclin-1 remained unchanged (SMD=1.22; 95%CI: -0.30, 2.73, p=0.115). Notably, significant heterogeneity existed regarding rapamycin's

impact on Beclin-1 expression (I^2=81.39, p=0.01), but not regarding Tunnel-positive cells (I^2=13.31%, p=0.27), p-mTOR (I^2=0.00%, p=1.00), and p-S6 (I^2=0.00%, p=0.90) (Figure 2).

3.2.2 Inflammation

Our analysis underscored substantial IL-1 reductions after rapamycin administration (SMD= -1.91; 95%CI: -2.61, -1.21, p<0.001). Importantly, no heterogeneity was observed regarding rapamycin's effect on IL-1 (I^2=0.00, p=0.80) (Figure 3).

3.2.3 Motor function (locomotion)

Analyzing Rapamycin's impact on motor function, grip test outcomes at 24 hrs (SMD= 0.45; 95%CI: -0.65, 1.55, p=0.424) and 72 hrs post-TBI (SMD= 0.68; 95%CI: -0.03, 1.39, p=0.061) exhibited no significant improvement. Remarkably, the grip test at 24 hrs post-TBI demonstrated heterogeneity (I^2=76.40%, p=0.02), while no significant heterogeneity was observed at 72 hrs post-TBI (I^2=44.02%, p=0.17) (Figure 4).

3.2.4 Neurological defect severity

Five articles examined the severity of neurological deficits. Employing the Galbraith plot, Jiang et al.'s study (30) was identified as an outlier; consequently, this study was omitted from the pooled analysis. Pooled data analysis found significant neurological severity score (NSS) improvements at 24h (SMD= -1.16; 95%CI: -1.69, -0.62, p<0.001; I^2=0.00%), 72h (SMD= -1.44; 95%CI: -2.00, -0.88, p<0.001; I^2=0.00%), and 168h post-TBI (SMD= -1.56; 95%CI: -2.44, -0.68, p<0.001; I^2=63.37%) (Figure 5).

Risk of bias

The allocation sequence was appropriately generated and implemented in six of the studies. All included studies used animals that were similar initially. However, none of the studies clearly disclosed allocation concealment, and there was no mention of random housing during the experiments in any of them. Investigators were blinded in three studies, while outcome assessors were blinded in all of the studies. It was unclear if a random selection process was used for outcome assessment in any of the studies. There did not appear to be any incomplete outcome data or other factors that could potentially cause bias. In conclusion, the overall quality of evidence from the included studies was considered low. (Table 2).

3.3. Publication bias

There was no evidence of publication bias in relation to the impact of rapamycin on any of the investigated outcomes (Figure 6).

3.4. Certainty of evidence

The assessment of the certainty of evidence using GRADE indicated moderate certainty for rapamycin's effects on TUNEL-positive cells, p-mTOR, p-S6, and IL-1, primarily due to a potential risk of bias. Conversely, there was low certainty for rapamycin's effects on Beclin-1, motor function, and neurological defect severity, largely due to potential bias and inconsistency. Overall, the level of evidence regarding the impact of rapamycin administration ranged from low to moderate quality (Table 3).

4. Discussion

Our study represents the first systematic review and meta-analysis investigating the therapeutic efficacy of rapamycin in rodent models of TBI. We observed significant improvements in apoptosis, inflammation, and neurological status following rapamycin administration in these rodent models. However, motor function recovery, as assessed by the grip test, did not show such improvement.

Apoptosis is the main cause of trauma-induced cerebral cell death, and apoptotic neuronal death is an important characteristic of secondary damage following TBI, contributing to nearly two-thirds of cellular deaths in animal models (31, 32). Previous research has shown that early therapeutic interventions targeting the apoptotic pathways are associated with a reduction in lesion size and cortical cell loss after TBI (33, 34). Recent studies have explored rapamycin's potential to mitigate the progression of apoptosis following cerebral ischemia injury and TBI (35). Numerous molecular components, such as mTOR, Beclin-1, and p-S6, participate in initiation and progression of autophagy. The two mTOR complexes, mTOR complex-1 (mTORC1) and mTOR complex-2 (mTORC2), play an important role in proliferation control (36). Beclin-1, also known as BECN1, is crucial for regulating autophagy and interacts with various binding proteins, exerting diverse physiological effects, including apoptosis, autophagy, and cellular metabolism (37). In addition, p62, commonly identified as sequestosome 1 (SQSTM1)/A170, is a multifunctional ubiquitin-binding protein that plays various roles in tumorigenesis, autophagy, and cell signaling pathways (38). The impact of enhanced autophagy in TBI is currently under investigation, with some evidence suggesting that mild increases in autophagy facilitate the degradation and recycling of unwanted cellular components, thereby promoting neuronal survival in TBI. In contrast, excessive autophagy may disrupt the regeneration process (39).

We demonstrated that expression of Tunnel-positive cells, p-S6, and mTOR, as autophagic and apoptotic markers declined significantly after rapamycin administration. However, evidence with high heterogeneity demonstrates no such effect on Beclin-1 expression. This could be due to the complex interactions within apoptosis pathways, the presence of other modulators influencing Beclin-1 expression, and the likelihood that rapamycin, as an indirect inhibitor of mTOR, affects pathways beyond those regulating Beclin-1 expression (40, 41).

Elevated levels of inflammatory cytokines, particularly TNF- and IL-1, in the damaged cortex are believed to contribute

to the severity of brain injuries (42-45). In an inflammatory context, TNF-, along with various other proinflammatory mediators, are predominantly produced by activated microglia (46). Animal studies have shown that the release of TNF- into the brain leads to brain inflammation, disruption of the blood-brain barrier (BBB), and recruitment of intracranial leukocytes (44). Also, IL-1 plays a crucial role in initiating and advancing a complex inflammatory cascade. High levels of IL-1 have been detected in both brain tissue and cerebrospinal fluid during the initial hours after a brain injury (47). Recent studies have demonstrated the association of mTOR kinase with microglia activation and the inflammatory process. Since rapamycin acts as an mTOR kinase inhibitor, it holds the potential for anti-inflammatory effects (48).

Our study is in line with these findings, showing a significant decrease in IL-1 levels. However, it is important to note that TNF- meta-analysis was not conducted due to variations in the sampling regions, but three studies reporting the effects of rapamycin on TNF- demonstrated a significant reduction in its levels (24, 28, 30).

It is reasonable to assume that the improvement in the severity of neurological defects, as assessed by the NSS test, can be attributed to the significant reduction in neuronal loss, enhanced neuro-regeneration, and reduced inflammation. In contrast, there was no improvement observed in motor function, assessed using the grip test, within the first 72 hours post-TBI. Notably, all the included studies evaluating grip tests employed a single-dose intracerebral administration of rapamycin, while those assessing NSS behavioral tests used multiple-dose intraperitoneal treatments.

It is noteworthy that only a limited number of underlying pathways were reported in the literature - with some degrees of heterogeneity present - emphasizing the need for caution in the interpretation of these findings. In addition, some variables were too scarce to be pooled, such as severity of TBI, the timing of treatment, the number and routes of administered doses, robust follow-up evaluations, and some outcomes were evaluated solely based on one method of assessment, such as grip test for motor function recovery and IL-1 for inflammatory markers; these considerations necessitate further experimental research in this field for the generalizability of results.

5. Conclusion

Our study demonstrated a significant decrease in apoptotic and inflammatory markers and improved neurological status in rodents with TBI. However, no such improvements were observed in motor function recovery. The low to moderate-level evidence from this study underscores the importance of conducting more comprehensive experimental investigations to further evaluate the effectiveness of rapamycin treatment in animal models of TBI.

6. Declarations

6.1. Acknowledgments

None.

6.2. Conflict of interest

The authors declare that they have no conflicts of interest.

6.3. Funding

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6.4. Authors' contribution

Study design: MY, RS Data gathering: MK, AA Analysis: MY Interpretation of results: MK, AA Drafting and revising: all authors All authors read and approved final version.

6.5. Using artificial intelligence chatbots

None.

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Table 1: Baseline characteristics of included studies

Author, year, country	Gender, strain, species, weight (gram)	No of controls/treated	Model, location, severity of injury	Treatment condition*, dose number, Injury to treatment interval(min)	Rapa dose (mg/kg), administration route	Follow-up (days)
Campolo,2021, Italy	Male, CD1, Mice, 20-25	20/20	CCI, Right hemisphere cortex, Moderate	Post, 2, 60&240	1, PO	1
Chen, 2019, China	Male, C57BL/6, Mice, 20-25	11/11	Weight drop, Left parietal cortex, Moderate	Pre/Post,2, 15 prior and 240 post	3, IP	7
Ding, 2014, China	Male, ICR, Mice, 28-32	34/34	Weight drop, Left anterior frontal area, Severe	Post, 1, 30	2, IP	7
Erlich,2007, Israel	Male, Sabra, Mice, 35	7/7	CHI, Left anterior frontal area, Severe	Post, 1, 240	0.5 or 1, IP	7
Gao,2020, China	Male, C57BL/6J, Mice, 25-30	7/7	CCI, Left parietal cortex, Moderate	Pre, 1, 30	0.003, ICV	3
Guo ,2013, China	Male, CD-1, Mice, 25-30	16/16	CCI, Left fronto-parietal cortex, Moderate	Pre, 1, 60	6, IP	3
Nikolaeva,2016, USA	NR, C57BL6, Mice, NR	6/6	CCI, Left parieto-temporal cortex, Moderate	Post, 1, 60	1, IP	1
Park, 2012, USA	Male, C57Bl/6, Mice, 30-32	18/12	CCI, Left parieto-temporal cortex, Severe	Post,1, 0	0.0016, ICV	10
Shi,2021, China	Male, SD, Rat, 200-250	6/6	CCI, 2 mm in front of the herringbone suture and 2 mm beside the midline of skull, Severe	Post, 2, 1440&4320	5, NR	3
Song, 2015, China	Male, ICR, Mice, 28-32	8/8	Weight drop, Left anterior frontal area, NR	Post, 1, 30	2, IP	3
Wang, 2017, USA	Male, SD, Rat, 85-95	8/8	Weight drop, Midline, Mild	Post, 1, 240	3, IP	7
Xu, 2014, USA	Male, C57/BL6, Mice, 25-30	18/18	CHI, Between the Coronal and Lambdoid sutures, Mild	Post,1, 0	0.0016, ICV	3

CCI: Controlled Cortical Injury, CHI: Closed Head Injury, NR: Not Reported, PO: Per Oral, IP: Intraperitoneal, ICV: Intracerebroventricular.
*Refers to rapamycin therapy administered either before (Pre) or after (Post)TBI.

Table 2: Risk of bias assessment of the included studies according to SYRCLE's risk of bias tool

Study	Item 1: Sequence generation	Item 2: Baseline characteristics	Item 3: Allocation concealment	Item 4: Random housing	Item 5: Blinding trial caregivers	Item 6: Random outcome assessment	Item 7: Blinding outcome assessors	Item 8: Incomplete outcome data	Item 9: Selective outcome reporting	Item 10: Other sources of bias
Campolo,2021	U	L	U	U	L	U	L	L	U	L
Chen, 2019	U	L	U	U	L	U	L	L	U	L
Ding, 2014	U	L	U	U	L	U	L	L	U	L
Erlich,2007	U	L	U	U	U	L	L	L	U	L
Gao,2020	L	L	U	U	U	U	U	L	U	L
Guo ,2013	L	L	U	U	L	U	L	L	U	L
Nikolaeva,2016	U	L	U	U	U	L	L	L	U	L
Park, 2012	U	L	U	U	L	U	U	L	U	L
Shi,2021	L	L	U	U	L	U	U	L	U	L
Song, 2015	L	L	U	U	L	L	L	L	U	L
Wang, 2017	L	L	U	U	U	U	L	L	U	L
Xu, 2014	L	L	U	U	U	U	U	L	U	L

L: low risk of bias, H: high risk of bias, U: unclear risk of bias.

Table 3: The certainty of evidence regarding the administration of rapamycin and the evaluated outcomes

Outcome	Number of analyses	Risk of bias	Imprecisiont	Inconsistency (I2 range)	Indirectness	Publication bias	Level of evidence
TUNEL positive cells	4	Serious	Not serious	Not serious	Not serious	Not serious	Moderate
p-mTOR	4	Serious	Not serious	Not serious	Not serious	Not serious	Moderate
Beclin-1	3	Serious	Not serious	Serious	Not serious	Not serious	Low
p-S6	4	Serious	Not serious	Not serious	Not serious	Not serious	Moderate
IL-1	3	Serious	Not serious	Not serious	Not serious	Not serious	Moderate
Motor Function	6	Serious	Not serious	Serious	Not serious	Not serious	Low
Neurological Defect Severity	10	Serious	Not serious	Serious	Not serious	Not serious	Low

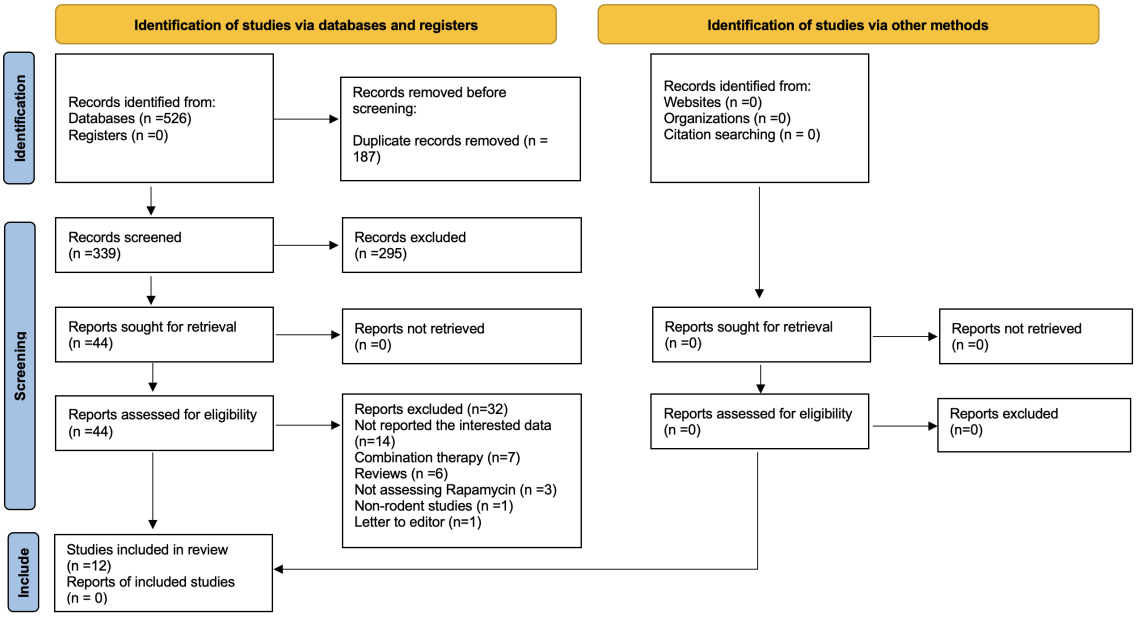

Figure 1: Prisma flow diagram of the present study.

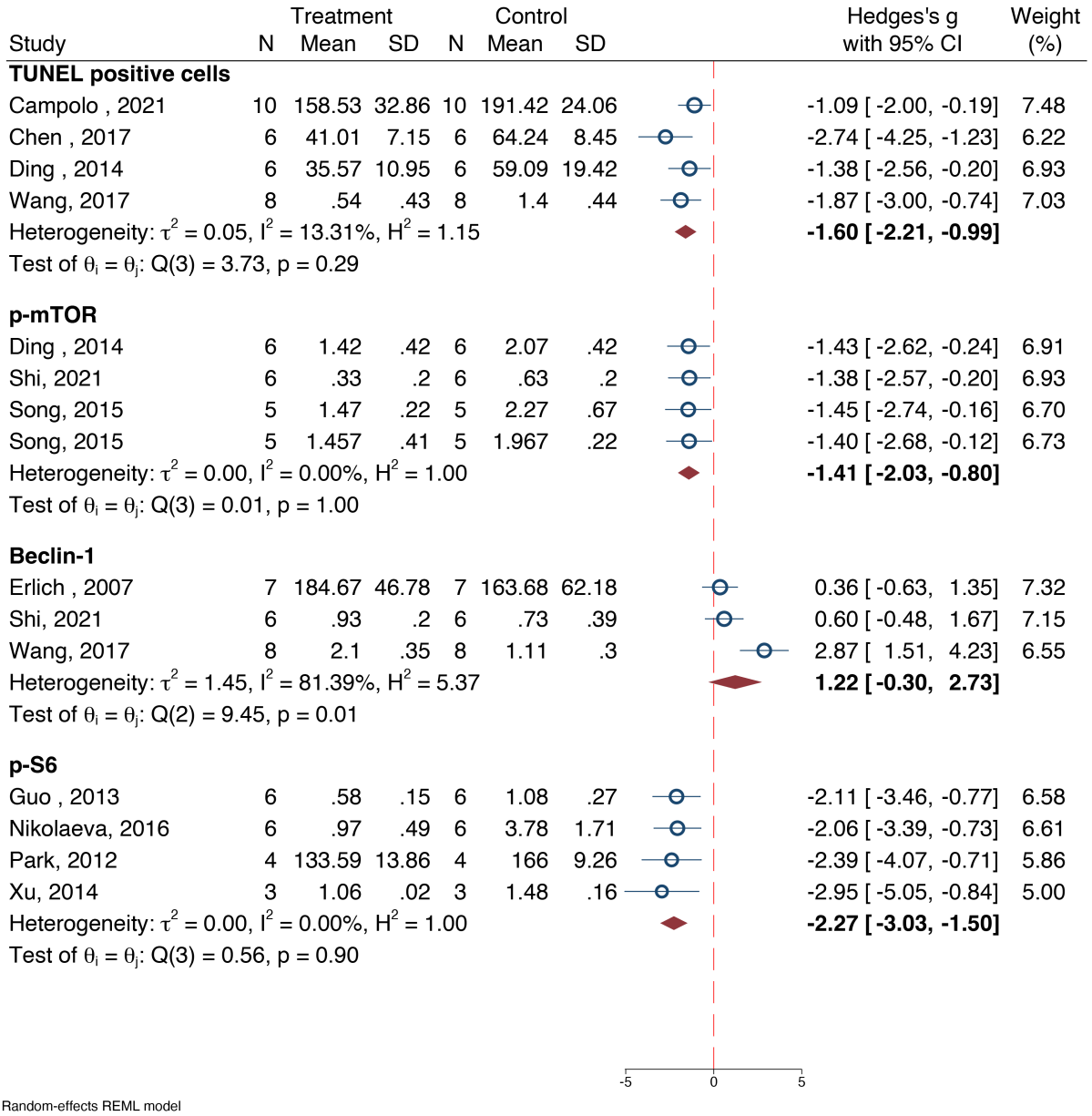

Figure 2: Forest plot of Rapamycin's impact on apoptotic and autophagic indexes in TBI individuals. SMD, standardized mean difference; CI, confidence interval.

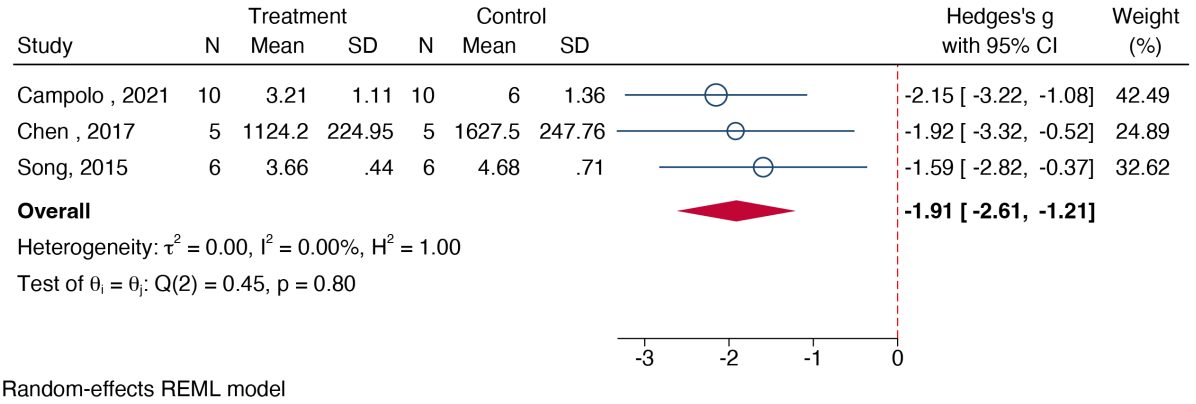

Figure 3: Forest plot of Rapamycin's impact on inflammatory index, IL-1, in TBI individuals. SMD, standardized mean difference; CI, confidence interval.

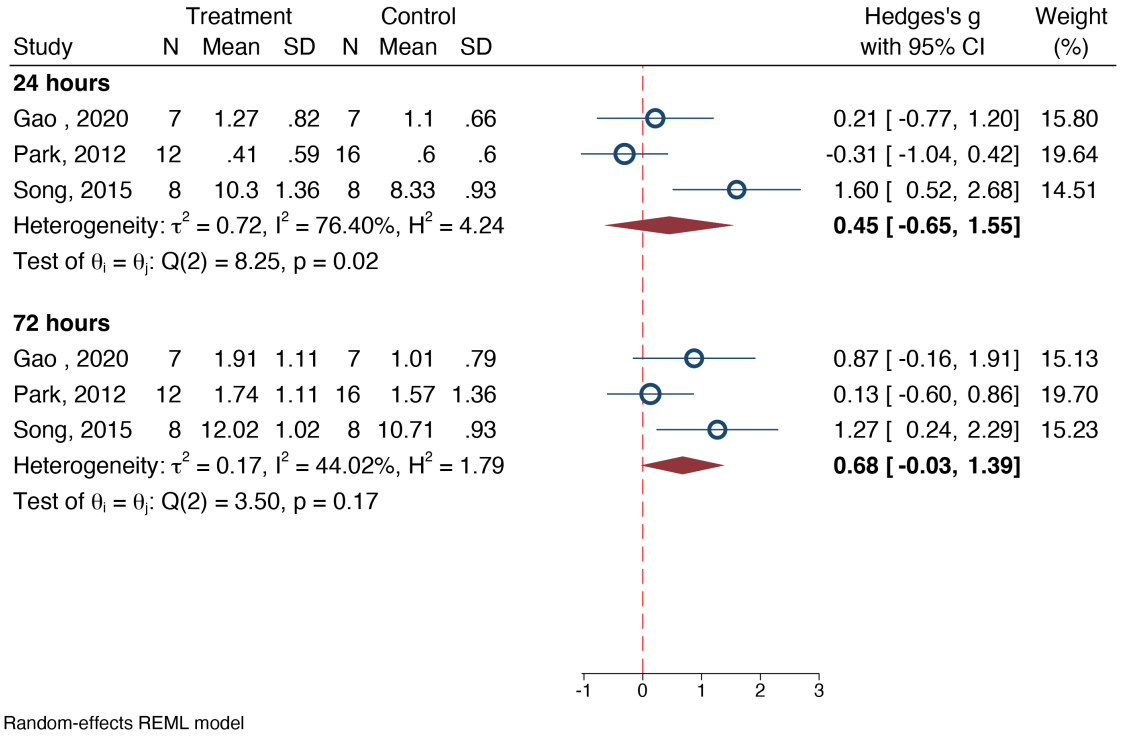

Figure 4: Forest plot of Rapamycin's impact on motor function index, grip test, in TBI individuals. SMD, standardized mean difference; CI, confidence interval.

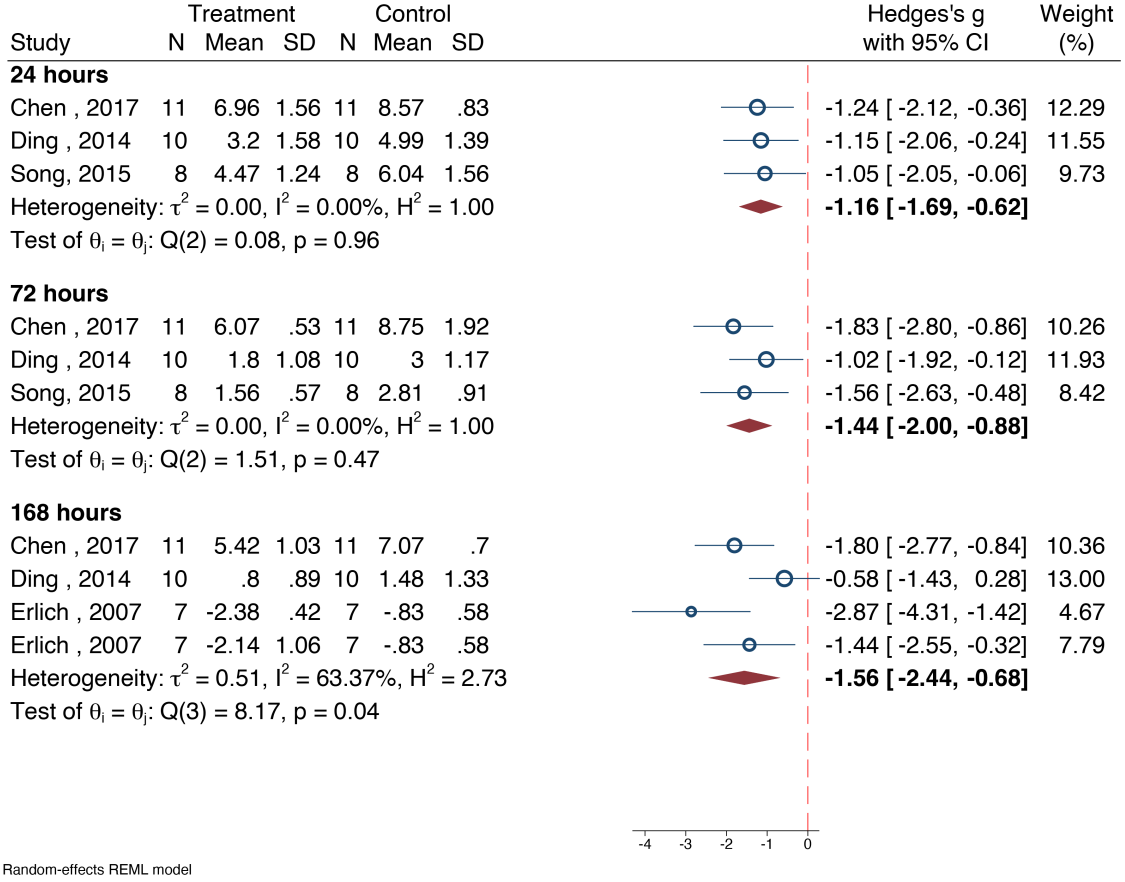

Figure 5: Forest plot of Rapamycin's impact on neurological defect severity, NSS test, in TBI individuals. SMD, standardized mean difference; CI, confidence interval.

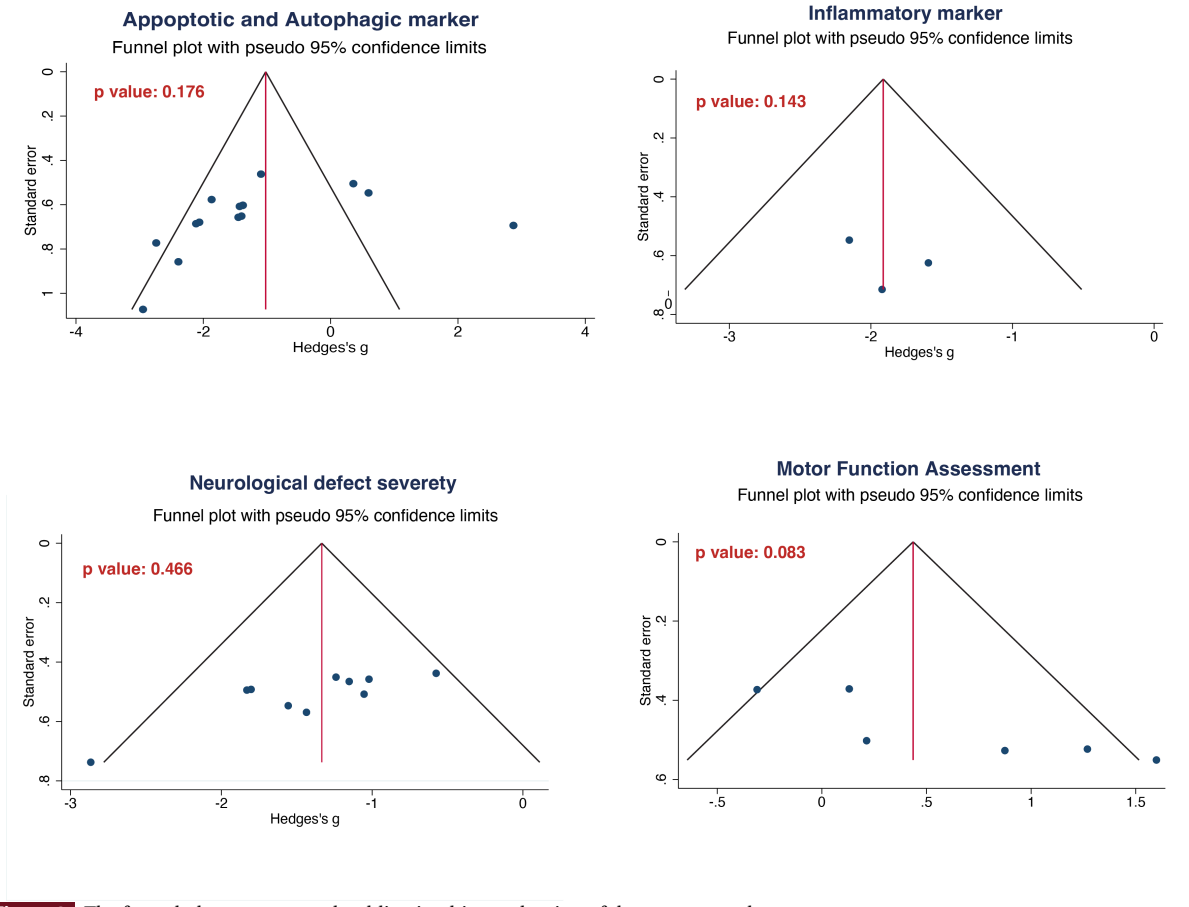

Figure 6: The funnel plots represented publication bias evaluation of the present study.