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REVIEW ARTICLE

Apelin as a Candidate for Hypertension Management; a Systematic Review and Meta-Analysis on Animal Studies

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Abstract: **Introduction:** Hypertension is a medical emergency that requires immediate medical attention. Recent studies have suggested that peripheral injection of Apelin may lower blood pressure. However, there is no comprehensive conclusion on the role of Apelin in treatment of hypertension. The aim of this systematic review was to evaluate the effects of Apelin on blood pressure in animal studies. **Methods:** Extensive search and data gathering were conducted using keywords related to blood pressure and Apelin on Medline, Embase, Scopus, and Web of Science databases at the end of July 2022. Two researchers screened and summarized the articles independently. Analysis was then conducted based on Apelin dose, route of administration, and follow-up. The findings were reported as standardized mean difference (SMD) with a 95% confidence interval (95% CI). **Results:** Data from 10 animal studies were included in the present systematic review. Time interval between Apelin administration and blood pressure assessment was 1 to 21 minutes. Findings showed that administration of Apelin immediately reduces mean arterial pressure (MAP) (SMD=-3.13; 95% CI: -4.43 to -1.82; p<0.001), systolic blood pressure (SBP) (SMD= -1.62; 95% CI: -2.22 to -1.02; p<0.001), and diastolic blood pressure (DBP) (SMD= -1.10; 95% CI: -1.59 to -0.62; p<0.001). On follow-up, the effects of Apelin on MAP (meta-regression coefficient=-2.46; p=0.002) and DBP (meta-regression coefficient= -0.16; p=0.012) decreased over time, while the blood pressure lowering effects of Apelin on SBP did not change during follow-up (meta-regression coefficient=-0.17; p=0.063). It was also found that by increasing the dose of Apelin, DBP and SBP further reduced. These findings suggest that the effect of Apelin on SBP (meta-regression coefficient=0.08; p=0.001) and DBP (meta-regression coefficient=0.059; p=0.007) is dose-dependent, and their correlation is significant. **Conclusion:** The present systematic review showed that peripheral administration of Apelin immediately reduces MAP, SBP and DBP in hypertensive animals. In contrast, central administration of Apelin increases these parameters.

Keywords: Hypertension; Apelin; Arterial Pressure; Blood Pressure

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1. Introduction

Hypertension is a major risk factor for cardiovascular diseases and stroke, two leading causes of death worldwide. It is currently estimated that 1.4 billion (31.1%) people are affected by high blood pressure (1, 2). Based on data-driven projections, more than 50 percent of the world's population will have hypertension within the next 30 years (1-5). This highlights the importance of blood pressure management as one of the most important priorities in health care. Risk factors such as obesity, psychological stress, alcohol consump-

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tion, high salt intake, sedentary lifestyle, and genetic predisposition are the leading causes of hypertension (5). Because of the multifactorial etiology, alternative modalities should be considered for the management of hypertension. Chronic mild inflammation during obesity is a potential risk factor of hypertension (3). Inflammation results in the release of adipokines from adipose tissues, which might be associated with hypertension.

High blood pressure leads to vascular oxidative stress, which subsequently results in vascular damage, remodeling, fibrosis, and decreased elasticity. As a result, many clinical trials and studies have shown that antioxidant therapies play an important role in the management of hypertension. However, antioxidants such as vitamin C, α-tocopherol (Vit E), and flavonoids did not have significant effects of lowering blood pressures (1, 6). Discovery of the vasodilatory effects of endogenous peptides such as Apelin presents a potential direction of blood pressure management in patients with essential hypertension (7).

Apelin is a vasoactive endogenous peptide produced from C-terminal of a 77-amino acid pre-proApelin. It is cleaved by enzymes to form different Apelin fragments (Apelin 13, 16, 17, 19, 36). The most active fragment is Apelin-13 and its receptor, APJ, is a member of the G-protein coupled receptors (8). Apelin and APJ are widely distributed in the cardiovascular system (9). The Apelin-APJ signaling is also important for proper development of the cardiovascular system and formation of blood vessels. Apelin- and APJ-knockout mice displayed abnormalities with cardiac contractility, pressure overload, and aging (10). Similarly, animals with Apelin and APJ abnormalities developed spontaneous hypertension (11). In pregnant preeclamptic women, Apelin and APJ expression in syncytiotrophoblasts and cytotrophoblasts were decreased compared with normotensive control. In animal models with preeclamptic hypertension, treatment with Apelin was shown to decrease blood pressure (12-15). It has also been shown that in patients with pulmonary hypertension, Apelin and APJ expression were decreased. In animal models of hypertension, treatment with Apelin was shown to reduce blood pressure (11, 16). Although Apelin's ability to temporarily lower blood pressure has been well documented, there are studies that have reported no changes or an increase in blood pressure (9, 15, 17, 18).

Despite many efforts to study endogenous peptides such as Apelin, there is no comprehensive conclusion on Apelin's effects on blood pressure. Thus, performing a meta-analysis and systematic review can be helpful for reaching an agreement. The aim of this study was to provide a more precise resource about the effects of Apelin on blood pressure.

2. Methods

2.1. Study design

This meta-analysis was designed to evaluate the effects of Apelin injection on blood pressure in hypertensive rats and mice. PICO was defined as: P; Animals (rats and mice) with hypertension through different models, I; Apelin injection, C; Comparison of Apelin-treated hypertensive animals with non-treated hypertensive animals, and O; The outcomes related to blood pressure based on changes in systolic blood pressure (SBP), diastolic blood pressure (DBP), and mean arterial pressure (MAP).

2.2. Selection criteria

In this systematic review and meta-analysis, all experimental studies that evaluated the effects of Apelin on hypertension and blood pressure were included. Since most studies were conducted on rats and mice, we included the same population in our study – without any sex or race/strain limitations. Exclusion criteria included studies without a control group, and studies that did not report desired data, including blood pressure level, type, or time of the Apelin injection. Also excluded were duplicate studies, review studies, human studies, and in-vitro studies.

2.3. Search strategy

Two reviewers separately conducted extensive search on Medline (via PubMed), ISI Web of Science, Embase, and Scopus in July 2022. The keywords used in searches were words related to blood pressure and Apelin. Keywords were selected as widely as possible so that no suitable study would be missed (Appendix 1).

Although only animal studies were included in the present meta-analysis, the animal studies filter (from the online databases) was not used in the search strategy. This was done in order not to miss any related studies since the online databases cannot always correctly differentiate between animal and human studies. Keywords used in the search strategy were obtained using MeSH section of PubMed database, Emtree network of Embase database, and search in related article titles.

Consulting with specialists in the field of hypertension was another method used to finalize the keywords. To find additional articles or unpublished data, manual search was performed in the bibliography of relevant studies and related articles. On the other hand, for searching in Gray Literature, three strategies have been followed. First, searching ProQuest database for dissertations; second, contacting the authors of related articles to access unpublished or forthcoming data; and third, using Google and Google Scholar search engines to find more literature.

2.4. Data gathering

Search in the mentioned databases was conducted and duplicate articles were removed, then two researchers started screening the articles independently. In the first step, the selection of articles was based on the title and abstract obtained from the databases, and in the second step, full text of possibly related articles was studied to select all related articles. All steps were accomplished separately by two researchers and in cases of disagreement the dispute was resolved through consultation with a third researcher. Data extracted from articles included in this systematic search were recorded in a checklist designed based on Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement guidelines. Extracted data included information related to the study design, the characteristics of all the samples (age, weight, sex, model of inducing hypertension), administration protocol, administration route (peripheral or central), number of samples studied, investigated outcomes (mean arterial pressure, systolic blood pressure, diastolic blood pressure), and follow-up duration. Since most experimental studies use graphs to report their findings; whenever necessary, data were extracted using Plot digitizer.

2.5. Risk of bias assessment

Quality control of the articles was performed using a checklist designed based on Systematic Review Centre for Laboratory Animal Experimentation (SYRCLE)'s risk of bias tool for animal studies (19). In cases of disagreement the dispute was resolved through consultation with a third researcher.

2.6. Statistical analysis

To conduct analysis, mean standard deviation of recorded data and number of samples in each group was recorded in STATS 14.0 statistical software. Then, using the 'Metan' command in this software, standardized mean difference (SMD) with a 95% confidence interval (CI) was calculated for each group. And finally, a pooled effect size was reported. Heterogeneity was evaluated based on I2. Either Random effect model' or 'Fix effect model' was used based on Heterogeneity. Since Apelin dose, follow-up duration, and administration protocol (peripheral or central) varied between different studies, studies were divided based on these variables and then analysis was performed. It is worth mentioning that 'Funnel plot' was used to identify publication bias using the Egger's test (20).

3. Results

The systematic search resulted in 1338 non-duplicate articles. After screening titles and abstracts, 28 articles were reviewed in detail. Ten articles (18, 21-29) were included in the present meta-analysis (Figure 1). All 10 of these studies were conducted on rats. Apelin-13 was used in 8 studies, Apelin-12 in 1 study, and Apelin-36 in 1 study. The route of Apelin administration in 8 studies was peripheral, 7 of them intravenous and 1 of them intraperitoneal. In the two remaining studies, the administration route was central. Model of hypertension induction was as follows: L-NAME injection in 3 articles, two-kidney-one-clip (2K1C) model in 3 articles, angiotensin II injection in 2 articles, and DOCA-salt injection in 1 article. In 1 article, spontaneously hypertensive rats were used. Follow-up duration varied from 0 minute to 21 days. Two articles measured blood pressure noninvasively, using tail cuff. The remaining 8 articles measured arterial pressure invasively by placing a catheter in the carotid artery. Induced hypertension in 3 articles was acute, while in the other 7 it was chronic (Table 1).

3.1. Meta-analysis

Effect of Apelin administration on mean arterial pressure (MAP)

In our analysis, studies were divided into two groups based on Apelin administration route: 1) peripheral injection and 2) central injection. Results of statistical analysis showed that peripheral Apelin injection decreases MAP (SMD=-3.13; 95% CI: -4.43 to -1.82; p<0.001), while central Apelin injection results in MAP elevation (SMD=3.55; 95% CI: 2.43 to 4.67; p<0.001) (Fig. 2). In the evaluation of the effect of total injected dose of Apelin on MAP, it was found that MAP does not alter with change in dose of Apelin, and there is no significant relationship between dose and beneficial effects of Apelin on MAP (meta-regression coefficient=0.11; p=0.454) (Fig. 5). On the other hand, according to the results of statistical analysis, by continuing the follow-up process, the effect of Apelin on MAP decreases over time (meta-regression coefficient=-2.46; p=0.002).

Effect of Apelin administration on systolic blood pressure (SBP)

As mentioned, the studies were divided into two groups based on Apelin administration route: 1) peripheral injection and 2) central injection. Results of statistical analysis showed that peripheral Apelin injection decreases SBP (SMD=-1.62; 95% CI: -2.22 to -1.02; p<0.001), while central Apelin injection results in SBP elevation (SMD=5.13; 95% CI: 1.86 to 8.41; p=0.008) (Fig. 3). In the evaluation of the effect of total injected dose of Apelin on SBP, it was found that with increase in dose of Apelin, SBP is further reduced, which suggests that its effect on SBP is dose-dependent, and their relation is significant (meta-regression coefficient=0.08; p=0.001) (Fig. 5). On the other hand, based on our statistical analysis, by continuing the follow-up process -and recording the changes in SBP- the effect of Apelin does not significantly change over time (meta-regression coefficient=-0.17; p=0.063).

Effect of Apelin administration on diastolic blood pressure (DBP)

Results of statistical analysis also showed that peripheral Apelin injection decreases DBP (SMD=-1.10; 95% CI: -1.59 to -0.62; p<0.001), while central Apelin injection results in DBP elevation (SMD=4.09; 95% CI: 2.11 to 6.07; p= 0.089) (Fig. 4). In the evaluation of the effect of total injected dose of Apelin on DBP, it was found that with increase in dose of Apelin, DBP is further reduced, which suggests that its effect on DBP is dose-dependent, and their relation is significant (meta-regression coefficient=0.059; p=0.007) (Fig. 5). Based on the results of statistical analysis, by continuing the follow-up process, the effect of Apelin on DBP decreases over time (meta-regression coefficient=-0.16; p=0.012).

Publication bias assessment

In publication bias assessment of the present study, it was revealed that there was no evidence of publication bias in any of the studied parameters including: the relation between Apelin administration and 1) mean arterial pressure (p<0.124), 2) systolic blood pressure (p=0.458) and 3) diastolic blood pressure (p=0.181).

4. Discussion

Results of our analyses indicate that peripheral Apelin administration decreases MAP, SBP and DBP, while central administration increases these parameters. Statistical analysis also shows that increasing dose of administration causes further reduction in SBP and DBP, but no significant change is seen in its effect on MAP. When Apelin administration was continuous, its effects persisted during follow-up.

The mechanisms by which Apelin changes blood pressure is not fully understood. Studies have shown that Apelin has a transient effect on blood pressure, which starts less than one minute after intravenous injection and can last up to 3-4 minutes before the blood pressure returns to its prior level (17, 22, 30-34). In contrast, it has been shown that Apelin micro-injection in the rostral ventrolateral medulla (RVLM) increases neural activity in this region, which in turn increases sympathetic activity and causes vasoconstriction that results in blood pressure elevation (35). Studies on the mechanism of vasodilatory effects of Apelin, when administered peripherally, have shown that Apelin increases the production of NO through increasing eNOS expression. As a result, Apelin dilates blood vessels through the endothelium-dependent pathway (36). As previously mentioned, the Apelin administration was divided into two groups: 1) peripheral administration, and 2) central administration. Results show that peripheral administration decreases MAP, while central administration increases MAP. Our findings show that the effect of Apelin on MAP did not change with changing the dose of Apelin and no significant relationship was observed between dose and its effects. Based on statistical findings, with continuing the follow-up process and recording the changes in blood pressure after the last administration, effect of Apelin diminishes over time. It appears that Apelin exerts short-term effects by increasing the production of NO, and in the case of intraventricular injection, it increases blood pressure by increasing vasopressin production and sympathetic vasoconstrictor activity.

It was shown that the administration of Apelin reduces SBP in the peripheral administration, while it increases SBP in the central route of administration. Studying the effect of total prescribed dose of Apelin on SBP revealed that by increasing the dose of Apelin, SBP is further reduced, and a significant relationship was observed between the dose and its effects. Additionally, our results indicate that by continuing the follow-up process and recording SBP after treatment with Apelin, the effect of Apelin does not change over time. From these findings, it can be inferred that the effectiveness of Apelin on SBP is not affected by time, but is more dose-dependent. This means that increasing the dose of Apelin further reduces SBP. Since p-value was close to the significance level, it is feasible that future studies can prove its effectiveness. Since Apelin increases myocardial contractility, it may elevate SBP. However, the activation of baroreceptors in aortic arch and carotid sinus subsequently decreases SBP. Apelin increases production of NO, which decreases SBP and thus, decreases cardiac afterload. Most likely, the duration of administration should be longer to exert long-term effects. Since Apelin decreases MAP, it makes sense that Apelin also reduces SBP by improving vasodilation.

Another indicator discussed in most of the studies was DBP. Peripheral Apelin administration increases DBP and its central administration decreases it. The effect of Apelin on DBP was dose-dependent, which means higher doses cause further reduction in DBP and it was statistically significant. According to the obtained results, continuation of the follow-up process and recording changes in DBP shows that the effectiveness of Apelin continues over time.

In the present research, none of the studied indicators, including the relationship between Apelin administration with MAP, SBP and DBP, showed evidence of publication bias so it can be concluded that Apelin is effective for controlling blood pressure.

Experts believe that a suitable medication to control blood pressure crisis should reduce blood pressure by 10 to 15% in the first hour and lead to an extra 10 to 15% decrease in the subsequent 4 hours to prevent hypoperfusion complications. Although, Apelin, as a rapid-onset medication, can decrease BP in a few minutes, its optimum dose for treating hypertension is not known. Therefore, we strongly recommend that future studies identify a dose of Apelin that can reduce blood pressure by 10-15% during the first hour.

5. Limitation

One of the most important limitations of the current study is presence of risk of bias in the included studies. Lack of reporting required data in the eligible studies for passing judgment about random sequence generation, allocation concealment, random housing, random outcome assessment, blinding of observers, incomplete outcome data, and selective outcome reporting are the main sources of high risk of bias. These items are generally not reported in experimental studies, and shows one of the basic problems in reporting of preclinical evidence. Another limitation of the current meta-analysis was the wide variation in the doses of Apelin prescribed in the studies. Although the variation in the prescribed dose was not a source of heterogeneity, several doses of this drug should be compared in future studies (dose-response gradient) and finally, the optimum dose should be suggested.

6. Conclusion

The present systematic review of preclinical evidence showed that peripheral administration of Apelin reduces MAP, SBP and DBP in hypertensive animals. On the other hand, central administration of Apelin increases these parameters.

7. Declarations

7.1. Acknowledgments

Not applicable.

7.2. Ethics approval

Not applicable.

7.3. Patient consent

Not applicable.

7.4. Informed consent

Not applicable.

7.5. Availability of data and materials

All data generated or analyzed during this study are included in this published article and its supplementary information file.

7.6. Permission to reproduce material from other sources

Not applicable.

7.7. Conflicting interests

The authors declare that they have no competing interests.

7.8. Funding

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7.9. Author contributions

Ideation and design: MY, and YA. Data collection: MM, MM, AM, PG, HAR. Analysis: MY. Drafting the work: MY, YA, MM. Revising draft critically for important intellectual content: All authors. The authors read and approved the final manuscript.

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Table 1: Characteristics of included studies

Author; year	Gender; species; strain; age	Dose (nmol/kg) / total number of administration / number administration/day / route	Interval time between induction of BP to treatment (minutes)	Model of HTN induction / dose of inducer / type of HTN	Method of BP assessment	Follow-up
Akcilar; 2013	Male; Rat; Wistar Albino; 8–10-wk	200 / 17 / 1 / IP	24	DOCA-salt treatment / 25 mg/kg / Chronic	Tail cuff BP	17 days
Ishida; 2004	Male; Rat; WKY SHR; 12-wk	3, 6, 15 / 1 / 1 / IV	0.25	L-NAME / 10 mg/kg / Acute	Intra-arterial catheter	5 minutes
Lee; 2005	Male; Rat; SHR Wistar; approximately 15-wk	15 / 1 / 1 / IV	0	Angiotensin II / 30 ng/kg / Acute	Intra-arterial catheter	1, 5, 10 minutes
Rostamzadeh; 2018	NR; Rat; Wistar; NR	40, 60 / 1 / 1 / IV	2688	2K1C / NA / Chronic	Intra-arterial catheter	1, 5, 10 minutes
Siddiquee; 2011	Male; Mouse; C57Bl/6j; 8-wk	15 / 21 / 1 / IV	504	L-NAME, Angiotensin II / 1.0 mg/mL drinking water, 1.0 μg/kg/day / Chronic	Tail cuff BP	21 days
Soltanihekmat; 2011	Male; Rat; Sprague–Dawley; NR	10, 20, 40/ 1 / 1 / IV	672	2k1c / NA / Chronic	Intra-arterial catheter	1, 5, 10 minutes
Tatemoto; 2001	Male; Rat; Wistar; 8-9-wk	7, 8, 20 / 1 / 1 / IV	0.16	L-NAME / 30 mgr per kg / Acute	Intra-arterial catheter	1, 4 minutes
Yeganeh-Hajahmadi; 2017	Male; Rat; Wistar; NR	20, 40 / 1 / 1 / IV	672	2k1c / NA / Chronic	Intra-arterial catheter	1, 4, 10 minutes
Zhang; 2014	Male; Rat; WKY SHR; 13-wk	4, 45, 450 / 15 / 1 / in PVN	0	SHR rats / NA / Essential	Intra-arterial catheter	1 minute
Zhao; 2018	Male; Rat; WKY SHR; 13-wk	4, 450 / 1 / 1 / in PVN	0	SHR rats / NA / Essential	Intra-arterial catheter	1 minute

2K1C: 2 kidneys one clip; BP: Blood pressure; HTN: Hypertension; IP: Intraperitoneal; IV: Intravenous; DOCA: Deoxycorticosterone acetate; L-NAME: L-NG-Nitro arginine methyl ester; NA: Not applicable; NR: Not reported; PVN: Paraventricular nucleus; SHR: Spontaneous hypertensive rat; WKY: Wistar Kyoto; wk: Weeks.

Table 2: Risk of bias assessment among the studies based on SYRCLE's tool

Domain	Sequence generation	Baseline characteristics	Allocation concealment	Random housing	Blinding of caregivers	Random outcome assessment	Blinding of observers	Incomplete outcome data	Selective outcome reporting	Other sources
Akcilar; 2013	Low risk	Low risk	High risk	High risk	Not reported	High risk	Not reported	Not reported	Cannot be determined	Low risk
Ishida; 2004	High risk	Low risk	High risk	High risk	Not reported	High risk	Not reported	Not reported	Cannot be determined	Low risk
Lee; 2005	High risk	Low risk	High risk	High risk	Not reported	High risk	Not reported	Not reported	Cannot be determined	Low risk
Rostamzadeh; 2018	Low risk	Low risk	High risk	High risk	Not reported	High risk	Not reported	Not reported	Cannot be determined	Low risk
Siddiquee; 2011	High risk	Low risk	High risk	High risk	Not reported	High risk	Not reported	Not reported	Cannot be determined	Low risk
Soltanihekmat; 2011	Low risk	Low risk	High risk	High risk	Not reported	High risk	Not reported	Not reported	Cannot be determined	Low risk
Tatemoto; 2001	High risk	Low risk	High risk	High risk	Not reported	High risk	Not reported	Not reported	Cannot be determined	Low risk
Yeganeh-Hajahmadi; 2017	Low risk	Low risk	High risk	High risk	Not reported	High risk	Not reported	Not reported	Cannot be determined	Low risk
Zhang; 2014	Low risk	Low risk	High risk	High risk	Not reported	High risk	Not reported	Not reported	Cannot be determined	Low risk
Zhao; 2018	High risk	Low risk	High risk	High risk	Not reported	High risk	Not reported	Low risk	Cannot be determined	Low risk

SYRCLE: Systematic Review Centre for Laboratory Animal Experimentation.

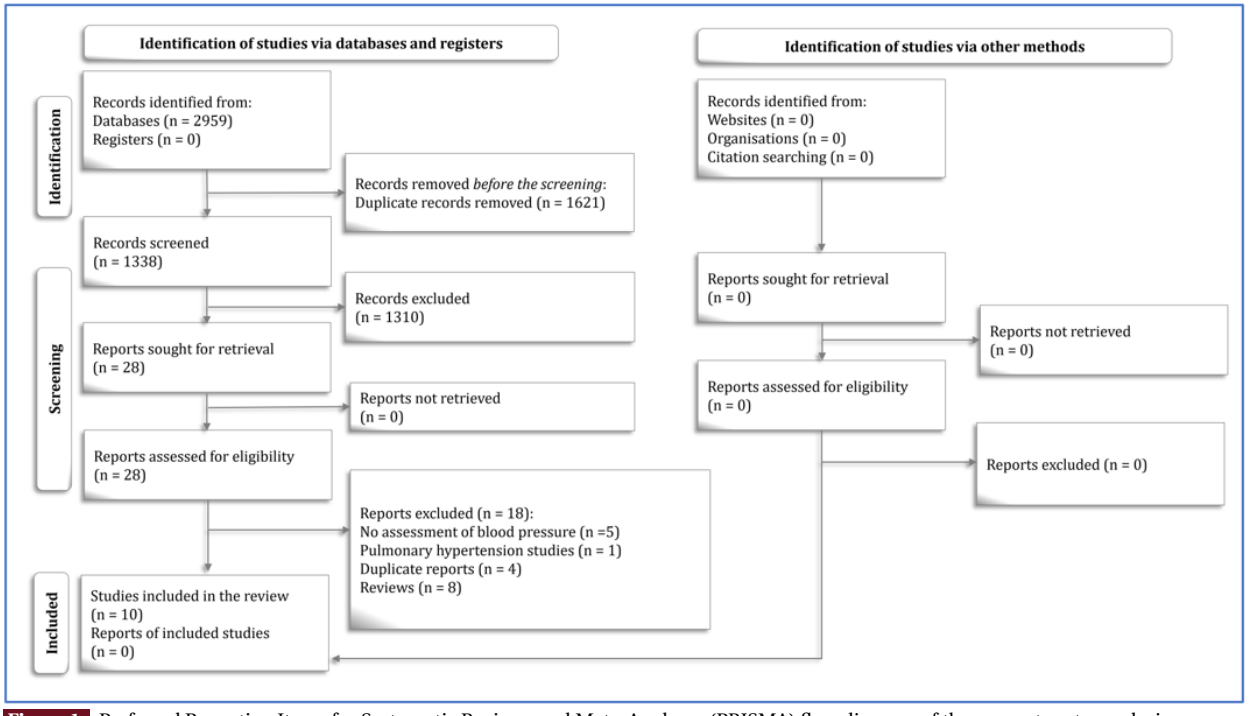


Figure 1: Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) flow diagram of the present meta-analysis

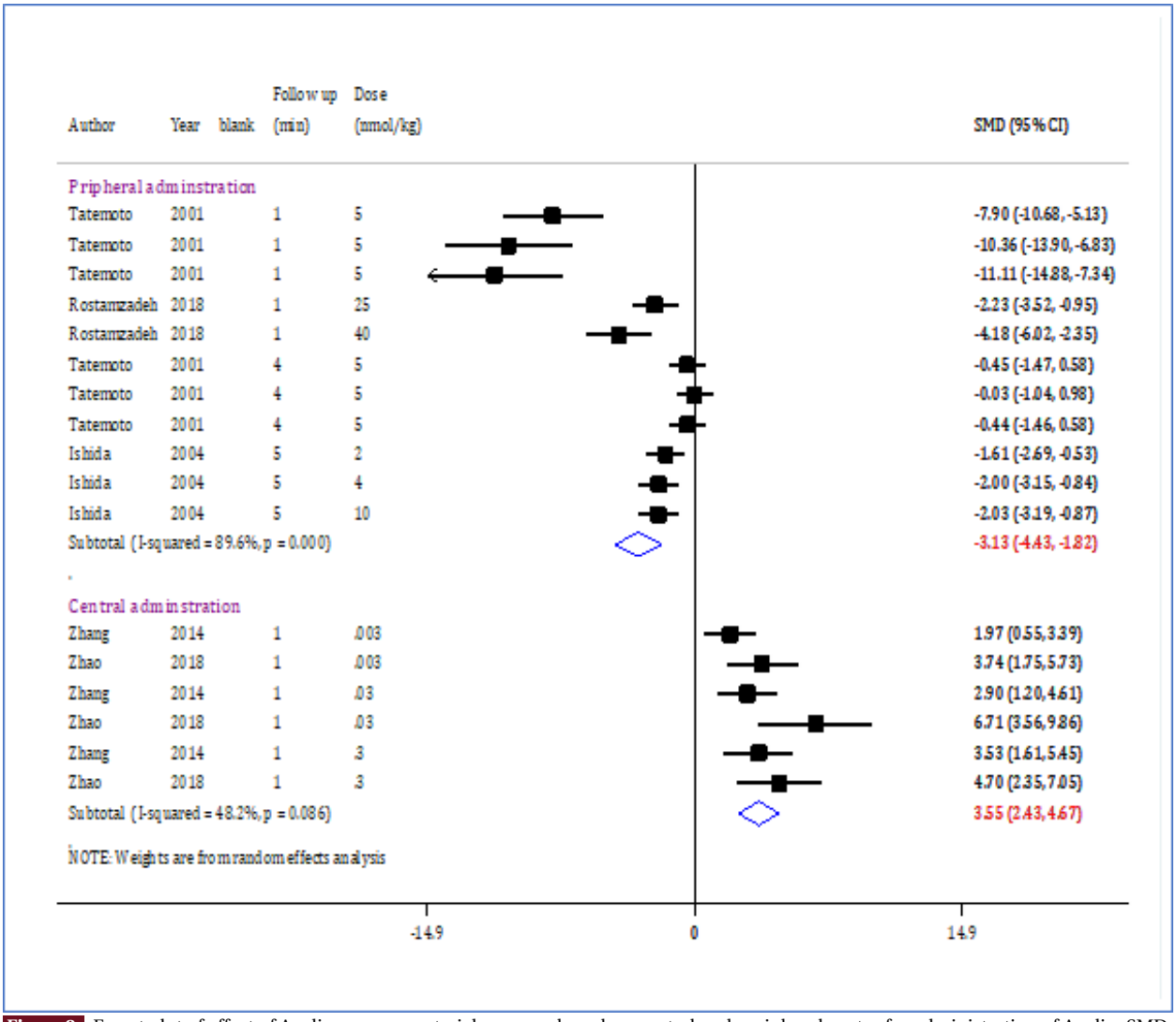


Figure 2: Forest plot of effect of Apelin on mean arterial pressure based on central and peripheral routes for administration of Apelin. SMD: standard mean difference; CI: confidence interval.

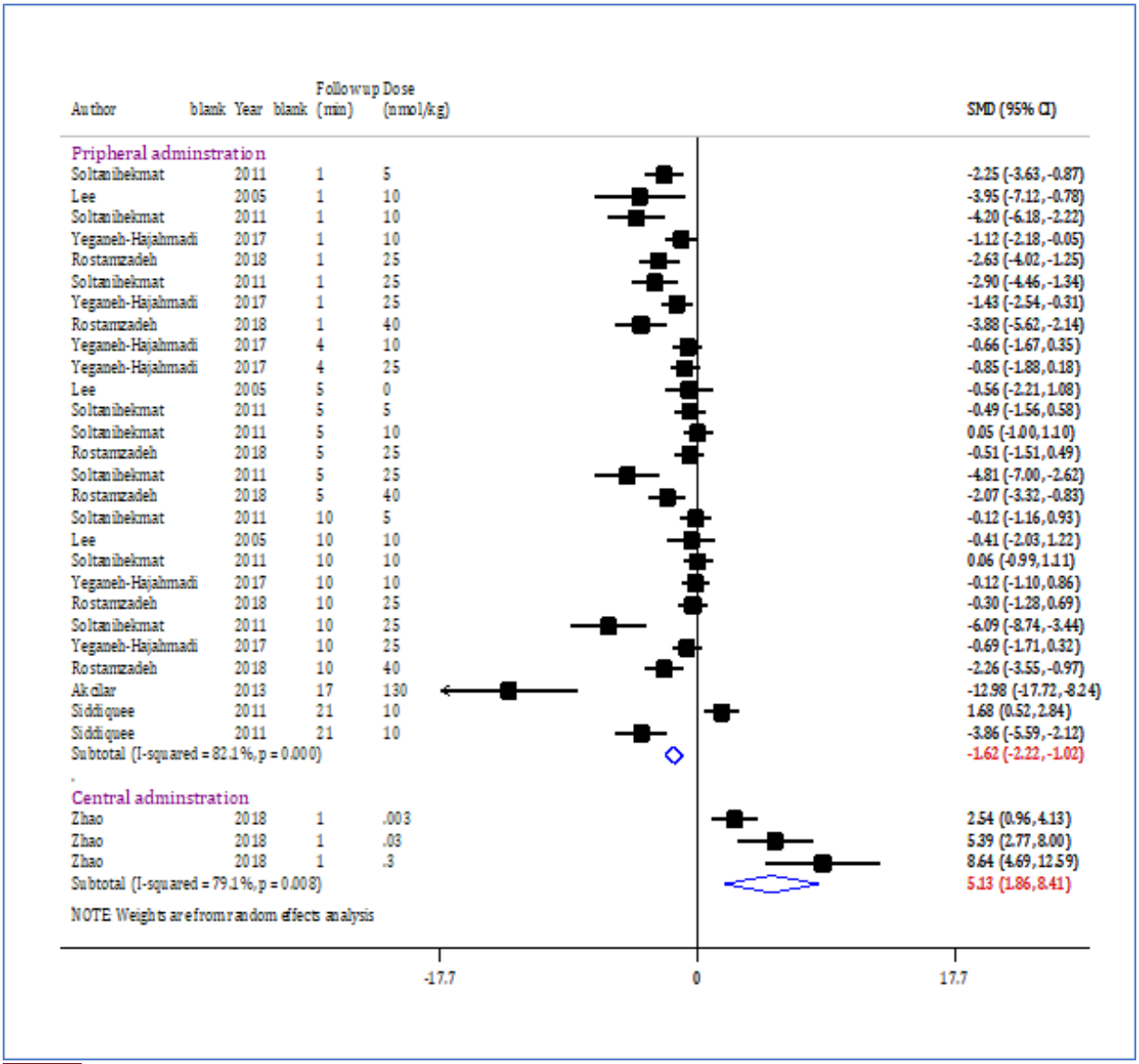


Figure 3: Forest plot of effect of Apelin on systolic blood pressure based on central and peripheral routes for administration of Apelin. SMD: standard mean difference; CI: confidence interval.

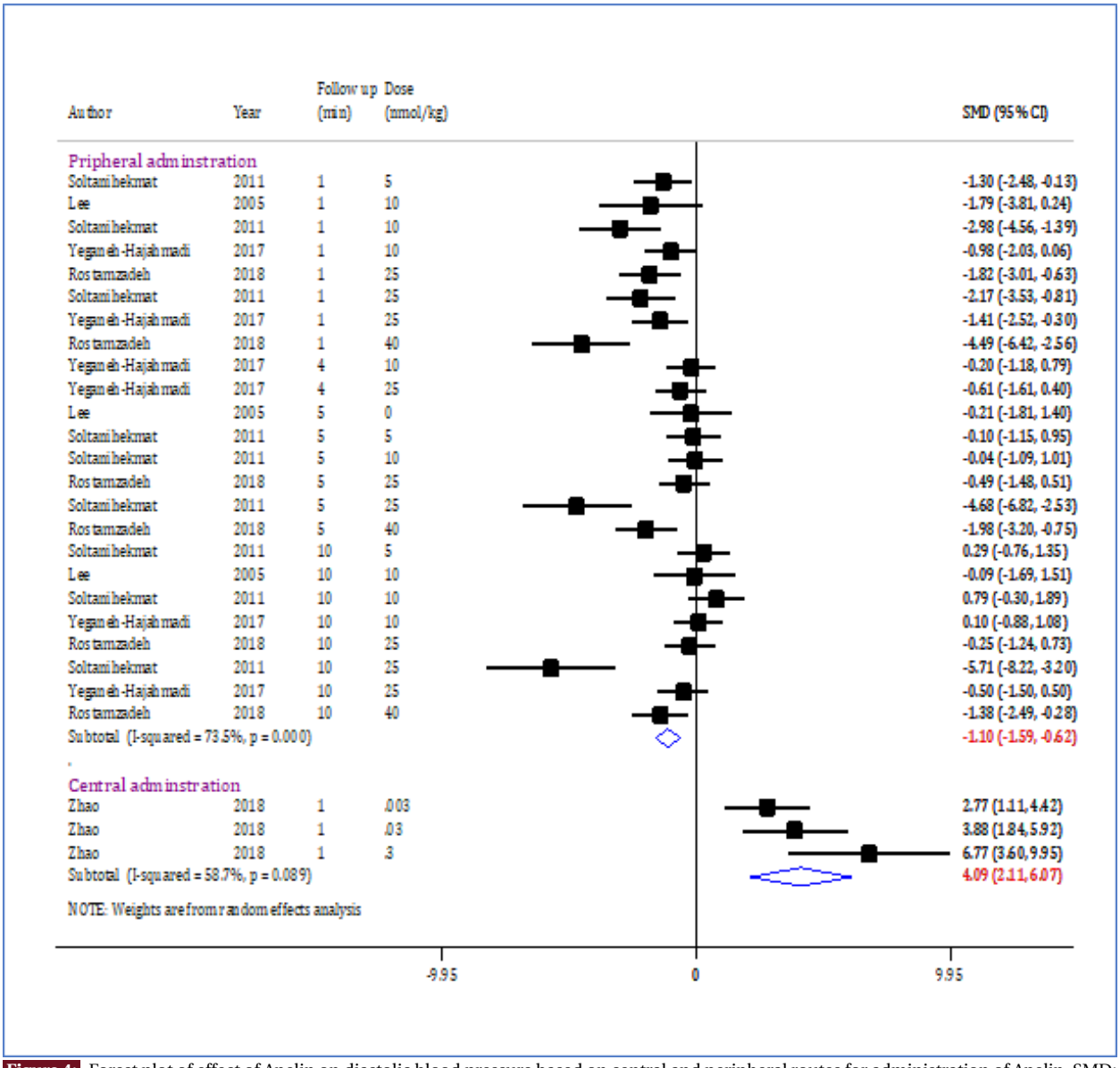


Figure 4: Forest plot of effect of Apelin on diastolic blood pressure based on central and peripheral routes for administration of Apelin. SMD: standard mean difference; CI: confidence interval.

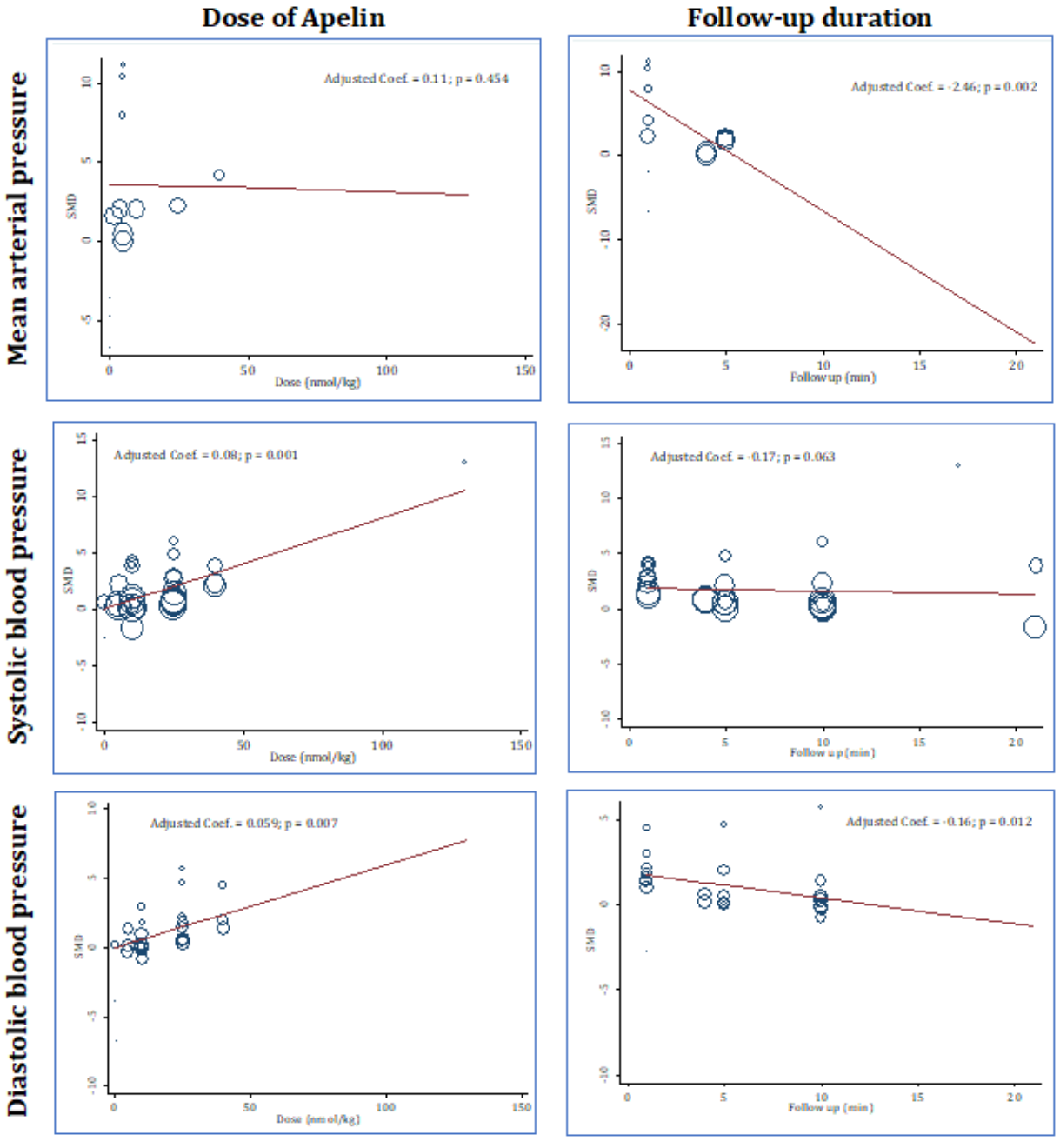
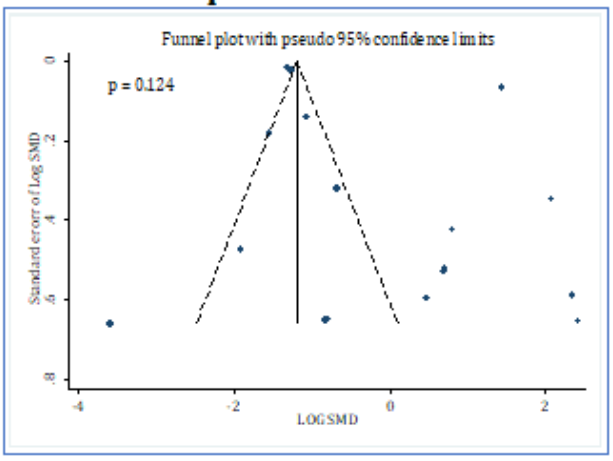
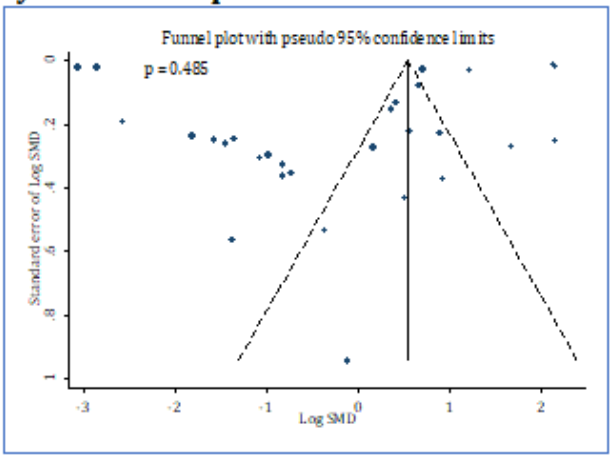


Figure 5: Meta regression analysis of effect of peripheral Apelin administration on blood pressure based on dose of Apelin and follow-up duration. SMD: standard mean difference.

Mean arterial pressure



Systolic blood pressure



Diastolic blood pressure

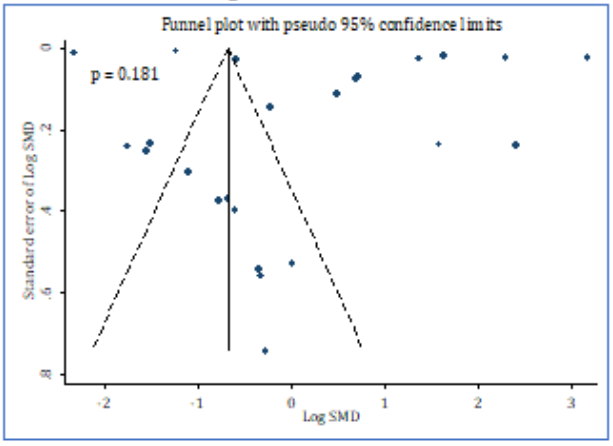


Figure 6: Publication bias in assessment of the effect of Apelin administration on blood pressure. SMD: standard mean difference.