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Research Article

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Mechanisms of *Atractylodes macrocephala* Koidz. on therapy to diarrheal disease: A systematical review and meta-analysis of preclinical evidence

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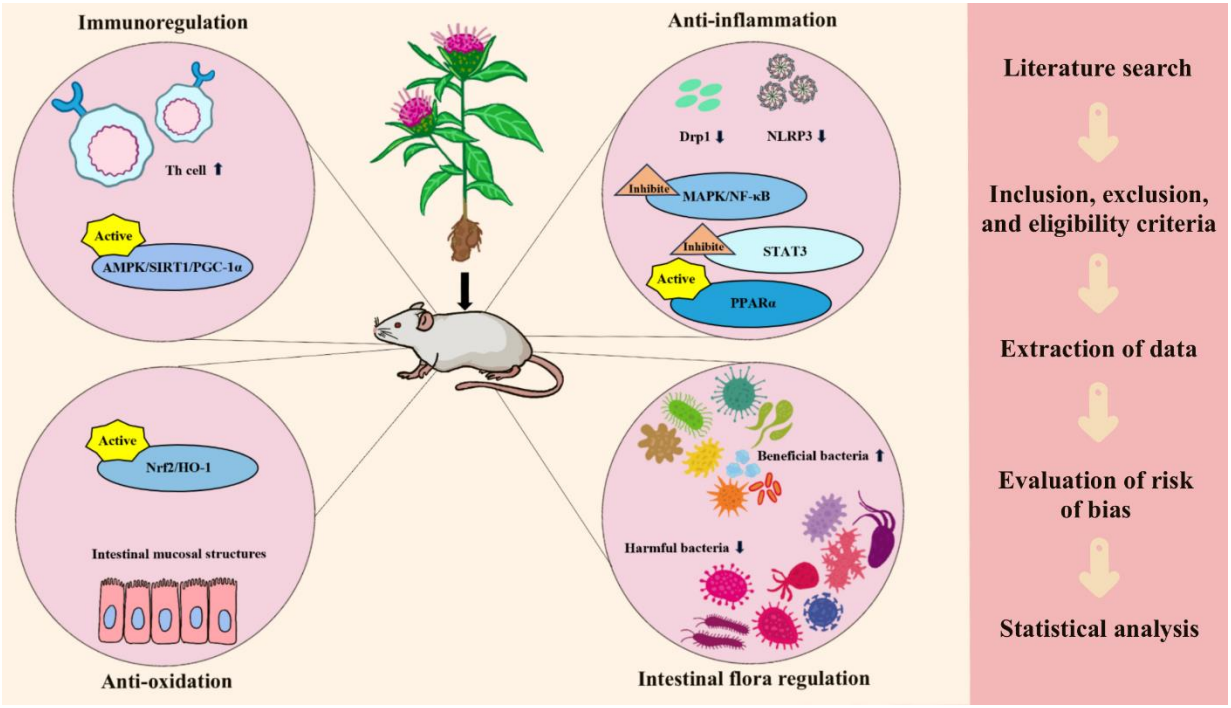
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Graphical abstract



Abstract

Background: *Atractylodes macrocephala* Koidz. (AMK) is a traditional Chinese medicine used for the treatment of various diseases. Atractylenolide and polysaccharide from *Atractylodes macrocephala* Koidz. (PAMK) are the representative bioactive components in AMK. There have been many in vivo studies on AMK for the treatment of diarrhea, the most common gastrointestinal disorder worldwide and the high mortality rate. The aim of this study is to assess the effectiveness of AMK in treating diarrhea using preclinical evidence and meta-analysis. Additionally, the primary mechanisms by which AMK acts against diarrhea are summarized.

Methods: We systematically searched PubMed, WOS, Embase, Cochrane Library, WanFang, CNKI, Cqvip and CBM databases and other databases from January 1, 2000, to March 10, 2023, to evaluate the animal experiments of AMK for the treatment of diarrhea. Finally, 23 animal experiments were included. Thirteen outcome measures, including overall condition(body weight, diarrhea score, disease activity index), immune-related indicators (colon length, spleen index, histopathological score, SIgA), pro-inflammatory factors(IL-1β，IL-6，IL-17,TNF-α), anti-inflammatory factors(IL-10) and MPO were obtained by using Review Manager 5.4 software for meta-analysis.

Results: Except for the indicator of spleen index, which was not significant difference, it was significantly decreased in the indicator of diarrhea score, disease activity index, histopathological score and significantly elevated in the indicator of body weight, colon length. It showed that AMK could improve immune function in diarrhea. Meanwhile AMK significantly down-regulated pro-inflammatory factors, IL-1β， IL-6，IL-17 , TNF-α and MPO, up-regulated anti-inflammatory factors IL-10, indicating that AMK may have anti-inflammation and anti-oxidation effects.

Conclusion: AMK exhibits protective effects against diarrhea through immunoregulation, anti-inflammation, anti-oxidation, and regulation of intestinal flora. These findings provide preclinical evidence supporting the application of AMK in diarrhea treatment.

Keywords: ***Atractylodes macrocephala*** **Koidz.; Diarrhea; Mechanisms; Systematic review; Meta-analysis**

1.Introduction

Diarrhea is a pathological process characterized by an increased frequency of bowel movements, loose or watery stools, and accompanied by gastrointestinal symptoms such as abdominal pain, nausea, and vomiting[1]. The etiology of diarrhea is complex and diverse, including infectious diarrhea, food intolerance, drug reactions, chronic gastrointestinal diseases, immune-related disorders, and others[2]. According to the Global Burden of Disease, diarrheal disease ranks third among infectious diseases that burden children under the age of 10 globally[3]. Diarrhea also stands as the eighth leading cause of death across all ages, exerting a profound impact on global health[3].

Diarrhea consistently induces alterations in the overall physical condition, immune-related indicators, inflammatory factors, oxidative indicators, and intestinal flora. First, diarrhea can cause changes in the overall physical condition, such as weight loss, higher diarrhea scores, and increased disease activity index[4]. Second, diarrhea can affect immune-related indicators, resulting in shorter colon length, decreased spleen index, higher histopathological scores, and reduced levels of secretory immunoglobulin A (SIgA) in patients[5]. Furthermore, immune system disruptions can trigger inflammatory responses in the gastrointestinal tract, characterized by abnormal expression of pro-inflammatory cytokines (IL-1β, IL-6, IL-17, TNF-α), and anti-inflammatory cytokine (IL-10)[6]. Lastly, diarrhea resulting from different factors can lead to imbalances in the intestinal microbiota to varying degrees[7].

Over the past few years, traditional Chinese medicine has demonstrated its efficacy and innovative approach in the treatment of diarrhea. As a traditional Chinese medicinal herb, AMK has a long history of use and is widely applied in traditional Chinese medicine. The medicinal application of AMK, particularly for nourishing the spleen and stomach, was first documented in the Shen Nong Ben Cao Jing around 200 AD. The medical expert of the Qing Dynasty, Huang Gongxiu considered AMK an essential medicine for spleen invigoration. According to the Materia Medica Seeks Truth , AMK is described as having a warm nature and being the primary medicine for invigorating the spleen[8]. It is noteworthy that the term "spleen" in Traditional Chinese Medicine differs significantly from its Western medicine counterpart. According to Traditional Chinese Medicine principles, when food enters the gastrointestinal tract, it undergoes transformation facilitated by the spleen's function[9]. This process involves the distribution of nutrients derived from the food throughout the body, converting them into essence, qi, blood, and bodily fluids. As a result, various organs, meridians, and channels in the body receive essential nourishment[10]. Consequently, the concept of the spleen in Traditional Chinese Medicine is fundamentally intertwined with the gastrointestinal system. To clarify, Traditional Chinese Medicine primarily focuses on enhancing the digestive and absorptive processes within the gastrointestinal system.

According to recent reports, AMK exhibits various biological activities, including improving gastrointestinal function, anti-inflammatory, anti-aging, antioxidant, antibacterial, and anticancer effects[11]. AMK demonstrates a range of pharmacological actions in the treatment of diarrhea, highlighting its distinctive

advantages. It effectively addresses diarrhea through its anti-inflammatory, anti-oxidant, and immunoregulatory mechanisms[12]. The effective active components of AMK, such as Atractylenolide and PAMK, have been increasingly recognized and investigated in both Chinese and Western medicine[13]. Consequently, research pertaining to these components has been progressively conducted. To the best of our knowledge, there is a dearth of literature documenting the utilization of AMK for treating diarrhea clinically, rendering a meta-analysis of pertinent clinical studies unfeasible.

Pharmacological research has extensively explored the potential of AMK in alleviating diarrhea in animal models. However, a comprehensive synthesis of the beneficial effects and underlying mechanisms of AMK in diarrhea remains lacking. Therefore, our study conducted a systematic review and meta-analysis of animal experiments, aiming to enhance the understanding of animal study efficacy and improve outcome estimation precision. This investigation serves to provide preclinical evidence supporting the application of AMK in the treatment of diarrhea.

2.Method

This systematic review aims to investigate the effectiveness of AMK in treating diarrhea based on preclinical trial data through meta-analysis. The meta-analysis in this study was conducted following the PRISMA checklist[14].

2.1 Strategy for literature search

A systematic literature search was conducted in PubMed, Web of Sciences (WOS), Embase, Cochrane Library, WanFang, China National Knowledge Infrastructure (CNKI) databases, Cqvip and China Biology Medicine (CBM) and other databases, from January 1, 2000, to March 10, 2023. The specific search items were used as follows: (Atractylodes macrocephala Koidz. OR Rhizoma Atractylodis Macrocephalae OR Atractylodis Macrocephalae Rhizoma OR Baizhu OR atractylodis rhizoma OR bighead atractylodes rhizome OR white atractylodes rhizome OR the rhizome of large-headed atractylodes OR rhizome of lagehead atractylodes OR atractylenolide I OR atractylenolide II OR atractylenolide III OR atractylodes polysaccharides) AND (diarrhea OR inflammatory bowel disease OR IBD OR ulcerative colitis OR Crohns disease OR functional diarrhea OR functional gastrointestinal disease OR irritable bowel syndrome) AND (animal OR rat OR mice).

("Atractylodes macrocephala Koidz." OR "Rhizoma Atractylodis Macrocephalae" OR "Atractylodis Macrocephalae Rhizoma" OR "Baizhu" OR "atractylodis rhizoma" OR "bighead atractylodes rhizome" OR "white atractylodes rhizome" OR "the rhizome of large-headed atractylodes" OR "rhizome of lagehead atractylodes" OR "atractylenolide I" OR "atractylenolide II" OR "atractylenolide III" OR "atractylodes polysaccharides") AND ("diarrhea" OR "inflammatory bowel disease" OR "IBD" OR "ulcerative colitis" OR "Crohns disease" OR "functional diarrhea" OR "functional gastrointestinal disease" OR "irritable bowel syndrome") AND ("animal" OR "rat" OR "mice")

2.2. Inclusion, exclusion criteria and eligibility criteria

The inclusion criteria for the studies were as follows: 1) randomized controlled trials examining the effects of AMK on diarrhea-related indicators; 2) experimental groups receiving AMK or its active ingredients for treatment; 3) control groups receiving saline or distilled water for treatment; 4) all rodent diarrhea models. There were no restrictions on age, sex, species, animal modeling methods, treatment dosage, or duration.

The following criteria were used to exclude studies: 1) experimental groups treated in combination with other drugs; 2) reviews, protocols, non-animal experiments (such as clinical trials, cell experiments, etc.), literature lacking extractable data or inaccessible full texts; 3) non-randomized controlled trials.

The main results were as follows: 1) overall condition (body weight, diarrhea score, disease activity index); 2) immune-related indicators (colon length, spleen index, histopathological score, SIgA); 3) pro-inflammatory factors (IL-1β, IL-6, IL-17, TNF-α); 4) anti-inflammatory factors (IL-10) and MPO.

2.3. Extraction of data

The articles were screened based on the inclusion and exclusion criteria. They were independently assessed and extracted by two researchers. The extracted information included: 1) name of the first author; 2) year of publication; 3) animal age; 4) weight; 5) species; 6) sex; 7) diarrhea inducer (administration mode and dose); 8) AMK treatment (administration mode, dose, administration time, sample size); 9) main results (overall condition, immune-related indicators, inflammatory factors, etc.); 10) mechanisms of action.

The outcome data from the articles are extracted, including animal sample size (N), mean, or standard deviation (SD). If the data are presented in graphical form, GetData v2.26 software is used for data extraction.

2.4. Evaluation of Risk of Bias

Two authors independently assessed the articles for inclusion and exclusion criteria and reached a consensus on which articles to include. We evaluated the methodological quality using SYRCLE's risk of bias in animal studies tool [15]. The evaluation included the following items: three for selection bias, two for performance bias, two for detection bias, one for attrition bias, one for reporting bias, and one for other bias. Each item was classified as "Low risk of bias," "Unclear risk of bias," or "High risk of bias."

2.5. Statistical analysis

The meta-analysis was performed using Review Manager 5.4 and R 4.3.0 software. The heterogeneity of included studies was assessed based on I^2 statistic, with values greater than 50% indicating high heterogeneity. When $I^2 > 50\%$ and $P \leqslant 0.1$, sensitivity and subgroup analyses were conducted to explore the sources of heterogeneity. For highly heterogeneous results, a random effects model was applied; otherwise, a fixed effects model was used.

A significance level of $P<0.05$ was used to determine statistical significance. Subgroup analysis and sensitivity analysis were conducted to investigate potential sources of heterogeneity. The subgroup analysis focused on several outcome measures, including body weight, diarrhea score, DAI, colon length, spleen index, histopathological score, IL-1β, IL-6, IL-17, TNF-α, and MPO, for which sufficient research reports were available. The variables considered in the subgroup analysis included types of AMK and animal species. Sensitivity analysis involved systematically removing each study and assessing the corresponding changes in the I^2 value.

3.Results

3.1. Process of inclusion

Initially, a total of 361 studies were obtained from 8 databases. Duplicate articles (110) were then filtered out based on titles and abstracts. We excluded 46 studies that clearly did not meet the initial screening criteria. Subsequently, the full text of the remaining 205 papers was read for secondary screening. Among these, 182 studies were excluded for at least one of the following reasons: combination with other drugs, non-animal studies, unavailability of full text, or missing key data. Finally, 23 papers were included for meta-analysis. [16,17-21,22-26]. The results based on the above search strategy are shown in Fig. 1.

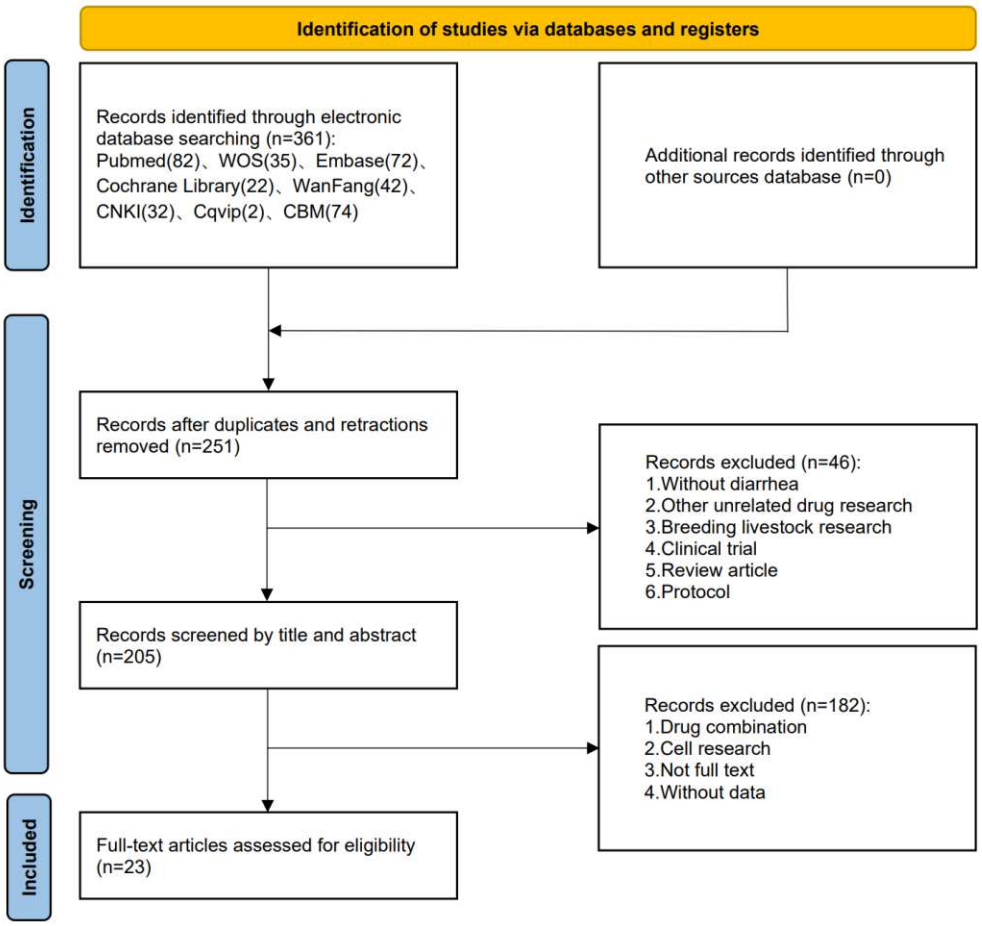


Fig. 1. Flowchart of literature screening process.

3.2. Features of inclusion

Our study selected twenty-three studies conducted between 2000 and 2023 using rat or mouse models of diarrhea. The findings of our study revealed: (1) C57BL/6 mice were the most common, appearing in 8 studies, 4 of which were C57BL/6J mice, followed by Sprague Dawley (SD) rats in 5 studies, Wistar rats in 3 studies, Kunming mice in 3 studies, BALB/c mice in 2 studies, and ICR mice in 2 studies. (2) 14 studies were male, 3 studies were female, 2 studies included both male and females, and 4 studies did not specify gender. (3) The number of animals in each group ranged from 5 to 20. (4) Rhubarb decoction and senna leaf decoction were used to construct spleen

deficiency diarrhea models in 9 studies, DSS and TNBS were used to construct ulcerative colitis models in 6 studies, DSS and TNBS were used to construct ulcerative colitis models in 6 studies, DSS，dinitrochlorophenon，dinitrochlorophenylethanol and acetic acid was used to construct ulcerative colitis models in 1 study, DSS was used to construct intestinal mucosal injury models in 1 study, and rotavirus fluid was used to construct viral diarrhea models in 1 study. (5) AMK and its bioactive components were administered by intragastric in 22 cases and by intervenous in 1 case, with a dose of 0.2-3750 mg/kg/d for 5-70 days. The specific results are shown in Table 1.

Table 1 Contents of 23 animal experiment.

Author name	Year	Age	Weight (g)	Species	Sex	Diarrhea inducer	Drug	Duration	Dosage	Sample size	Outcome measures	Mechanism of action
Yang et al.	2022	10 weeks	20±2	C57BL/6J mice	male	3% DSS solution free drinking, 7d	PAMK	7d	100/200/400 mg/kg, i.g.	20	a,c,d,e,f,k,l	inhibite the activation of IL-6/ STAT3 signaling
Qu et al.	2022	6-8 weeks	20±2	BALB/c mice	male	3.5% DSS solution free drinking, 7d	bran-fried Atractylodes lancea	7d	516.6 mg/kg, i.g.	12	a,c,d,g,h,k	regulate the intestinal flora
Qin et al.	2021	5-6 weeks	20±2	C57BL/6J mice	male	AOM 10 mg/kg i.p., once＋tap water free drinking, 5d＋4% DSS solution free drinking, 7d＋tap water free drinking, 14d＋4% DSS solution free drinking, 7d＋tap water free drinking, 14d	Atractylenolide I	70d	25/50 mg/kg, i.g.	16	a,g	inhibit the activation of Drp1 and NLRP3 inflammasomes
Han et al.	2022	8 weeks	20-23	C57BL/6J mice	male	3% DSS solution free drinking, 7d	Atractylenolide III	7d	5/10 mg/kg, i.v.	12	a,c,d,f,h,k	activate the AMPK/SIRT1/PGC-1α signaling pathway
Kai et al.	2022	4 weeks	18.19 ±0.95	C57BL/6 mice	male	distilled water free drinking, 7d＋3%	AMP	14d	100mg/kg, i.g.	10	a,d,g,h,k	prevente the overexpression of

						DSS solution free drinking, 7d						proinflammatory cytokines and regulate the intestinal flora
Feng et al.	2020	/	20±2	C57BL/6J mice	male	distilled water free drinking, 3d+2.5% DSS solution free drinking, 7d	AMP	10d	10/20/40 mg/kg, i.g.	8	a,b,d,f,g,k	regulate the intestinal flora, fecal metabolism and plasma metabolism
Ren et al.	2021	8 weeks	22-25	C57BL/6 mice	male	TNBS 150mg/kg en., 7d	Atractylenolide III	7d	5/10/20 mf/kg, i.g.	6	a,c,d,g,k,l	regulate oxidative stress via the FPR1 and Nrf2 pathways and regulate the intestinal flora
Xiao et al.	2023	/	200-220	Wistar rats	male	rhubarb decoction 20ml/kg i.g., bid, 10d	EAR	7d	10 ml/kg, i.g.	6	a,g,i,k,m	enhance anti-inflammatory ability, improve intestinal mucosal injury and restore and shape intestinal microenvironment
Shi et al.	2019	/	140±20	Wistar rats	/	rhubarb decoction 20ml/kg i.g., bid, 10d	EEDAR	7d	10 ml/kg, i.g.	10	a,b,i,k	regulate intestinal inflammatory response and gut microbiota
Lin et al.	2022	/	20±2	BALB/c mice	male	3.5% DSS solution free drinking, 7d	EEAR	7d	555/1110 mg/kg, i.g.	12	a,c,d,e,g,h,k,l	inhibite the inflammatory response and maintain the intestinal barrier function via modulation of MAPK/NF-κB pathways
Heo et al.	2023	8 weeks	/	C57BL/6 mice	male	2.5% DSS solution free drinking, 14d	Atractylodin	14d	40 mg/kg, i.g.	/	a	anti-inflammatory effects by activating PPARα
Wang et al.	2019	/	20±2	Kunming mice	male and female	5-FU 40mg/kg i.p., 5d	AMO	11d	0.01ml/g, i.g.	6	a,b,e,f,g,h,j,k	inhibite intestinal pathology and modulate gut microbial structure

						lard 10ml/kg i.g., on odd-numbered days; forced swimming and fed cabbage						
Ma et al.	2019	6 weeks	200±20	SD rats	male	(freely) on even-numbered days, 14d ＋senna leaf decoction 10ml/kg i.g., 7d	raw and bran-fried Atractylodis Rhizoma	7d	3750 mg/kg, i.g.	10	a,h.k	secretion and expression of cytokines and gastrointestinal hormones
Han et al.	2017	6 weeks	/	C57BL/6 mice	female	4% DSS solution free drinking, 7d	AMK	7d	100 mg/kg, i.g.	8	a,c,d,f,g,k	inhibite inflammatory mediators via inactivated ERK as well as repressed NF-κB and STAT3 and enhance antioxidative activities via HO-1 induction
Liu et al.	2023	3 weeks	/	ICR mice	male	10% folium sennae 0.4ml i.g., 7d	EEAR/EEDAR	7d	3.69/14.76 g/kg, i.g.	/	a,b,g,h,i,k	remodel the intestinal flora
Han et al.	2022	/	210-245	SD rats	male	raw Dahuang water decoction 20ml/kg i.g., 8d	water decoction and PAMK	8d	3.6/1 g/kg, i.g.	5	a,b	regulate the intestinal flora
Zhou et al.	2019	4 days	/	Kunming mice	/	rotavirus fluid 200 μl i.g., 2d	Atractylenolide III	5d	0.1/0.2/0.4 g/kg, i.g.	/	a,b	/
Du et al.	2020	/	18-22	Kunming mice	/	9% folium sennae 0.3ml/10g i.g., 10d	AMK	7d	20 g/kg, i.g.	10	a,e	/
Shi et al.	2019	/	140-160	Wistar rats	/	raw Dahuang water decoction 20ml/kg i.g., bid, 10d	EEAR/EEDAR	7d	10 ml/kg, i.g.	10	a,b,i,k	improve intestinal inflammation and repair intestinal damage
Ma et al.	2018	/	18-22	ICR mice	male	folium sennae 0.5ml/20g i.g., 7d	EEAR/EEDAR	7d	14.94 g/kg, i.g.	/	a,b	/
Zhu et al.	2014	/	187±9.78	SD rats	female	2% dinitrochlorophenone 0.3ml gtt., 14d＋0.1% dinitrochlorophenylethanol 0.25ml en.,	AMK	14d	2 ml, i.g.	10	f,h,i,j	regulate Th cell

						1d＋10% acetic acid 1ml en., 1d 2% dinitrochlorophenone 0.3ml gtt., 14d＋						inhibit inflammatory
Ye et al.	2014	/	187± 9.78	SD rats	female	0.1% dinitrochlorophenyl ethanol 0.25ml en., 1d＋10% acetic acid 1ml en., 1d	AMK	14d	2 ml, i.g.	10	f,h,j	infiltration and tissue destruction
He et al.	2010	/	180-220	SD rats	male and female	Dahuang water decoction 4ml/200g i.g., qod, 42d	Atractylenolide	7d	0.2/0.44/0.88 mg, i.g.	6	e,m	anti inflammation and inhibition of gastrointestinal motility

i.g. intragastric; i.p. intraperitoneally; i.v. intravenous; en. enema; gtt. Drops; a. body weight; b. diarrhea score; c. disease activity index (DAI); d. colon length; e. spleen index; f. histopathological score; g. interleukin (IL)-1β; h. IL-6; i. IL-10; j. IL-17; k. tumour necrosis factor (TNF)-α; l. myeloperoxidase (MPO); m. SIgA

3.3. Assessment of study quality

The results of the quality assessment, conducted using SYRCLE's risk bias tool, are presented in Fig. 2. Four studies did not report the use of randomized methods for grouping experimental animals. No studies explicitly reported "random sequence generation," "allocation concealment," "random housing," "blinding of participants and personnel," "random selection of animals in the evaluation results," or "blinding of outcome assessment." The vast majority of studies (95.7%) reported "baseline characteristics." Twelve studies (52.2%) reported "incomplete outcome data," and all studies showed a low risk of bias in "selective reporting." Two studies may have had "other bias."



Fig. 2. Deviation chart (A) and bias summary (B) incorporating research risks. Low riskof bias: '＋' Unclear risk of bias: '－' High risk of bias:'×'

3.4. Main results: effect of AMK on diarrhea

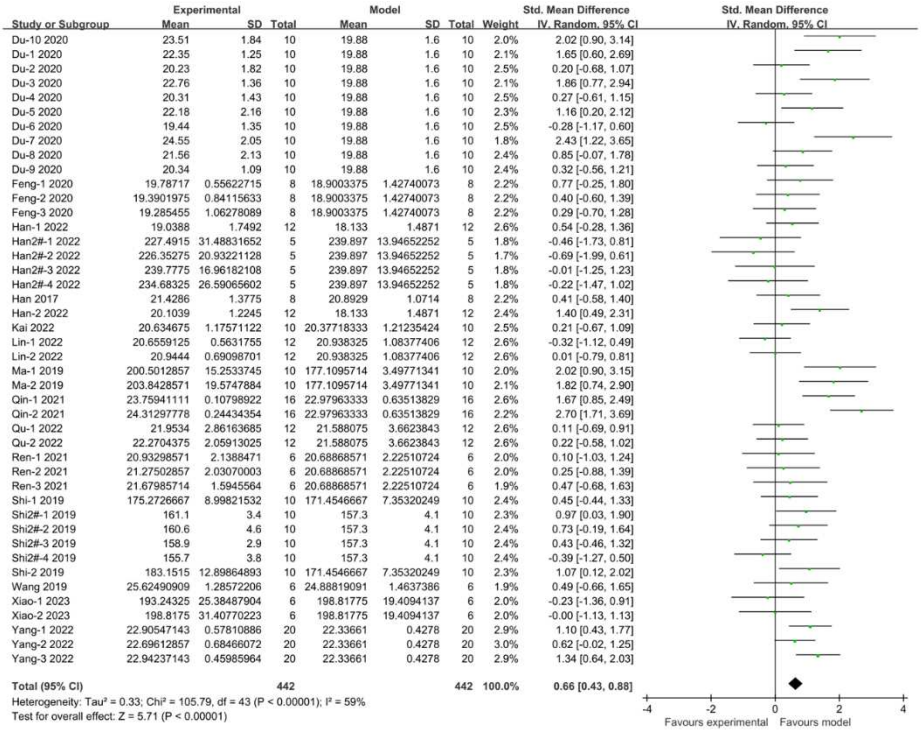
3.4.1. Overall condition

Due to the statistical heterogeneity, body weight and DAI were analyzed by random effect model: body weight I^2=59%, DAI I^2=52%. No statistical heterogeneity was found in diarrhea score in this meta-analysis, and the diarrhea score was analyzed by fixed effect model: diarrhea score I^2=0% (Fig. 3).

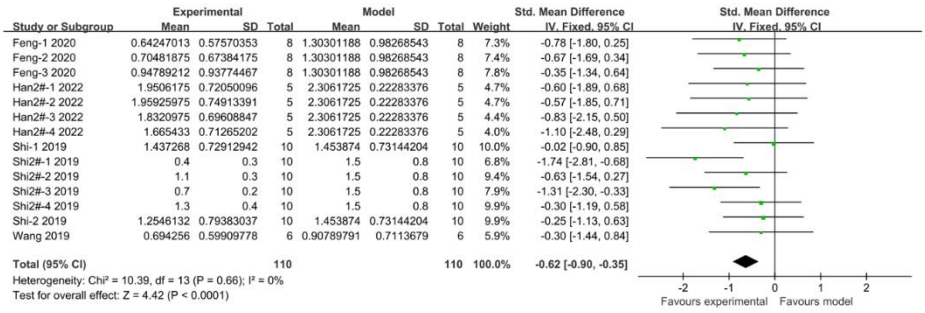
Body weight was reported in 16 studies, there are in total 442 administration animals and 442 model animals, compared with the model group, the body weight could be significantly elevated by AMK [SMD=0.66, 95% CI: 0.43 to 0.88, P<0.00001] (Fig.

3A). Diarrhea score was reported in 5 studies, there are in total 110 administration animals and 110 model animals, compared with the model group, the diarrhea score could be significantly decreased by AMK [SMD=−0.62, 95% CI: −0.90 to −0.35, P<0.0001] (Fig. 3B). DAI was reported in 6 studies, there are in total 158 administration animals and 158 model animals, compared with the model group, the DAI could be decreased by AMK [SMD=−0.45, 95% CI: −0.79 to −0.10, P=0.01] (Fig. 3C).

A



B



C

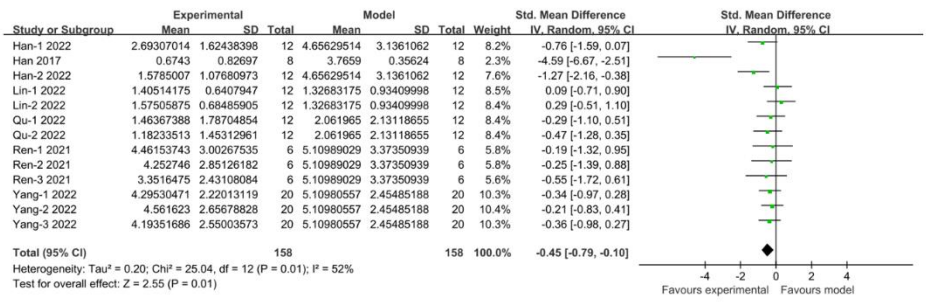


Fig. 3. Forest plots of the effect of AMK on body weight (A), diarrhea score(B)

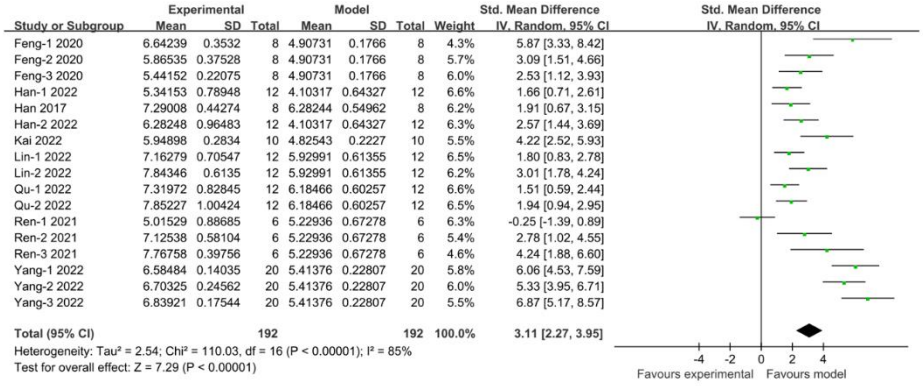
and DAI (C) in diarrhea animal group.

3.4.2. Immune-related indicators

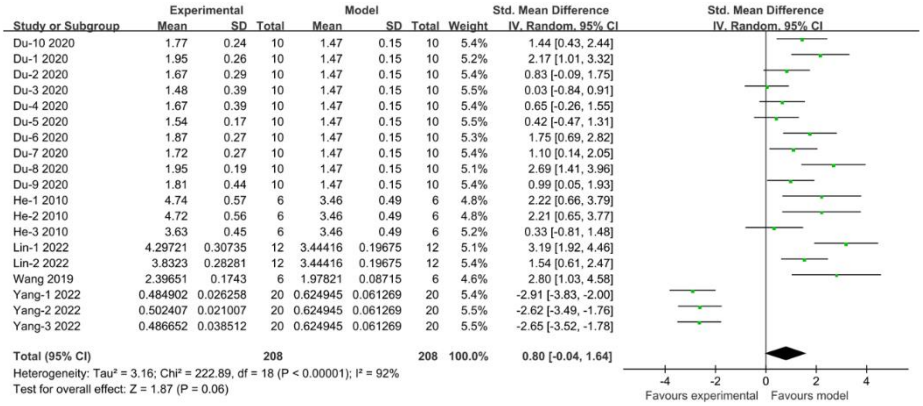
Immune-related indicators such as colon length, spleen index，histopathological score and SIgA was detected. Due to the significant heterogeneity, all immune-related indicators were analyzed by random effect model: colon length I^2=85%, spleen index I^2=92%, histopathological score I^2=94%, SIgA I^2=79%. (Fig. 4).

Colon length was reported in 8 studies, there are in total 192 administration animals and 192 model animals, compared with the model group, the colon length could be significantly elevated by AMK [SMD=3.11, 95% CI: 2.27 to 3.95, P<0.00001] (Fig. 4A). Spleen index was reported in 5 studies, there are in total 208 administration animals and 208 model animals, compared with the model group, the spleen index could be elevated by AMK [SMD=0.80, 95% CI: －0.04 to 1.64, P=0.06] (Fig. 4B). Histopathological score was reported in 5 studies, there are in total 128 administration animals and 128 model animals, compared with the model group, the histopathological score could be decreased by AMK [SMD=－8.22, 95% CI: －10.90 to －5.54, P<0.00001] (Fig. 4C). SIgA was reported in 2 studies, there are in total 30 administration animals and 30 model animals, compared with the model group, the SIgA could be significantly elevated by AMK [SMD=2.46, 95% CI: 0.78 to 4.14, P=0.004] (Fig. 4D).

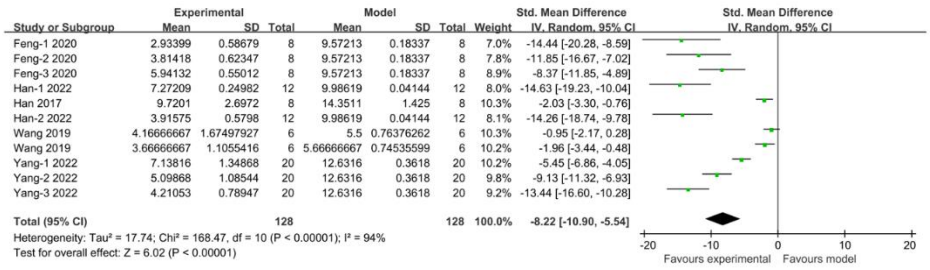
A



B

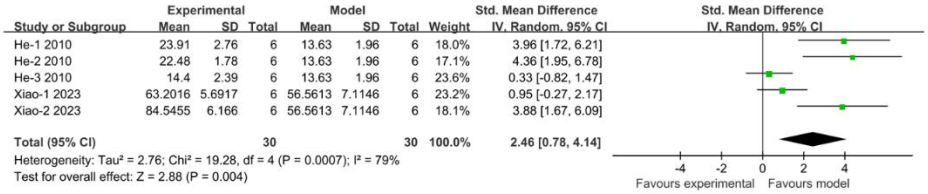


C



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D



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280 Fig. 4. Forest plots of the effect of AMK on colon length (A), spleen index (B),
281 histopathological score (C) and SIgA (D) in diarrhea animal group.

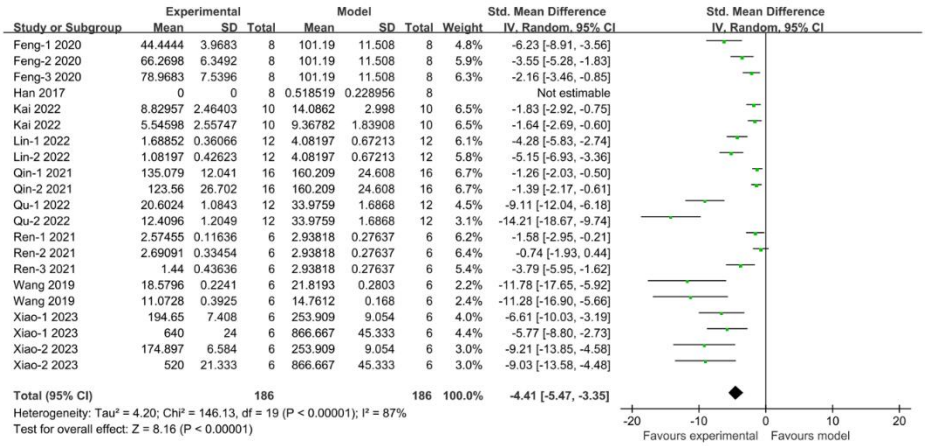
282 3.4.3. Pro-inflammatory factors

283 Due to the significantly heterogeneity, IL-1β, IL-6, IL-17 and TNF-α were
284 analyzed by random effect model: IL-1β I^2=87%, IL-6 I^2=90%, IL-17 I^2=83%, TNF-α
285 I^2=88%. (Fig. 5).

286 IL-1β was reported in 9 studies, there are in total 186 administration animals and
287 186 model animals, compared with the model group, the IL-1β could be significantly
288 decreased by AMK [SMD=－4.41, 95% CI: －5.47 to －3.35, P<0.00001] (Fig. 5A).
289 IL-6 was reported in 8 studies, there are in total 144 administration animals and 144
290 model animals, compared with the model group, the IL-6 could be significantly
291 decreased by AMK [SMD=－5.74, 95% CI: －7.27 to －4.21, P<0.0001] (Fig. 5B).
292 IL-17 was reported in 3 studies, there are in total 32 administration animals and 32
293 model animals, compared with the model group, the IL-17 could be decreased by AMK
294 [SMD=－6.97, 95% CI: －10.56 to －3.37, P=0.0001] (Fig. 5C). TNF-α was
295 reported in 3 studies, there are in total 318 administration animals and 318 model
296 animals, compared with the model group, the TNF-α could be decreased by AMK
297 [SMD=－3.40, 95% CI: －4.14 to －2.66, P<0.00001] (Fig. 5D).

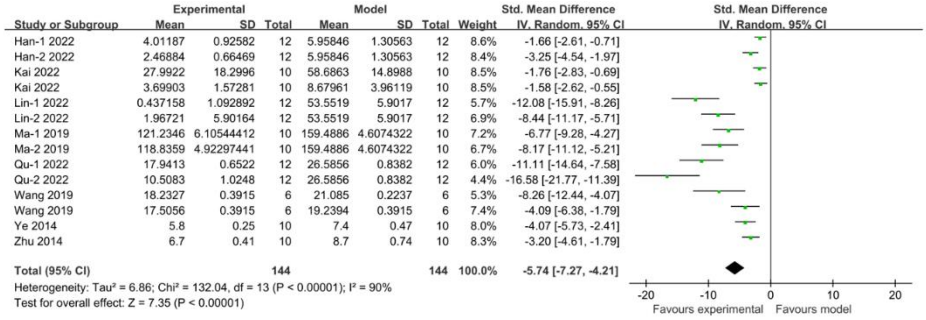
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A



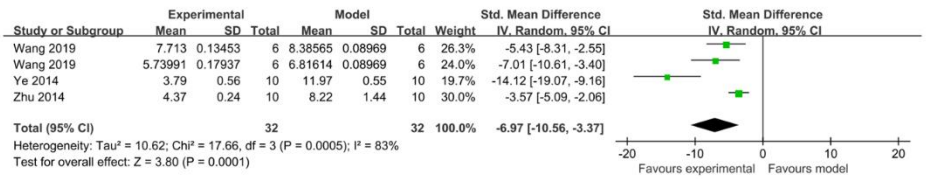
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B



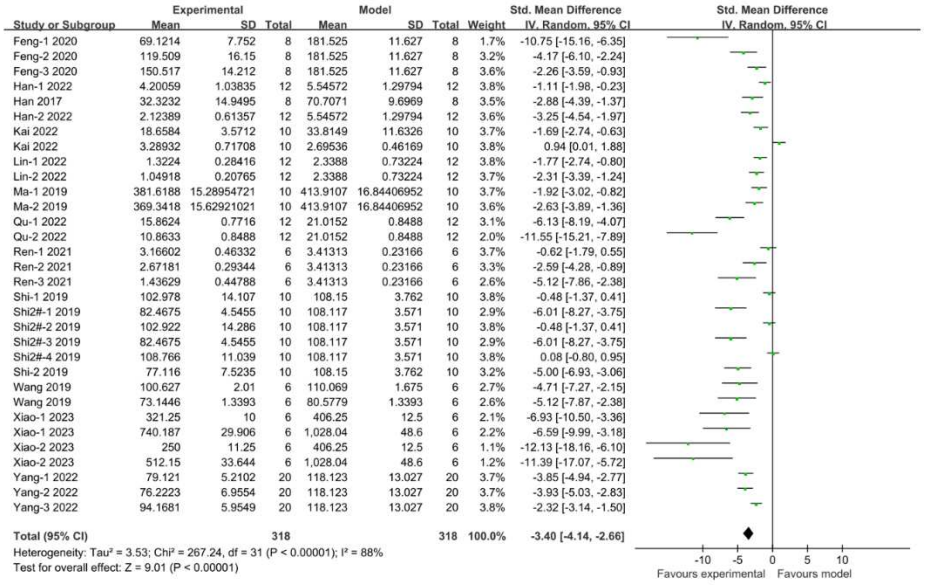
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C



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D



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Fig. 5. Forest plots of the effect of AMK on IL-1β (A), IL-6 (B), IL-17 (C) and TNF-α (D) in diarrhea animal group.

3.4.4. Anti-inflammatory factors

Due to the significantly heterogeneity, IL-10 was analyzed by random effect model: IL-10 I^2=81%. (Fig. 6).

IL-10 was reported in 4 studies, there are in total 94 administration animals and 94 model animals, compared with the model group, the IL-10 could be significantly elevated by AMK [SMD=1.19, 95% CI: 0.40 to 1.99, P=0.003] (Fig. 6).

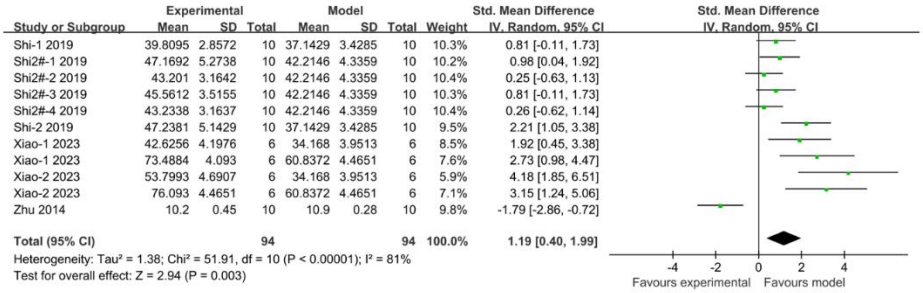


Fig. 6. Forest plot of the effect of AMK on IL-10 in diarrhea animal group.

3.4.5. MPO

No statistical heterogeneity was found in MPO in this meta-analysis, and the MPO was analyzed by fixed effect model: MPO I^2=0% (Fig. 7).

MPO was reported in 3 studies, there are in total 102 administration animals and 102 model animals, compared with the model group, the MPO could be significantly decreased by AMK [SMD=－2.06, 95% CI: －2.41 to －1.71, P<0.00001] (Fig. 7).

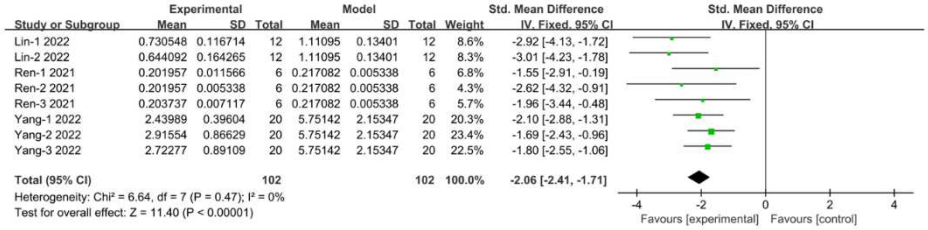


Fig. 7. Forest plot of the effect of AMK on MPO in diarrhea animal group.

3.5. Subgroup analyses

3.5.1. Grouped by AMK category

According to the category of AMK, the results were divided into four subgroups: PAMK, Atractylenolide I, Atractylenolide III and others.

Subgroup analyses of body weight were performed in 16 studies. Meta-analysis heterogeneity of body weight subgroup: PAMK I^2=41%, Atractylenolide I I^2=60%, Atractylenolide III I^2=2%, and others I^2=59%. The body weight could be significantly elevated by AMK in subgroups [SMD=0.46, 95% CI: 0.09 to 0.83, P=0.01; SMD=2.15,

95% CI: 1.14 to 3.15, P<0.0001; SMD=0.65, 95% CI: 0.36 to 0.94, P<0.0001]. Subgroup analyses of diarrhea score were performed in 5 studies. Meta-analysis heterogeneity of diarrhea score subgroup: PAMK I^2=0% and others I^2=36%. The diarrhea score could be significantly decreased by AMK in subgroups [SMD=－0.67, 95% CI: －1.10 to －0.23, P=0.003; SMD=－0.60, 95% CI: －0.95 to －0.24, P=0.001]. Subgroup analyses of DAI were performed in 6 studies. Meta-analysis heterogeneity of DAI subgroup: PAMK I^2=0%, Atractylenolide III I^2=0%, and others I^2=79%. The DAI could be significantly decreased by Atractylenolide III [SMD=－0.69, 95% CI: －1.14 to －0.24, P=0.003]; No significant differences in PAMK [SMD=－0.30, 95% CI: －0.66 to 0.06, P=0.10]; No significant differences in others AMK [SMD=－0.60, 95% CI: －1.52 to 0.32, P=0.20]. Subgroup analyses of colon length were performed in 8 studies. Meta-analysis heterogeneity of colon length subgroup: PAMK I^2=75%, Atractylenolide III I^2=79%, and others I^2=0%. The colon length could be significantly elevated by AMK in subgroups [SMD=4.79, 95% CI: 3.35 to 6.04, P<0.00001; SMD=2.01, 95% CI: 0.69 to 3.33, P=0.003; SMD=1.95, 95% CI: 1.48 to 2.42, P<0.00001]. Subgroup analyses of spleen index were performed in 5 studies. Meta-analysis heterogeneity of spleen index subgroup: PAMK I^2=0% and others I^2=62%. The spleen index could be significantly decreased by PAMK [SMD=－2.72, 95% CI: －3.23 to －2.21, P<0.00001]; The spleen index could be significantly elevated by others AMK [SMD=1.40, 95% CI: 0.96 to 1.84, P<0.00001]. Subgroup analyses of histopathological score were performed in 5 studies. Meta-analysis heterogeneity of histopathological score subgroup: PAMK I^2=84%, Atractylenolide III I^2=0%, and others I^2=0%. The histopathological score could be significantly decreased by AMK in subgroups [SMD=－10.01, 95% CI: －13.03 to －6.99, P<0.00001; SMD=－14.44, 95% CI: －17.65 to －11.23, P<0.00001; SMD=－1.59, 95% CI: －2.35 to －0.84, P<0.0001]. Subgroup analyses of IL-1β were performed in 9 studies. Meta-analysis heterogeneity of IL-1β subgroup: PAMK I^2=68%, Atractylenolide I I^2=0%, Atractylenolide III I^2=66%, and others I^2=73%. The IL-1β could be significantly decreased by AMK in subgroups [SMD=－2.67, 95% CI: －3.79 to －1.54, P<0.00001; SMD=－1.33, 95% CI: －1.87 to －0.78, P<0.00001; SMD=－1.80, 95% CI: －3.30 to －0.30, P=0.02; SMD=－7.99, 95% CI: －9.94 to －6.05, P<0.00001]. Subgroup analyses of IL-6 were performed in 8 studies. Meta-analysis heterogeneity of IL-6 subgroup: PAMK I^2=0%, Atractylenolide III I^2=74%, and others I^2=86%. The IL-6 could be significantly decreased by AMK in subgroups [SMD=－1.67, 95% CI: －2.41 to －0.93, P<0.0001; SMD=－2.40, 95% CI: －3.95 to －0.84, P=0.003; SMD=－7.79, 95% CI: －9.96 to －5.61, P<0.00001]. Subgroup analyses of TNF-α were performed in 3 studies. Meta-analysis heterogeneity of TNF-α subgroup: PAMK I^2=91%, Atractylenolide III I^2=77%, and others I^2=89%. The TNF-α could be significantly decreased by AMK in subgroups [SMD=－2.95, 95% CI: －4.42 to －1.48, P<0.0001; SMD=－2.24, 95% CI: －3.53 to －0.94, P=0.007; SMD=－4.10, 95% CI: －5.21 to －2.99, P<0.00001]. Subgroup analyses of MPO were performed in 3 studies. Meta-analysis heterogeneity of MPO subgroup: PAMK I^2=0%,

Atractylenolide III I^2=0%, and others I^2=0%. The MPO could be significantly decreased by AMK in subgroups [SMD=－1.85, 95% CI: －2.29 to －1.42, P<0.00001; SMD=－1.96, 95% CI: －2.83 to －1.10, P<0.00001; SMD=－2.96, 95% CI: －3.82 to －2.10, P<0.00001].

3.5.2. Grouped by animal species

According to animal species, the results were divided into two subgroups: rats and mouse.

Subgroup analyses of body weight were performed in 16 studies. Meta-analysis heterogeneity of body weight subgroup: rats I^2=55%, mouse I^2=61%. The body weight could be significantly elevated by AMK in subgroups [SMD=0.43, 95% CI: 0.01 to 0.85, P=0.04; SMD=0.75, 95% CI: 0.48 to 1.02, P<0.00001]. Subgroup analyses of diarrhea score were performed in 5 studies. Meta-analysis heterogeneity of diarrhea score subgroup: rats I^2=45%, mouse I^2=0%. The diarrhea score could be significantly decreased by AMK in subgroups [SMD=－0.43, 95% CI: －1.01 to －0.25, P=0.001; SMD=－0.62, 95% CI: －1.03 to －0.21, P=0.003]. Subgroup analyses of spleen index were performed in 5 studies. Meta-analysis heterogeneity of spleen index subgroup: rats I^2=63%, mouse I^2=93%. The spleen index could be significantly elevated by AMK in rats subgroup [SMD=1.49, 95% CI: 0.15 to 2.83, P=0.03]; No significant differences in mouse subgroup [SMD=0.67, 95% CI: －0.27 to 1.16, P=0.16]. Subgroup analyses of IL-1β were performed in 9 studies. Meta-analysis heterogeneity of IL-1β subgroup: rats I^2=0%, mouse I^2=87%. The IL-1β could be significantly decreased by AMK in subgroups [SMD=－7.12, 95% CI: －8.98 to －5.26, P<0.00001; SMD=－3.81, 95% CI: －4.88 to －2.74, P<0.00001]. Subgroup analyses of IL-6 were performed in 8 studies. Meta-analysis heterogeneity of IL-6 subgroup: rats I^2=76%, mouse I^2=92%. The IL-6 could be significantly decreased by AMK in subgroups [SMD=－5.25, 95% CI: －7.31 to －3.19, P<0.00001; SMD=－6.00, 95% CI: －7.95 to －4.05, P<0.00001]. Subgroup analyses of IL-17 were performed in 3 studies. Meta-analysis heterogeneity of IL-17 subgroup: rats I^2=94%, mouse I^2=0%. The IL-17 could be significantly decreased by AMK in mouse subgroup [SMD=－6.05, 95% CI: －8.30 to 3.79, P<0.00001]; No significant differences in rats subgroup [SMD=－8.57, 95% CI: －18.89 to 1.75, P=0.10]. Subgroup analyses of TNF-α were performed in 3 studies. Meta-analysis heterogeneity of TNF-α subgroup: rats I^2=90%, mouse I^2=87%. The TNF-α could be significantly decreased by AMK in mouse subgroup [SMD=－3.90, 95% CI: －5.35 to 2.44, P<0.00001; SMD=－3.21, 95% CI: －4.06 to －2.66, P<0.00001].

Table 2 Subgroup analysis based on (A) AMK categories and (B) species of animals.

A

Subgroups / Outcomes	PAMK				Atractylenolide I				Atractylenolide III				Other				Subgroups Differences
	SMD/MD	95% CI	P	I^2	SMD/MD	95% CI	P	I^2	SMD/MD	95% CI	P	I^2	SMD/MD	95% CI	P	I^2	P
Body weight	0.46	[0.09,0.83]	0.01	41%	2.15	[1.14,3.15]	<0.0001	60%	0.62	[0.17,1.08]	0.007	2%	0.65	[0.36,0.94]	<0.0001	59%	0.02
Diarrhea score	-0.67	[-1.10,-0.23]	0.003	0%	-	-	-	-	-	-	-	-	-0.60	[-0.95,-0.24]	0.001	36%	0.81
DAI	-0.30	[-0.66,0.06]	0.10	0%	-	-	-	-	-0.69	[-1.14,-0.24]	0.003	0%	-0.60	[-1.52,0.32]	0.20	79%	0.40
Colon length	4.79	[3.55,6.04]	<0.00001	75%	-	-	-	-	2.01	[0.69,3.33]	0.003	79%	1.95	[1.48,2.42]	<0.00001	0%	0.0001
Spleen index	-2.72	[-3.23,-2.21]	<0.00001	0%	-	-	-	-	-	-	-	-	1.40	[0.96,1.84]	<0.00001	62%	<0.00001
Histopathological score	-10.01	[-13.03,-6.99]	<0.00001	84%	-	-	-	-	-14.44	[-17.65,-11.23]	<0.00001	0%	-1.59	[-2.35,-0.84]	<0.0001	0%	<0.00001
IL-1 β	-2.67	[-3.79，-1.54]	<0.00001	68%	-1.33	[-1.87，-0.78]	<0.00001	0%	-1.80	[-3.30，-0.30]	0.02	66%	-7.99	[-9.94，-6.05]	<0.00001	73%	<0.00001
IL-6	-1.67	[-2.41,-0.93]	<0.0001	0%	-	-	-	-	-2.40	[-3.95.-0.84]	0.003	74%	-7.79	[-9.96,-5.61]	<0.00001	86%	<0.00001
TNF- α	-2.95	[-4.42,-1.48]	<0.0001	91%	-	-	-	-	-2.24	[-3.53,-0.94]	0.0007	77%	-4.10	[-5.21,-2.99]	<0.00001	89%	0.09
MPO	-1.85	[-2.29,-1.42]	<0.00001	0%	-	-	-	-	-1.96	[-2.83,-1.10]	<0.00001	0%	-2.96	[-3.82,-2.10]	<0.00001	0%	0.08

409

B

Subgroups	Rats				Mouse				Subgroups Differences
Outcomes	SMD/MD	95% CI	P	I^2	SMD/MD	95% CI	P	I^2	P
Body weight	0.43	[0.01,0.85]	0.04	55%	0.75	[0.48,1.02]	<0.00001	61%	0.21
Diarrhea score	-0.63	[-1.01,-0.25]	0.001	45%	-0.62	[-1.03,-0.21]	0.003	0%	0.98
Spleen index	1.49	[0.15,2.83]	0.03	63%	0.67	[-0.27,1.16]	0.16	93%	0.32
IL-1β	-7.12	[-8.98,-5.26]	<0.00001	0%	-3.81	[-4.88,-2.74]	<0.00001	87%	0.003
IL-6	-5.25	[-7.31,-3.19]	<0.00001	76%	-6.00	[-7.95,-4.05]	<0.00001	92%	<0.00001
IL-17	-8.57	[-18.89,1.75]	0.10	94%	-6.05	[-8.30,-3.79]	<0.00001	0%	0.64
TNF-α	-3.90	[-5.35,-2.44]	<0.00001	90%	-3.21	[-4.06,-2.66]	<0.00001	87%	0.42

410

411 **3.6. Sensitivity analysis**

412 After individually excluding studies, we identified Yang[19] as the source of
413 heterogeneity. After excluding this study, the results showed statistical significance,
414 with a significant heterogeneity of the spleen index (I^2 = 62%). The results
415 demonstrated statistical significance[SMD=1.40, 95% CI: 0.96 to 1.84, P＜0.00001].

416 Additionally, after individually excluding studies, we observed no significant
417 differences in other results showing significant heterogeneity. However, the source of
418 this heterogeneity remains unclear.

419 **3.7. Publication bias**

420 Publication bias was observed in colon length, spleen index, histopathological
421 score, IL-1β, IL-6, and TNF-α. The funnel plot, used to assess the risk of bias, exhibited
422 asymmetry (Fig. 8).

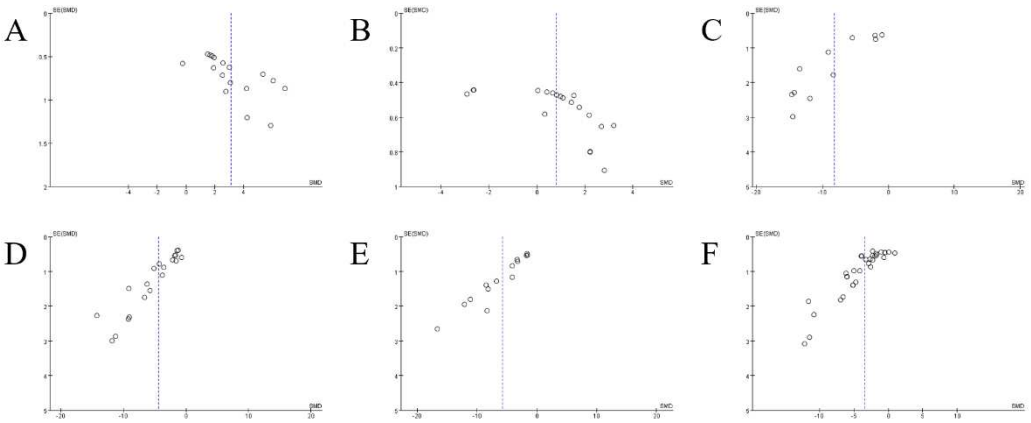


Fig. 8. Funnel plots (A) colon length (B) spleen index (C) histopathological score (D) IL-1 β (E) IL-6 (F) TNF- α .

4.Discussion

Article advantages. This article tackles the specific clinical query concerning the efficacy of AMK in diarrhea treatment by employing a rigorous and standardized literature search methodology. Adhering to consistent inclusion criteria, we meticulously selected pertinent studies and conducted a comprehensive and critical review of the cited literature. Stringent criteria were applied during literature selection to uphold the objectivity of the acquired data. Employing meta-analysis, we performed quantitative assessments of specific data, ensuring the credibility and dependability of our findings. In the era of evidence-based medicine, systematic reviews and meta-analyses serve as sources of objective, high-quality evidence to inform clinical decision-making. Our study meticulously screened and incorporated literature that met our predefined criteria, thereby upholding reference quality while quantifying data, summarizing underlying mechanisms, and synthesizing conclusions. In doing so, we present objective evidence for the prospective advancement of this research.

Article limitations. The quality of the included randomized controlled trials in this study is generally low, as indicated by the assessment conducted using the SYRCLE bias risk tool. This raises concerns about the authenticity of the findings. Insufficient high-quality research hampers data generalization and the evaluation of explicit correlations. Additionally, many animal research studies simply mention being "randomized" without providing specific details about the randomization method, particularly its implementation and the use of blinding procedures. Proper implementation of blinding is crucial during the intensive and measurement stages of animal studies to minimize biases associated with performance and testing, thus enhancing result reliability. Therefore, further investigation in this area is warranted to address these concerns. Furthermore, substantial heterogeneity exists among the studies due to variations in animal species and administration routes. To deepen our understanding in this field, it is recommended to include safety or toxicity assessments and carefully consider the limitations of the experimental approach.

Subgroup analysis. We conducted a subgroup analysis to investigate the sources of heterogeneity based on the treatment of AMK and animal species, including body weight, diarrhea score, DAI, colon length, spleen index, histopathological score, IL-1β, IL-6, IL-17, TNF-α, and MPO. AMK was classified into four subgroups based on its category: PAMK, Atractylenolide I, Atractylenolide III, and others. The observed heterogeneity varied across these subgroups, suggesting that different types of AMK may contribute to the heterogeneity. In some subgroups, heterogeneity with an I^2 value of less than 50% may be attributed to bias arising from small sample sizes (Table 2A). Animals were divided into two subgroups based on their species: rats and mice. The results showed a reduction in IL-1β levels from 87% (Fig. 5A) to 0% (Table 2B) in the rats subgroup, while the IL-17 levels in the mouse subgroup decreased from 83% (Fig. 5C) to 0% (Table 2B). However, the limited sample size prevented us from confirming these findings. Furthermore, statistically significant variations were observed in body weight, colon length, spleen index, histopathological score, IL-1β, and IL-6 among the

subgroups categorized by different types of AMK. Similarly, significant differences were noted in IL-1β and IL-6 across the subgroups classified by different animal species. Hence, we propose that the inclusion of distinct AMK types and animal species in our study may contribute.

Sensitivity Analysis. After excluding the study conducted by Yang [19], we observed heterogeneity in the spleen index during this meta-analysis. Upon thorough review and comparison with other studies analyzing the spleen index, we found a notable distinction between Yang's study and the others, primarily regarding the age of the mice used. Yang's study utilized 10-week-old mice, whereas the majority of other studies predominantly employed 3-8-week-old rats or mice. Consequently, the variation in animal age can account for the heterogeneity observed in the results.

Mechanisms. The potential mechanisms underlying the protective effects of AMK in the treatment of diarrhea can be summarized as follows(Fig. 9): (1) Immunoregulation: To establish a stable intestinal immune state, it is crucial to enhance the regulatory functions of immune cells, particularly Th cells, to ensure a balanced immune system[27]. AMK has the potential to enhance the effectiveness of diarrhea treatment by modulating cytokine secretion and expression [24], regulating the response of Th cells [26], activating the AMPK/SIRT1/PGC-1 α signaling pathway[18] to improve colon length and histopathological score, elevating spleen index, and enhancing SIgA levels. (2) Anti-inflammation: Excessive production and abnormal activation of inflammatory factors can result in an inflammatory response in the intestinal mucosa, leading to impaired barrier function and an imbalance in fluid secretion and absorption, ultimately causing diarrhea[28]. AMK has the potential to alleviate intestinal mucosal inflammation by inhibiting the activation of Drp1 and NLRP3 inflammasomes[17], suppressing NF-κB and STAT3 signaling[19,25], modulating the MAPK/NF-κB pathway[22], activating PPARα[23], reducing the secretion of pro-inflammatory factors such as IL-1β, IL-6, IL-17, and TNF-α, and enhancing the secretion of IL-10. (3) Anti-oxidation: Antioxidation is a factor that helps prevent the worsening of diarrhea[29]. Oxidative stress disrupts the body's oxidation-reduction balance, leading to the production and accumulation of numerous reactive oxygen species[30]. These reactive oxygen species can damage intestinal mucosal cells and related structures, promote inflammation, and worsen diarrhea[31]. AMK could improve diarrhea aggravated by oxidative stress factors such as MDA, GSH and SOD by regulating oxidative stress through the FPR1 and Nrf2 pathways[20] and enhancing antioxidative activities through HO-1 induction[25]. (4) Intestinal flora regulation: A homeostatic intestinal flora promotes nutrient absorption, resists pathogenic microorganisms, and maintains immune balance[32]. Imbalances in the intestinal flora can cause intestinal mucosal damage, increased inflammation, and contribute to the occurrence and worsening of diarrhea[33]. AMK restores intestinal flora balance by increasing beneficial bacteria abundance and suppressing harmful bacteria growth[16,20].

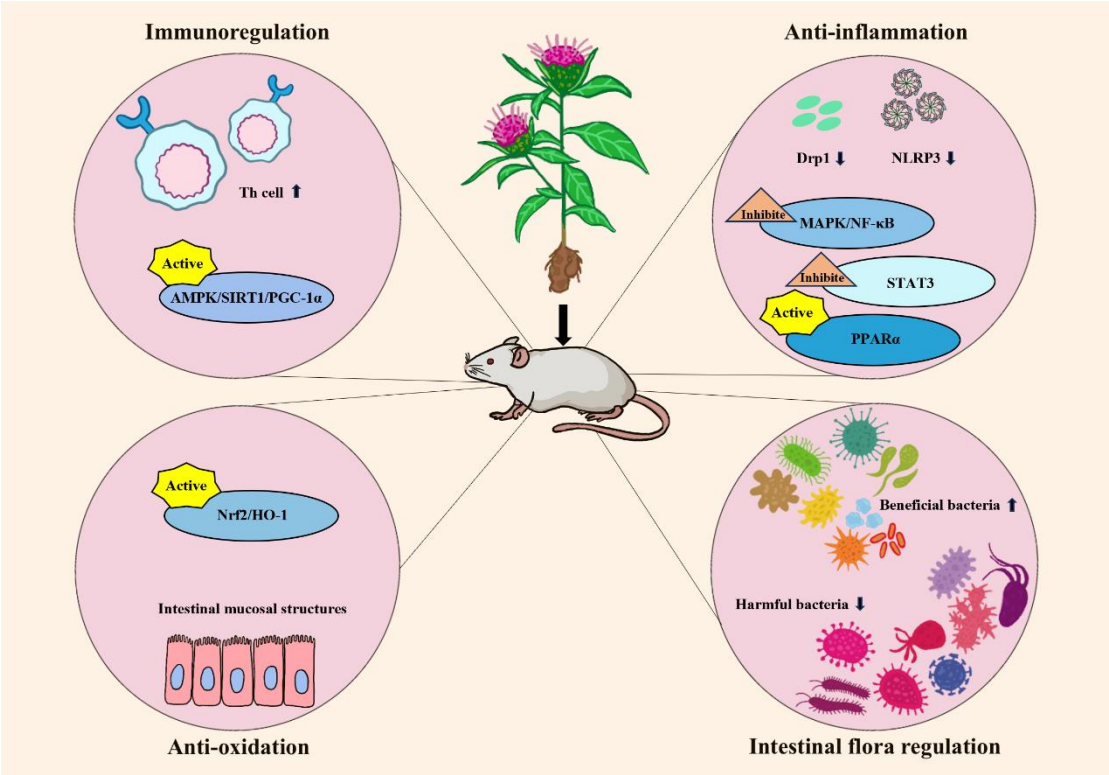


Fig. 9. The schematic diagram of the mechanism of action of AMK in the treatment of diarrhea.

5. Conclusion

In conclusion, our systematic review and meta-analysis indicate that AMK has the potential to alleviate diarrhea through mechanisms such as immunoregulation, anti-inflammation, anti-oxidation, and regulation of intestinal flora. This evidence-based research provides a foundation for the future application of AMK in the treatment of diarrhea in clinical settings.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

Pancen Ran: Conceptualization, Methodology, Visualization, Writing-Original Draft. Fengxian Jiang: Formal analysis, Investigation, Writing-review & editing. Jingjiang Lai: Investigation, Validation. Yang Shu: Software, Data Curation. Jing Zhao: Investigation, Writing-review & editing. Zhizhao Zhang: Software, Data Curation. Fangyan Hu: Investigation, Validation. Juqiong Wang: Term, Validation, Writing-review & editing. Jian Wei: Conceptualization, Validation, Writing-review & editing. Guobin Fu: Supervision, Project administration, Funding acquisition, Writing-review & editing. All authors read and approved the final manuscript.

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Not applicable.

559 **Abbreviations**

Abbreviation	Stands for
AMK	Atractylodes macrocephala Koidz.
PAMK	polysaccharide from Atractylodes macrocephala Koidz.
AMP	Atractylodes macrocephala polysaccharide
EAR	ethanolic extract of rice water-fried Atractylodis Rhizoma
EEAR	ethanolic extract of Atractylodis Rhizoma
EEDAR	ethanolic extract of deep-fried Atractylodis Rhizoma
DSS	dextran sulfate sodium
AOM	azoxymethane
TNBS	trinitrobenzene sulfonic acid
AMO	Atractylodes macrocephala essential oil
SD	Sprague Dawley
IL-1 β	Interleukin-1 β
IL-6	Interleukin -6
IL-10	Interleukin -10
IL-17	Interleukin -17
TNF- α	Tumor necrosis factor alpha
MPO	myeloperoxidase
SIgA	secretory immunoglobulin A

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