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The role of Ozone therapy in improving cognitive function in animal models of cognitive impairment: a systematic review

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SUMMARY: *Cognitive dysfunction icn mildly retarded, on other muddledness that affect Affects cognitive ability. With the increase of life expectancy worldwide, mainstream consciousness trends dysfunction now it's growing, placing burdens on patients, families and society, making the search for methods to intervention before disease occurs cognitive dysfunction a topic of great interest. Current treatments have limited efficacy and accessibility. Recently, medical ozone clinical benefits are significant a safe, be becoming to and effective treatment for many conditions. Prior studies indicate that this form of therapy may affect cognitive function by it inhibits free radical damage, programmed cell death and astrocyte activation, promotes hippocampal neurogenesis and acetylcholine synaptic remodeling, supported by in vivo evidence. However, there is a lack of systematic evaluation and meta-analyses. The review identified 749 citations, of which, 3 studies were included. Build the first model evaluation inspect known to our role on Ozone treat in improving cognitive function in animal models of cognitive impairment. This study integrates the current evidence have sth. to do with potential the role ozone therapy in enhancing cognitive function, and provides inspiration and support for future research directions.*

KEYWORDS: *cognitive function; neurological diseases; dementia; ozone; animal models*

1 Introduction

Cognitive dysfunction encompasses mild cognitive impairment, dementia, and other cognitive dysfunctional disorders affecting higher cognitive functions such as reasoning, memory, and judgement. As the life expectancy of the global population increases, the number of people with cognitive dysfunction also increases, placing a burden not only on the individual but also on their families and society [1]. Current pharmacological and non-pharmacological treatments have limited efficacy and accessibility, with there being no treatments being widely utilized in clinical practice to improve cognitive function. Ozone is a safe treatment, economical in effective remedy method that is currently in terms of treatment options manage art range of diseases, from slow and infectious diseases to vascular and orthopedic issues, as well as skin lesions and dentistry, yielding excellent results [2-7]. Previous experiments have

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indicated ozone could potentially have protective and therapeutic effects on peripheral nerves [8-11]. There is also evidence suggesting that ozone may also modulate neurodegenerative changes during aging [12, 13]. Prior research have demonstrated that ozone may impact cognitive function through various pathways, such as reducing oxidative stress, apoptosis, and inflammatory responses [14-17]. Discuss perceptions of ozone therapy function, it may be useful to study its effect on mice models.

A systematic comment on PROSPERO (CRD42023389148) and start with the first plan Reporting Items for Systematic Reviews reach Meta-Analyses direction of guidance [18].

2 Methods

2.1 Search strategy

Database Conduct a search PubMed (MEDLINE), EMBASE, Scopus of Web from a scientific point of view January 2023. Keywords included: “trioxygen”, “ozone”, “cognition”, “cognitive disorder”, “Cognitive Dysfunction”, “cognitive impairment”, “dementia”, “Alzheimer disease”, “neurocognitive”, “cognitive decline”, “cognitive performance”. For specific search terms, see supplementary material Online Resource 1. In addition to database retrieval, a "snowballing" use methods to identify relevant articles from each included article's reference section.

2.2 Study selection

After removing duplicate records, screening was completed independently by two reviewers (LS and LW). Titles, abstracts and full expression screened in order to eligibility. Reach a consensus through consultation and discussion, or consult senior researchers to resolve disputes (KXY).

If the study meets the following criteria, it will be judged to meet the requirements:(1) controlled studies with a separate control group; (2) used healthy animals without any neurological/genetic defects or animals with induced neurological/genetic defects to model cognitive impairment; and (3) assessed outcome measures including behavioral tests, pathological changes or related mechanisms changes.

Studies were excluded if they: (1) were Summary of the results of the meeting, diagnosis and treatment implementation plan, review studies, cultural and political commentary, letters, or book chapters; (2) were in vitro studies; (3) did not include ozone therapy treatment; or (4) the report of the results of the study was incomplete.

2.3 Data extraction

LS as well as LW using the data extraction template pre-set in Microsoft Excel spreadsheet, each researcher carried out the data extraction and integration work separately. The detailed information extracted included:

Key attributes of the study included: title, first author's name, publication date, geographic region of study, cohort naming, length of study period, average or median follow-up duration, and definition and acquisition of outcome measures.

Data extracted included:

(1) Research characteristics: First author, title, journal of publication, year and country of publication, study design, animals species, and sample size.

(2) Experimental details: ozone generation, model building, successful modelling tests, administration route, behavioral tests, other assessments, statistical software and P-value.

(3) Grouping and administration of drugs.

Correspondin authors were contacted for missing information. Resolve differences properly through communication or by consulting experienced researchers (KXY). As a relatively new field with high methodological heterogeneity and a small sample, no meta-analysis was planned. Eligible articles were grouped and synthesized based on the results. EndNote was used for reference management.

For quantitative coding, ozone dose, behavioral improvement and methodological quality were standardized using the following equations:

$$D_i = (C_i \times V_i)/m_i \tag{1}$$

$$R_{\mathrm{PFT},i} = \frac{T_{\mathrm{model},i} - T_{\mathrm{ozone},i}}{T_{\mathrm{model},i}} \times 100\% \tag{2}$$

$$Q_i = \Sigma_{k=1}^{10} I_i k, I_i k \in 0{,}1 \tag{3}$$

In Eq. (1), D_i denotes ozone dose per unit body mass, C_i denotes ozone concentration, V_i denotes administration volume, and m_i denotes animal body mass. Eq. (2) records the relative reduction in platform-finding time when lower PFT indicates better spatial learning performance. Eq. (3) defines the CAMARADES SyRF score from ten binary reporting and design items.

2.4 Quality assessment

LS as well as LWTthe Comarade system evaluation tool (SyRF) was used to complete the quality assessment of each included study in an independent manner [19, 20] by judging product quality an article by ten ratings: (1) peer reviewed; (2) temperature contorl; (3) After randomization, the subjects were divided into the treatment group and the control group respectively; (4) the study was conducted with a blind design ischaemia; (5) Conduct restrictive analysis based on the results; (6) Anesthetics were selected to exclude those with significant neuroprotective effects; (7) The study model was constructed using aged, diabetic, or hypertensive animals; (8) Scientific calculations for sample size were completed; (9) Strict adherence to animal welfare regulations; (10) Disclosure statements for potential conflicts of interest must be provided. rated as good (7-10 points), fair (4-6 points) or poor quality (1-3 points).

3 Results

3.1 Search results

The PRISMA flow diagram is presented in Figure 1. Database searches yielded 749 citations, with no additional studies from manual searches. Database results included Scopus (n=350), Web of Science (n =264), PubMed (n = 118), Cochrane (n = 11) and Embase (n = 6). Remove duplicate content, 479 Screen the title and abstract of the study .yielding 24 potentially eligible studies. The total is 21reason the article was excluded is case report (n = 1), review (n = 4), or irrelevance (n = 16). 3 articles were determined to be eligible for the study.

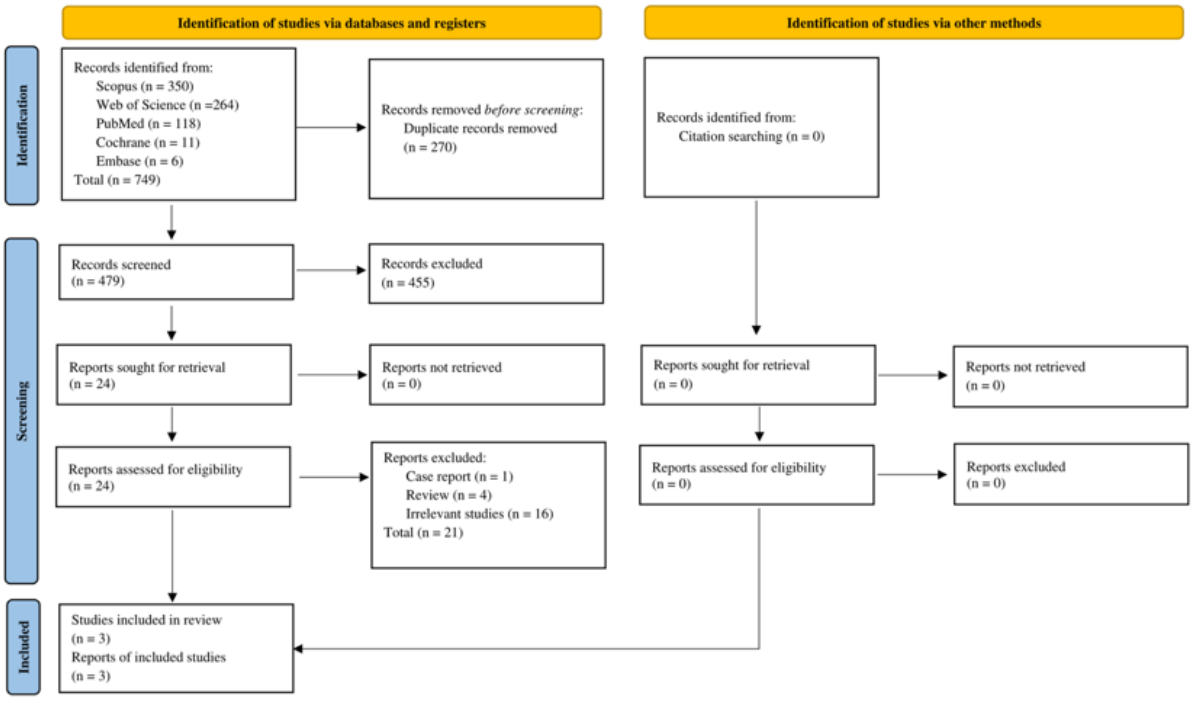

Figure 1: A flowchart summary of the systematic review literature search process

3.2 Study characteristics

Included studies were published between in English between 2018-2022. Subjects were rats (n=64) [21], rats (n=55) [22] and mice (n=60) [23]. Models included were: right carotid artery slik litigation and hypoxic treatment for 2h (n=48), Experimental mice with APP and PS1 double transgene characteristics (n=33); and sleep deprivation (SD) 16h/day for 4 weeks (n=36). In Lin et al., APP/PS1 mice develop cognitive impairment after 5 months of age. A detailed summary of the characteristics of the study is presented in Table 1; experimental details are summarized in Table 2 and Table 3. General information about experimental animals. All three papers used male experimental animals. Regarding age, two papers used 5-month-old experimental animals, and the other used neonatal rats that were 7 days old. The two papers that used rats employ Sprague-Dawley rats and Wistar rats, respectively, and the papers that employ mice used Experimental mice with APP and PS1 double transgene characteristics.

Ozone was generated OZOMED Basic (Kastner-Praxisbedarf-GmbH, Rastatt, Germany) for ozone production, or the EVOZONE basic Plus (Reutlingen, Germany). The concentration of ozone and the administered dose also varied. In Resitoglu et al., a concentration indicated 25 μg/mL was used and a dose indicated 1 mg/kg in the ozone 1 group or 2 mg/kg in the ozone 2 group was administered. In Lin SY et al., a concentration of 30 μg/mL or 50 μg/mL was used and a dose of 10 ml/kg, which is 0.3 mg/kg or 0.5 mg/kg. In Yan YN et al., the concentration used was 30 μg/mL and the dose administered was 1.1 mg/kg. The duration of administration also varied, with one study being a one-time administration and the other two studies being long-term administrations lasting more than 20 days.

Morris water maze (MWM) was used to confirm successful models in all studies. Resitoglu et al. used a single injection drug method, while the other two studies used once-daily administration for several consecutive days, and one for 24 consecutive days and one for 28 consecutive days.

Table 1: Study characteristics.

First author	Title	Journal	Year	Study Design	Animals Species	Sample size (n)	Country
Resitoglu, B.	The efficacy of ozone therapy in neonatal rats with hypoxic ischemic brain injury.	Bratislava Medical Journal	2018	Independent controlled studies	rats	64	Turkey
Lin, S. Y.	Ozone Inhibits APP/Aβ Production and Improves Cognition in an APP/PS1 Transgenic Mouse Model.	Neuroscience	2019	Independent controlled studies	mice	55	China
Yan, Y. N.	Intraperitoneal ozone injection prevents REM sleep deprivation-induced spatial learning and memory deficits by suppressing the expression of Sema3A in the hippocampus in rats.	Iran J Basic Med Sci	2022	Independent controlled studies	rats	60	China

Table 2: Experimental details.

First author, year	Ozone generation	Model building	Successful modeling tests	Administration Route	Behavioral tests	other tests	Statistical software	P-value
Resitoglu, B. 2018	EVOZONE basicPlus (Reutlingen, Germany)	HIBI model	MWM	Administered intraperitoneally	MWM	/	SPSS v.11.5	<0.05
Lin, S. Y. 2019	Ozone therapy device (OZOMED Basic; Kastner-Praxisbedarf-GmbH,Rastatt, Germany)	AD model	MWM	Administered intraperitoneally	MWM Open field test	Western blot Histopathological examination	SPSS23.0	<0.05
Yan, Y. N. 2022	Ozone therapy device (OZOMED Basic; Kastner-Praxisbedarf-GmbH,Rastatt, Germany)	REM Sleep deprivation model	MWM	Administered intraperitoneally	MWM Open field test	Western blot	GraphPad 8.0.1	<0.05

MWM: morris water maze; HIBI: hypoxic ischemic brain injury; AD: Alzheimer's disease; REM: rapid eye movement.

Table 3: Grouping and administration of drugs

First author, year	Total numbers (numbers in each group)	Group	Drug dose	Frequency& Duration	Ozone Concentration
Resitoglu, B. 2018	64(16)	Sham group	/	Once	/
		Control group	0.4 mL of saline		/
		Ozone 1 group	1ug/g ozone		25 μg/mL
		Ozone 2 group	2ug/g ozone		25 μg/mL
Lin, S. Y.2019	33(11)	Control group	Without ozone injection	Once a day & 24 consecutive days	/
		Ozone 1 group	0.3ug/g ozone		30 μg/mL
		Ozone 2 group	0.5ug/g ozone		50 μg/mL
	22(11)	WT control group	Without ozone injection		/
		WT ozone group	0.3ug/g ozone		30 μg/mL
Yan, Y. N. 2022	60(12)	Control group	/	Once a day & 28 consecutive days	/
		Wide platform group	/		/
		SD group	Saline		/
		Ozone group	1.1 ug/g		30 μg/mL
		Midazolam group	Midazolam 2 ug/g		/

WT:wild-type; SD: sleep deprivation.

3.3 Morris water maze

First, MWM confirmed no significant group differences at baseline in all models and studies.

Next, MWM confirmed the validity of the modeling. Animals that underwent modeling had statistically significantly longer platform finding times (PFTs) than non-modeled animals, demonstrating cognitive deficits in the modeled animals. The conclusions of the three studies reveal respective modeling made the experimental animals cognitively dysfunctional. In Resitoglu et al., modeled animals performed worse on all test days (first day: $p = 0.313$; Page 2: $p = 0.014$; day 3: $p = 0.037$; day 4: $p = 0.042$). In Lin SY et al., model building group had longer PFTs than the sham group in both tests after modeling (trial 2: $p < 0.01$; trial 3: $p < 0.01$). Yan et al. showed model building series had longer PFTs than control group in both tests after model building (trial 2: $p < 0.05$).

Finally, MWM confirmed that ozone has an ameliorative effect on cognitive dysfunction. In Resitoglu et al., the ozone 2 group performed better than the amodel building series on both day 2 and day 4 tests (day 2: $p = 0.428$ day 4: $p = 0.207$). In Lin SY et al., the ozone 2 group

had a shorter PFT than the model building group in the test (trial 3: $p < 0.01$). Yan et al. demonstrated that ozone 2 group had a shorter PFT than the model building group in the test (trial 3: $p < 0.05$). In addition, in Resitoglu et al., where multiple tests were performed, the ozone 2 group had a shorter PFT than the model building group (trial 3: $p < 0.05$). The PFT on day 4 was significantly shorter in the ozone 2 group than on day 1 ($p < 0.001$).

3.4 Neuropathological changes

Histopathological examination allows the observation of neuronal cell morphology in order to recognise the closely related Aβ senile plaque.In Resitoglu et al., The number of apoptotic neurons in the thalamic nuclei, hippocampus and parietal cortex was detected using the TUNEL method. The results showed that the number of apoptotic neurons in the right hemisphere the values of ozone group 1 and ozone group 2 were significantly lower than those of the control group ($p < 0.001$ and $p < 0.001$, respectively). The number of right hemisphere apoptotic neurons was Significantly lower than the latter ozone 2 group than in the ozone 1 group ($p < 0.001$). The number of apoptotic neurons in left hemisphere was significantly lower ozone concentration in ozone group 1 and ozone group 2 was significantly higher than that in the control group ($p = 0.025$ and $p = 0.001$, respectively).In Lin SY et al., The number of Ab plaques in APP/PS1 mice showed a downward trend as detected by immunohistochemistry.Consistent with this, concentrations of soluble Aβ1-40 and Aβ1-42 in brain tissue and peripheral blood were determined by an Elisa assay. Aβ1-42 was significantly reduced in the serum ozone-treated group compared with the AD group ($p = 0.015$). In Yan YN et al., Neuronal necrosis in the form of nuclear consolidation and partial lysis was clearly observed by hematoxylin and eosin staining in the model group, but no obvious neuronal cytoplasmic vacuolisation or irregular nuclei were found in the ozone-treated group.

3.5 Other results

In addition to MWM, other experimental and histological examinations were performed. Western blot and histopathological examinations were performed in the studies by Lin et al. and Yan et al. The results of Western blot revealed that proteins associated with cognitive impairment were reduced after ozone treatment, suggesting that ozone has an ameliorative effect on cognitive impairment at the protein level. The results of Western blot in the study of Lin et al. are as follows. Serum levels of Ab1-42 were significantly lower in the ozone 1 and ozone 2 groups compared to the control group (p=0.015; control-ozone 1: p =0.010; control-ozone 2: p =0.008). Compared with the wild-type (WT) group, the expression of APP protein in prefrontal cortex and hippocampus was significantly increased in the control group ($p < 0.001$); Dunnett's post-hoc test showed that the expression level of APP in prefrontal cortex was significantly reduced in transgenic mice after ozone administration ($p = 0.014$; control-ozone 1: $p = 0.012$; control-ozone 2: $p = 0.032$). Yan YN et al. showed the expression level of model construction group Sema3A was significantly higher than that of the control group ($p<0.05$). Sema3A is a gene that has it has been shown to be associated with cognitive impairment in this study. After ozone treatment the expression of Sema3A in the ozone group was significantly lower than the model building group ($p < 0.01$). Histopathologic examination showed the neuronal morphology and plaque area in the brains of the animals. In the results of both papers, the histopathological findings improved after treatment with ozone. Lin SY et al. found that the plaque area increased in the control group compared to the WT group ($p < 0.05$); and the plaque area decreased in both ozone 1 of ozone 2 groups compared to the control group ($p < 0.05$; 4; control-ozone 1: $p < 0.05$; control-ozone 2: $p < 0.05$). Yan YN et al. found the SD group showed degraded nuclei of neurons showed nodular lesions, some of which were dissolved and necrotic. In the hippocampus and prefrontal cortex tissues

of SD group, the number of glial cells surrounding neurons increased significantly.. However, the morphology of neurons in the ozone group was the same as that of the control group. These results suggest that ozone has an ameliorative effect on the changes brought about by cognitive impairment at the organoleptic level.

Yan YN et al. also performed RNA-sequencing and quantitative PCR. Quantitative PCR assayed gene expression was consistent with RNA-sequence results. The results were analyzed after gene ontology (GO) classification and functional enrichment, and Sema3A was found to be a cognitively relevant gene. The expression of Sema3A compared with the control group, it was significantly upregulated in the model construction group and significantly downregulated in the ozone group compared with the model building group.

3.6 Animal Welfare Aspects

All included studies were concerned with animal welfare issues and were approved by the ethics committee. Two of the studies provided information on access to mouse purchase, access to food and water, lighting conditions, of housing. Both studies used a 12-hour light/12-hour dark cycle (lights on at 7 a.m.), free water of mouse chow access, and housing in plastic cages. Lin SY et al. controlled the temperature at 22±2°C and provided humidity of 50±5%, while Yan YN et al. controlled temperature at 25°C.

3.7 Risk of bias

The quality of the studies included in the evaluation was assessed according to the SyRF standard, and the results showed a gradient difference from low to high. Two studies reached the good level and one study was at the general level. See Table 4 for details of the specific scoring rules.

Table 4: Quality characteristics of included studies.

First author, year	1	2	3	4	5	6	7	8	9	10	Score	Grade
Resitoglu, B. 2018	√	√	√	×	×	√	√	×	√	√	7	Good
Lin, S. Y.2019	√	√	√	×	×	×	√	×	√	√	6	Fair
Yan, Y. N. 2022	√	√	√	×	×	√	√	×	√	√	7	Good

4 Discussion

A variety of medications are available to treat cognitive impairment, including aducanumab, lecanemabi, fisetin, palmitoylethanolamine-oxazoline, rehmannioside A [24-29]. Dietary interventions such as walnuts and açai berry also offer [30, 31]. The FDA granted accelerated approval to two antibody-based drugs, aducanumab and lecanemab, in 2021 and 2023, respectively [32, 33]. However, the use of aducanumab has been controversial due to its limited coverage to only patients enrolled in clinical trials [34]. The recently approved lecanemab is the first Alzheimer's disease (AD) drug to gain full FDA approval in 20 years, but is currently highly overpriced. A previous meta-analysis found that antipsychotic medication was less effective than symptomatic treatment in relieving cognitive dysfunction, with no significant targeted medications [35]. Oxidative stress and neuroinflammatory responses are prominent links in the study of various dementia and cognitive dysfunction disorders.

Ozone exerts its neuroprotective effects by modulating the inflammatory response by stimulating the endogenous antioxidant system and improving blood flow to the brain, and

can antioxidant and inhibit inflammatory responses. In animal models of brain, liver, kidney and heart ischemia, ozone pretreatment has been shown prevent tissue damage caused by ischemia-reperfusion through other mechanisms such as inhibition of oxidative stress and inflammatory responses [36-40]. Ozone also exerts its resist inflammation and other the efficacy during the treatment processneocoronavirus [41].

Ozone therapy has been g accepted physicians in various fields since the twentieth century due to its safety, convenience, and cheapness [42]. The core mechanism of ozone therapy is activate the antioxidant reduction system through controlled lower levels of oxidative stress, which in turn reduces the overall level of oxidative stress[43]. Ozone is able to trigger signalling molecules through the regulatory activity Reactive oxygen species and so on activate antioxidant pathways at the cellular level.Ozone also has a regulatory effect on the immune system, which ultimately influences disease progression[44]. Regarding the safety of ozone therapy, it has not been studied in the papers produced in this search. Although ozone therapy has been used safely in the treatment of some diseases [45-47], further evidence is needed for its safety in the treatment of many diseases [48, 49]. Given that ozone was initially recognized as an environmentally harmful gas, its safety in medical applications is a common concern[50]. In Jacobs' 1982 paper, the incidence of adverse effects of whole-body medical triple-oxygen therapy was shown to be only 0.0007%, the main symptoms are nausea, headache and physical fatigue.[51]. Although there have been cases of death from direct intravenous injection of the gas, this method of use itself is not recommended. There have been a number of other complications reported, most of which were due to mishandling or there was no causal relationship between medical ozone injections and adverse events, and most of which disappeared within a few days without the need for special treatment[52-55]. High concentrations of ozone can cause damage to the organism, but regulated use of low concentration ozone therapy is relatively safe. Although regulated ozone therapy can also cause complications, most of these complications resolve on their own. As with other medical treatments used, ozone therapy may pose There are certain risks, specifically substantially reduced if the ozone therapist is well prepared both Theory and practice [56].

Animal models are considered the first step in exploration primary disease mechanisms and evaluating tSafety and effectiveness of treatment, and the ideal animal model needs to mimic as closely as possible the pathophysiological conditions of human cognitive impairment. Currently, there are various ways to create models of cognitive loss. This includes surgically, such as suturing the right carotid artery to create hypoxic ischemic brain injury models. This model has been widely used in previous studies for its ability to reduce cerebral blood flow, which in turn causes cerebral ischaemia and hypoxia as well as further complex pathophysiology such as oxidative stress and inflammation[57, 58]. Modelling can also be done by altering life circumstances, for example using a modified multiplatform to create a rapid eye movement sleep deprivation model. Sleep-deprived animals exhibit degree of oxidative stress increased, which led to the destruction of mitochondrial enzyme complex structure and function activity, increased acetylcholine esterase activity, and histopathological changes associated with hippocampal and thalamocortical regions of the brain[59-61]. Direct genetic modification is also one of the modelling approaches, such as the use of modified APP/PS1 double transgenic mice (AD mice). The APP/PS1 transgenic mouse model has been shown to be associated with core pathological processes in Alzheimer's disease, including amyloid beta plaque accumulation, neuronal loss, astrocyte activation and aberrant tau phosphorylation[62-64]. For the study of the potential mechanism of cognitive impairment, a variety of animal models have been used in related exploration work by simulating pathological states. Bilateral permanent occlusion of the common carotid artery is a well-

established method to study the effects of chronic cerebral underperfusion on cognitive dysfunction in rats, including White matter and hippocampus were damaged neurons [65]. At the end of the modeling process, its results should be verified, such as through administering MWM and open field test. After ozone treatment the effect of ozone treatment should be verified by behavioral measurements and histological monitoring.

Three studies were included in this review, all of which involved cognitive impairment animal models. Endpoint evaluation included MWM, open field test, western blot, and histopathological examination. Among them, MWM was used in all these studies. With more than 10,000 MWM-related publications in PubMed [66], and it being utilized in recent cognitive impairment studies [67-69], MWM is widely used as an important endpoint to determine the efficacy of drugs for cognitive impairment.

All three studies used MWM to determine the success of modeling. The final results all demonstrated the ability of ozone treatment to improving cognitive function in animal models with cognitive impairment. The results of the systematic evaluation based on the included studies showed that ozone treatment improved PFTs in MWM, reduced the expression of cognitive impairment-related genes and related proteins, and reduced plaque deposition in brain tissue. Therefore, ozone intervention in model animals with cognitive impairment has definite efficacy in terms of its ability to reduce neurological damage. However, the comparison of ROS levels and inflammatory molecules levels in experimental animals in vivo is lacking, and the beneficial effects of ozone are considered to be related to the activation of antioxidant and anti-inflammatory mechanisms in vivo.

CAMARADES SyRF was used in this paper to validate the quality of the articles. As with any research tool, CAMARADES SyRF may have some biases or limitations that may affect the reliability and interpretation of the results. Studies were rigorously screened for quality at the time of inclusion. When data synthesis was performed, more complete raw data were obtained and used as much as possible. In the interpretation of the results, differences between different study methods were considered and discussed as thoroughly as possible.

Limitations include the small number of included studies and incomplete data, making meta-analyses difficult to perform. This review compares the effects of ozone on cognitive deficits in rodents as assessed by water maze experiments and neuropathological experiments. The comparison is purely about presenting similarities and differences in change induced by ozone, as these studies show many differences in terms of species, models, age, and so on. Therefore, due to the nature of the methods and data from these studies, it was not possible to conduct a meta-analysis. Currently searched studies are also lacking in exploring the effects of long-term sustained treatment with ozone, as well as the effects on older and female animals. Further research is also needed on the effectiveness of treatment at different stages of cognitive dysfunction and on the effects of ozone pretreatment. Compared with in vitro studies, the number of studies on molecular mechanisms is significantly less ozone action the limitations of animal models make it difficult to achieve more scientific comparison of research data, and the lack of safety data further affects the integrity of the study outcomes, such as adverse events, in preclinical literature. Difference across studie s in models, method of ozone formulation and treatment regimens also affect reliability. More studies are needed to build evidence for ozone therapy in animal models of cognitive impairment. Currently searchable studies contain only rodents and lack studies on mammals.Despite the shortcomings of the research, this systematic review can still provide a macro perspective for related fields role of ozone therapy for cognitive impairment in animal models and to assess the methodological quality of research in this area. Future animal model studies better methodological reporting and more should be provided standardised reporting of results to improve their quality. As a field that still leaves a large gap, it is hoped that this review will

draw the attention of researchers and provide direction and a basis for further research.

In conclusion, current limited evidence suggests that ozone therapy may improve cognitive function in animal models, warranting further research. Safety and adverse effects should also be a focus in future studies. Thorough pleclinical evaluation of ozone therapy for cognitive impairment will facilitate its translation into clinical studies if the results continue to be promising, offering another possible treatment for cognitive dysfunction.

Supplementary materials

Table S1: Comprehensive search strategy employed in each database

Source	Search Strategy	Hits Retrieved
PubMed	(trioxygen OR ozone) AND (("Cognition Disorders"[MeSH Terms:noexp] OR "Cognitive Dysfunction"[MeSH Terms:noexp] OR "Dementia"[MeSH Terms:noexp] OR "Alzheimer Disease"[MeSH Terms] OR "dementia, vascular"[MeSH Terms] OR ("Cognitive Dysfunction"[Title/Abstract] OR "cognitive impairment*"[Title/Abstract] OR "cognitive disorder*"[Title/Abstract] OR "cognitive decline*"[Title/Abstract] OR "Dementia"[Title/Abstract] OR "alzheimer*"[Title/Abstract])disorder*"[Title/Abstract] OR "cognitive decline*"[Title/Abstract] OR "Dementia"[Title/Abstract] OR "alzheimer*"[Title/Abstract]))	118
Cochrane	(trioxygen OR ozone) AND (cognit* OR "Dementia" OR "alzheimer*")	11
Scopus	((trioxygen OR ozone) AND (cognit* OR "Dementia" OR "alzheimer*")	350
Embase	(TRIOXYGEN OR OZONE) AND (COGNI* OR 'COGNITIVE AGING'/EXP OR 'COGNITION'/EXP OR 'ALZHEIMER DISEASE'/EXP OR 'ALZHEIMER* OR 'MILD COGNITIVE IMPAIRMENT'/EXP OR 'MILD COGNITIVE IMPAIRMENT')	6
Web of Science	(trioxygen OR ozone) AND (cognit* OR "Dementia" OR "alzheimer*")	264

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