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Natural products improve healthspan in aged mice and rats: A systematic review and meta-analysis

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ABSTRACT

Over the last decades a decrease in mortality has paved the way for late onset pathologies such as cardiovascular, metabolic or neurodegenerative diseases. This evidence has led many researchers to shift their focus from researching ways to extend lifespan to finding ways to increase the number of years spent in good health; "healthspan" is indeed the emerging concept of such quest for ageing without chronic or disabling diseases and dysfunctions. Regular consumption of natural products might improve healthspan, although the mechanisms of action are still poorly understood. Since preclinical studies aimed to assess the efficacy and safety of these compounds are growing, we performed a systematic review and meta-analysis on the effects of natural products on healthspan in mouse and rat models of physiological ageing. Results indicate that natural compounds show robust effects improving stress resistance and cognitive abilities. These promising data call for further studies investigating the underlying mechanisms in more depth.

1. Introduction

The great advances in medicine and in drug development that have characterised the past century have led to an overall increase in human life expectancy in Westernized countries (World health statistics, 2019). For example, between 2000 and 2016 global life expectancy has increased by 5.5 years, from 66.5 to 72.0 years, with women living longer than men (World health statistics, 2019). The decrease in mortality, however, has paved the way for late-onset pathologies such as cardiovascular, metabolic or neurodegenerative diseases, including Alzheimer's disease. This trend has led many researchers to shift from researching ways to extend life towards finding ways to increase the number of years spent in good health; "healthspan" is indeed the emerging concept of such quest for ageing without chronic or disabling diseases and dysfunctions (Campisi et al., 2019; Fuellen et al., 2019; Luyten et al., 2016).

Abbreviations: 5-HT, 5-hydroxytryptamine or serotonin; Ach, Acetylcholine; AChE, Acetylcholinesterase; BDNF, Brain-Derived Neurotrophic Factor; BrdU, Bromodeoxyuridine; CaMKII-α, Calcium/calmodulin-dependent protein kinase II subunit α; CAT, Catalase; ChAT, Choline acetyltransferase; CREB, cyclic adenosine monophosphate-responsive element-binding; CSD, Contextual serial discrimination; DA, Dopamine; DCX, Doublecortin; DG, Dentate Gyrus; GAP-43, Growth-Associated Protein-43; GR, Glutathione Reductase; GSH-Px or GPx, Glutathione Peroxidase; HO-1, Heme Oxygenase 1; HPA, Hypothalamic-Pituitary-Adrenal; i.p., intraperitoneal; LHP, Lipid Hydroperoxides; MDA, Malondialdehyde; mTOR, mammalian Target of Rapamycin; MWM, Morris Water Maze; n.s., not specified; NA, Noradrenaline; NE, Norepinephrine; NGF, Nerve Growth Factor; NRF2, Nuclear Factor Erythroid 2-Related Factor 2; OBDT, Operant Brightness Discrimination Task; PA, Passive Avoidance; pCREB, phosphorylated Cyclic Adenosine Monophosphate-Responsive Element-Binding; pPKA, phosphorylated Protein Kinase A; PSD95, Postsynaptic Density Protein 95; PWM, Passageway Water Maze; RAM, Radial Arm Maze; ROS, Reactive Oxygen Species; SNAP 25, Synaptosomal-Associated Protein 25; SOD, Superoxide Dismutase; SYP, Synaptophysin; TBARS, Thiobarbituric acid-reactive substances; VAChT, Vesicular Acetylcholine Transporter; w/w, weight/weight.

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While the concept of lifespan is relatively easy to operationalize, as it can be quantified in years and has a hard end point, it is much more difficult to operationalize concepts such as health or healthspan (Kaeberlein, 2018). The latter, in particular, has been broadly defined as the portion of life characterised by lack of (major) diseases, and by a state of physiological, cognitive and physical wellbeing. However, there is a lack of accepted or validated metrics to measure it (Kaeberlein, 2018; Luyten et al., 2016). Healthspan should be considered as a continuous variable that changes in a dynamic way throughout life (Kaeberlein, 2018). Starting from the literature, we have recently provided a conceptual framework for the most prominent features contributing to a definition of health that are shared between humans and model organisms, with the final goal to integrate different operationalizations of health and to lay the groundwork for precision interventions. We defined biomarkers of health by their ability to predict future health better than chronological age and defined healthspan pathways as those molecular features of health that relate to each other by belonging to the same molecular pathway. This terminology has been loosely adopted from previous work (Fuellen et al., 2019). Health was operationalized in terms of stress resistance and homeostasis (summarized as physiological function), strength and locomotion (physical function), cognition (cognitive function) and reproduction (Fuellen et al., 2019). These features are shared among humans and the most common model organisms, such as invertebrates (*C. elegans*) and rodents (rats and mice) (Fuellen et al., 2019) and can be targets of intervention.

A growing body of research suggests that regular consumption of natural products found, among others, in fruits, vegetables, herbs, seeds or mixtures may have a pivotal role in promoting healthy ageing. These compounds are purported to provide extra health benefits, in addition to their basic nutritional value, by boosting stress resistance through activating adaptive stress response signalling pathways in cells (Mattson and Cheng, 2006; Trewavas and Stewart, 2003). Of notice also "not nutritional" plant extracts are widely used in traditional medicine and have clear advantages when compared to official drugs. In fact, many of them may hold clear geroprotective potential, including protection from genotoxic stress, anti-inflammatory activity, wound healing and cytotoxicity promotion toward cancer cells (Budovsky et al., 2014). Nutraceuticals based upon these compounds have thus attracted the interest of the scientific community, with the main aim of staving off various aspects of ageing, e.g. by promoting stress resistance, allowing for the prevention and/or treatment of different conditions, including cognitive decline (Tewari et al., 2018).

Notwithstanding the growing interest in this class of compounds, rigorous clinical trials addressing their specific effects are largely lacking, or show biases related to diet, genetic background or other interfering variables (Marx et al., 2017). Although the in vitro models might provide useful information on specific mechanisms possibly underlying human diseases, they fail to replicate the complex in vivo biology of the ageing process. To this regard, animal models may represent a useful tool to investigate these issues (see Lee et al., 2014; Tewari et al., 2018 and references therein for a comprehensive review). Notwithstanding an exponential increase in publications related to the effects of nutraceuticals on lifespan, so far - at least to the best of our knowledge - no systematic review has been carried-out aimed at assessing the effectiveness of natural compounds on healthspan, which we have tried to define operationally in a rigorous manner (Fuellen et al., 2019).

Among all animal models, rodents represent an interesting bridge between the easy to handle invertebrates (e.g. *C. elegans*) and humans. In addition, cognitive abilities, in particular memory retention, can be reliably tested in rodents (Fuellen et al., 2019; Luyten et al., 2016). We choose specifically to focus on rodent models (rat and mouse) of physiological ageing (see Methods), rather than models of specific diseases of ageing, as natural compounds contained in nutraceuticals are mostly meant to prevent ageing, rather than to cure specific disorders of ageing. To this regard, ageing should be considered as an overall risk factor for a wide range of life-threatening pathologies affecting the quality of life. Thus, treatments able to delay the ageing process may indeed hold the potential to improve healthspan (Budovsky et al., 2006). We selected papers containing the terms “learning and memory” (cognition), “stress resistance”, “metabolic homeostasis”, “grip strength and locomotion” (physical performance), leaving out other search terms such as “reproduction” as this is not a measure that can be assessed in old subjects (Fuellen et al., 2019). We focused on the term “metabolic homeostasis” rather than generically “homeostasis” since a large body of evidence supports a direct link among energy utilization, metabolism and the process of ageing (Finkel, 2015). Further to our search, we performed targeted meta-analyses, when appropriate.

2. Methods

2.1. Review protocol

The systematic search was conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines (Liberati et al., 2009; Moher et al., 2009). The protocol (based on SYRCLE’s systematic review protocol format for animal intervention studies; de Vries et al., 2015; **Supplementary item 1**) was registered in the PROSPERO registry on March 8th 2019 (registration number: CRD42019125286).

2.2. Literature search and study selection

A systematic literature search was conducted in three online databases (PubMed, Scopus, and Web of Science). The search was aimed at identifying relevant studies examining the effects of natural compounds in affecting specific aspects of healthspan, namely cognition (learning and memory), stress resistance, metabolic homeostasis, or physical performance (grip strength and locomotion) in mouse and rat models of physiological ageing. Searches were conducted on August 31st, 2019.

Titles and abstracts of retrieved studies were examined independently by two authors (CM, AB). Exclusion criteria were: i) language different from English; ii) non-original studies (e.g. reviews, editorials, commentaries), and no full-text articles (e.g. meeting abstracts); iii) interventions different from the administration of natural compounds or a mixture of them; iv) all species different from mouse and rat; v) mice $<$ 18 months of age, rats $<$ 24 months of age at the time of testing; vi) in vitro and in silico studies; vii) presence of concomitant interventions; viii) self-administration through operant conditioning.

The full-text of the potentially eligible studies not available from the three databases used was requested from corresponding authors by email. If there was no response to our initial email after a minimum of five business days, we sent a second reminder email to the corresponding author (also through ResearchGate). Full-texts retrieved were independently assessed for eligibility by two authors (CM, MB). Any disagreement between them over the eligibility of particular studies was solved through discussion or by consulting additional investigators (AB, FC).

Exclusion criteria applied during the full-text screening were as above for the abstract, with the addition of the following: ix) no full-text available; x) no relevant outcomes reported, i.e., outcomes other than cognition (learning and memory), stress resistance, metabolic homeostasis, physical performance (grip strength and locomotion); xi) absence of age-matched animals as comparator/control.

2.3. Data extraction

2.3.1. Qualitative synthesis

The full-text articles of the studies eligible for qualitative data extraction were independently assessed by two reviewers (CM, MB). Any disagreement between them was solved through discussion, or by consulting additional investigators (AB, FC). The data extracted included the following categories: i) bibliographic details (i.e., 1st

author, country, year of publication, journal); ii) animal characteristics (i.e., species, strain, age, sex); iii) study design characteristics (i.e., number of treated and control subjects); iv) intervention characteristics (i.e., compound, dose(s), vehicle, treatment duration, route of administration). Outcome measures extracted were: i) in vivo and ex vivo measures of cognition (learning and memory); ii) stress resistance; iii) metabolic homeostasis; iv) physical performance (grip strength and locomotion). Detailed information on the measures extracted within each category are reported in the full protocol **(Supplementary item 1)**. We retrieved data on the direction of the variation in subjects receiving natural compounds when compared with age-matched controls, i.e., statistically significant improvement, deterioration, no changes. When a study provided data for the administration of multiple doses, and/or using multiple tests, all such data were extracted.

2.3.2. Quantitative synthesis (meta-analysis)

Outcomes eligible for quantitative synthesis (meta-analysis) were in vivo measures of cognition (learning and memory) and measures of oxidative stress whose assessment was reported in 10 or more studies (18 studies for learning, 22 for memory, 12 for oxidative stress). As for ex vivo measures of cognition, these ranged widely from neurotransmitters to synaptic molecules, up to biomarkers of neurogenesis and neuronal proliferation. Such heterogeneity prevented us from performing a quantitative synthesis. Quantitative data extracted to enable the computation of standardized effect sizes were: the number of animals, the aggregate value of effect (i.e., the mean) and the standard deviation (SD) as a measure of group variance. For studies not reporting these measures, we contacted by email the corresponding author to request data of interest; a reminder email was sent after a minimum of five business days. If no response was received after a minimum of 10 business days, measures were extracted from graphs using a digital screen ruler (de Vries et al., 2015) by two independent reviewers (CM, MB). Discrepancies were identified and solved through discussion (an agreement was considered to be reached when measures from the two assessors showed a Lin's Concordance Correlation Coefficient greater than 0.95). In order to assess the accuracy of our measurements through comparisons with the real values, data were extracted from graphs also for those studies reporting the exact values of mean and SD on tables. When data extraction from graphs was not possible, the study was excluded from the meta-analysis. Final number of studies included in the quantitative synthesis was 21 for memory retention and 11 for stress resistance. Papers addressing learning were not included in the quantitative synthesis as data extraction from graphs was not possible and authors did not reply when asked to provide the raw data.

When the outcome was measured in different groups of animals receiving different doses, data obtained after administration of the highest dose were included in the main analysis. For two studies (Nitta et al., 1995; Ward et al., 2002), in which the same dose was used but with different treatment durations, the longest treatment was selected. A sensitivity analysis was performed including measures for the lowest dose/shortest treatment duration (see below). In case multiple behavioural tests were used to assess memory retention in the same group of animals, outcomes obtained by means of the Morris water maze (MWM) (Morris, 1984) were included in the main analysis. This task was selected since it is the most-often used in our sample of studies, and since it is particularly suitable to test memory deficits also in old rodents (Berry et al., 2008; Berry and Cirulli, 2013). In fact, it neither involves the use of punishments (e.g., electric shocks) nor of strategies such as food or water deprivation to motivate the animal to solve the task, all conditions that might potentially bias the results, particularly in aged animals (see Risk of bias comments). In addition, even very old rodents (especially mice) which would be poorly motivated to explore a dry environment (e.g., an arena, with or without objects) would swim to find a way to escape from the aquatic environment (Brandeis et al., 1989; Morris, 1984; Vorhees and Williams, 2014). When measures were obtained by other behavioural tests, those from the most-often used in our sample of studies were included in the main analysis (a sensitivity analysis was performed including measures from the other tests, see below).

Measures of oxidative stress were extracted from the selected studies and included in the quantitative synthesis; in this case, the most common parameters were included. When measures were taken from different brain areas, those from the hippocampus were selected, whenever possible, in order to ensure greater homogeneity, and because this brain area has been specifically implicated in memory retention, especially spatial memory (see Discussion).

Effect size calculations were based on the comparison between the group receiving natural compounds - or a mixture of them - and the control group (age-matched animals). The intervention effect for each individual treated-control comparison was expressed as Standardized Mean Difference (SMD; difference in mean between treated and control groups on pooled standard deviations, SD). The individual SMDs were pooled to obtain an overall SMD and 95 % confidence interval (95 % CI; indicating a range within which the true effect lies with 95 % certainty). If a study tested different compounds, the groups were analysed as if they were performed in separate studies. Whenever a control group served more than one treated group, we corrected the total number of control animals in the meta-analysis by dividing the number of animals in the control group by the number of intervention groups served.

Heterogeneity among results was explored by conducting subgroup analyses by animal model (mouse vs. rat) and by body region (peripheral organs vs. central nervous system). Nevertheless, as animal studies are usually heterogeneous with respect to other factors (e.g., strain, procedures, etc.; Hooijmans, IntHout et al., 2014; Hooijmans, Rovers et al., 2014; Vesterinen et al., 2014), a random-effect model was used to compute both the overall effect size and the separate effect sizes for the different subgroups, in order to take into account anticipated heterogeneity that cannot be explained. Since there were few studies in each group, we pooled the estimate of tau square across the subgroups (Rubio-Aparicio et al., 2017).

In order to test the robustness of our findings, sensitivity analyses were performed by repeating the primary meta-analysis, substituting range of values for decisions (such as selecting the highest dose) that may be considered arbitrary, specifically by substituting measures taken after the administration of the lowest dose and by means of non-selected tests.

We calculated the I^2 statistic for each analysis as a measure of the proportion of the overall variation that may be attributed to between-study heterogeneity (Higgins et al., 2003; Higgins and Thompson, 2002). Specifically, we considered an I^2 of less than 40 % as low, between 30 and 60 % as moderate, between 50 and 90 % as substantial, and between 75 and 100 % as considerable (Higgins et al., 2019).

To assess potential publication bias, a funnel plot of study effect sizes against standard errors was visually inspected for asymmetry resulting from a relative lack of small studies with small effect sizes (i.e., those most likely to be non-significant and to remain unpublished). Asymmetry was also statistically tested with Egger's bias test (Egger et al., 1997) with $p < 0.05$ indicating asymmetry.

Statistical analyses were performed using Comprehensive Meta-Analysis Version 3.0. (www.meta-analysis.com). Statistical significance was set at $p < 0.05$.

2.4. Assessment of the risk of bias

To assess the internal validity of the included studies, we used the SYRCLE's risk of bias tool for animal studies (C R Hooijmans et al., 2014). The SYRCLE's risk of bias tool is based on the Cochrane Risk of Bias tool and has been adjusted for aspects of bias that play a specific role in animal intervention studies. A 'yes' score indicates low risk of bias; a 'no' score indicates high risk of bias; and a 'unclear' score indicates unknown risk of bias. Two independent investigators (AB, FC) performed quality assessment of all included studies. Disagreements

were resolved by discussion or by consulting a third reviewer (CM).

3. Results

3.1. Search results

The search on the effects of natural products in promoting health-span in mouse and rat models of physiological ageing resulted in 1328 bibliographic records across three databases (619 for Scopus 381 for PubMed, 328 for Web of Science). The study selection process is summarized in Fig. 1 by using the PRISMA flow diagram. After excluding 85 non-English language studies and duplicates, 710 were left. Following the title and abstract screening, 592 studies were removed based on predetermined exclusion criteria. In the second selection phase, based on the full-text screening of the remaining 118 articles, 39 studies were included in the systematic review; among these 21 studies were included in the meta-analysis of the effect of natural compounds on memory retention, while 11 studies were included in the meta-analysis of the effect of these compounds on stress resistance measures.

3.2. Study characteristics

The characteristics of the included studies are described in Table 1. The characteristics among these studies varied considerably. The included studies were published between 1986 and 2019; most of these were conducted in China (n = 11), six studies in Japan, four in India, three in Korea, three in Germany, two in Iran, two in USA, two in France, one study was conducted in Egypt, one in Malaysia, one in Poland, one in Brazil, one in Greece and one in Israel.

The type of natural compound administered varied greatly among the studies considered, as well as the doses and the duration of the treatment, which ranged from four days to 12 months. Moreover, the characteristics of administration route differed substantially among the studies. Eighteen studies administered the compounds by intragastric gavage, nine by adding it to the chow, five by adding it to the drinking water; five studies indicated the oral route without further specifications, one study involved intraperitoneal injection, and one study did not specify the administration route.

Twenty-one studies used rats from different inbred and outbred stains: Wistar (n = 11), Sprague-Dawley (n = 5) and Fisher (n = 5); the remaining 18 studies used mouse inbred and outbred strains: C57Bl/6 (n = 10), ICR (n = 3), BALB/c (n = 2), Swiss (n = 1), Kunming (n = 1); only one study did not indicate the mouse strain used.

With regard to the sex of the experimental subjects, most of the studies used exclusively males (n = 33), four studies used exclusively females, one study involved both females and male subjects, and in one study the subjects' sex was not specified. The sample sizes of the included studies ranged from five to 20 experimental subjects per treatment group.

With regard to the outcomes of interest to us, 34 studies focused on cognition. Eighteen of these evaluated both in vivo and ex vivo parameters, 13 only in vivo parameters, and three only ex vivo measures. The assessment of the in vivo parameters was carried-out through specific behavioural tests such as the MWM, the PWM, the RAM, the PA, the CSD, the OBDT, the T-maze and the Y-maze tests. The ex vivo analyses were based upon quantification of mRNA and proteins involved in

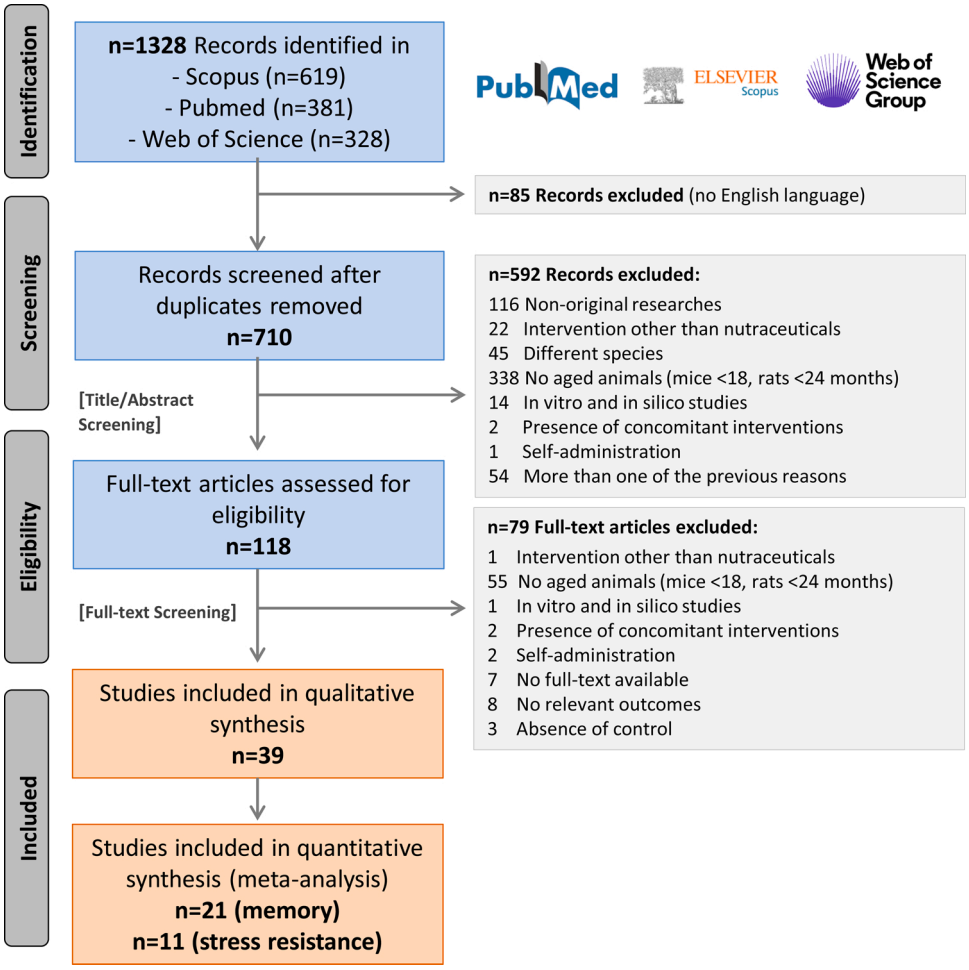

Fig. 1. Flow diagram of the study identification and selection process.

Table 1
Characteristics of the included studies.

Study	Country	Compound	Dose(s)	Treatment duration	Route of Admin.	Species	Strain	Age*	Sex	n treated	n ctrl	Outcomes
Chen et al., 2019	China	Liuwei Dihuang (LWDH)	0.432 g/kg	12 weeks	Drinking water	Mouse	BALB/c	24	M	8	8	Stress resistance
Zhao et al., 2019	China	Guilingji	37.5;70;150 mg/kg	4 weeks	Oral (n.s.)	Rat	Sprague-Dawley	24	M	10;10;10§	10	Cognition (*in vivo- ex vivo*); Stress resistance
Bensalem et al., 2018	France	Polyphenol-rich extract from grape and blueberry	500 mg/kg	14 weeks	Diet	Mouse	C57Bl/6	19	M	9-20	9-20	Cognition (*in vivo- ex vivo*)
Lee et al., 2017	Korea	Red ginseng extract Black ginseng extract	200 mg/kg 200 mg/kg	16 weeks	Oral (n.s.)	Mouse	ICR	22	M	5 5	5	Cognition (*ex vivo*)
Asseburg et al., 2016	Germany	Grape Skin extract	200 mg/kg	6 months	Diet	Mouse	C57Bl/6	19	M	20	20	Cognition (*in vivo- ex vivo*); Stress resistance
Beracochea et al., 2016	France	Wild blueberry full spectrum powder Wild blueberry extract	1000 mg/kg 50 mg/kg	75 days	Gavage	Mouse	C57Bl/6	18	M	15 15	15	Cognition (*in vivo*); Physical performance
Gray et al., 2016	USA	*Centella asiatica*	2 mg/ml	5 weeks	Drinking water	Mouse	C57Bl/6	21	M F	13 13	12 12	Cognition (*in vivo- ex vivo*); Stress resistance
Hosseini-Sharifabad et al., 2016	Iran	*Boswellia serrata* gum resin	100 mg/kg	8 weeks	Gavage	Rat	Wistar	26	M	10	10	Cognition (*in vivo*)
Lee & Oh, 2016	Korea	Red ginseng	200 mg/kg	3 months	Diet	Mouse	C57Bl/6	24	M	6	6	Cognition (*in vivo*); Stress resistance
Osman et al., 2016	Egypt	*Ginko biloba* leaf extract	100 mg/kg	28 days	Gavage	Mouse	n.s.	25	M	15	15	Cognition (*ex vivo*)
Saoji et al., 2016	India	*Centella* extract	300 mg/kg	4 days	Gavage	Mouse	Swiss	24	M	6	6	Cognition (*in vivo*)
Zhang, et al., 2015	China	Icariin *Epimedium* flavonoids	0.02% 0.06%	12 months	Diet	Mouse	C57Bl/6	24	M	11 9	9	Cognition (*in vivo*); Stress resistance; Physical performance
Guo et al., 2014	Japan	Coffee	n.s.	4 weeks	Drinking water	Mouse	C57Bl/6	27	M	10	10	Stress resistance; Physical performance
Liu et al., 2014	Korea	Multi-herbal formula Chong-Myung-Tang (CMT)	50;100;200 mg/kg	14 days	Gavage	Mouse	ICR	18	M	8;8;8§	8	Cognition (*in vivo- ex vivo*)
Taridi et al., 2014	Malaysia	Tocotrienol-rich Fraction	200 mg/kg	3 months	Gavage	Rat	Wistar	24	M	9	9	Cognition *(in vivo)*; Stress resistance; Physical performance
Yang et al., 2014	China	Ginsenoside Rg1	6 mg/kg	12 months	Gavage	Mouse	C57Bl/6	24	n. s.	8	8	Cognition (*in vivo- ex vivo*)
Paula-Freire et al., 2013	Brazil	Roots of *Heteropterys tomentosa* A. Juss Branches of *Heteropterys tomentosa* A. Juss	50 mg/kg 50 mg/kg	80 days	Gavage	Rat	Wistar	25	M	14 14	14	Cognition (*in vivo*)
Rastogi et al., 2012	India	Bacosides (steroidal saponins from *Bacopa monnieri*)	200 mg/kg	3 months	Gavage	Rat	Wistar	27	F	6	6	Cognition (*in vivo- ex vivo*); Stress resistance; Physical performance
Papandreou et al., 2011	Greece	Saffron	60 mg/kg	7 days	i.p.	Mouse	BALB/c	20	M	8	8	Cognition (*in vivo- ex vivo*); Stress resistance
Mizoguchi et al., 2010	Japan	Yokukansan	3% (w/w)	3 months	Diet	Rat	Fisher	24	M	10-12	10-12	Cognition *(ex vivo)*; Physical performance
Pyrzanowska et al., 2010	Poland	*Curcuma longa* extract	10;50 mg/kg	60 days	Diet	Rat	Wistar	24	M	8;8§	7	Cognition (*in vivo- ex vivo*); Stress resistance
Li et al., 2009	China	Green tea catechins	40;80;160 mg/kg	6 months	Drinking water	Mouse	C57Bl/6	20	F	15;15;15§	14	Cognition (*in vivo- ex vivo*); Physical performance
Song et al., 2009	China	*Scutellaria baicalensis* Georgi	35;70;140 mg/kg	15 days	Gavage	Rat	Sprague-Dawley	25	M	14;14;14§	14	Cognition *(in vivo);* Stress resistance
Li et al., 2008	China	Fuzhisan (mix)	0.3;0.6;1.2 g/kg	30 days	Gavage	Rat	Wistar	24	M	8;8;8§	8	Cognition (*in vivo- ex vivo*)
Zhang et al., 2008	China	Tenuifolin (extracted from *Radix Polygalae*)	0.02;0.04;0.08 g/kg	15 days	Gavage	Mouse	Kunming	24	M	10;10;10§	10	Cognition (*in vivo- ex vivo*)
Sarkaki et al., 2007	Iran	Grape seed extract	100 mg/kg	30 days	Gavage	Rat	Wistar	24	M	15	15	Cognition (*in vivo- ex vivo*)
Velavan & Begum 2007	India	*Asparagus racemosus*	500 mg/kg	4 weeks	n.s.	Rat	Wistar	26	M	6	6	Metabolism
Wang et al., 2007	China	Tetrahydroxystilbene glucoside	30;60 mg/kg	3 months	Gavage	Rat	Sprague-Dawley	24	M	9;7§	13	Cognition (*in vivo- ex vivo*)
Zhang et al., 2007	China	Essential oil from *Acorus gramiineus*	0.02;0.04;0.08 g/kg	30 days	Gavage	Rat	Sprague-Dawley	25	M	10;10;10§	10	Cognition (*in vivo- ex vivo*)
Balu et al., 2005	India	Grape seed extract	100 mg/kg	30 days	Gavage	Rat	Wistar	26	M	6	6	Cognition *(in vivo);* Stress resistance
Kou et al., 2005	China	Danggui-Shaoyao-San (mix)	125;250;500 mg/kg	3 months	Oral (n.s.)	Mouse	ICR	18	F	12;12;12§	12	Cognition (*in vivo- ex vivo*)
Topic et al., 2002	Germany	Zingicomb	1;10 mg/kg 10 mg/kg	10 days 5 months	Gavage	Rat	Wistar	24	M	5;6§ 6#	5 5#	Cognition *(in vivo)* Stress resistance
Ward et al., 2002	USA	*Ginkgo biloba* extract	100 mg/kg	28 days	Gavage	Mouse	C57Bl/6	21	M	17	17	Cognition (*in vivo- ex vivo*)

(*continued on next page*)

Table 1 (*continued*)

Study	Country	Compound	Dose(s)	Treatment duration	Route of Admin.	Species	Strain	Age*	Sex	n treated	n ctrl	Outcomes
				70 days						14#	14#	Cognition *(in vivo)*
Ninomiya et al., 2001	Japan	Hachimi-Jio-Gan	7%	10 weeks	Diet	Rat	Fisher	30	M	8	8	Metabolism; Physical performance
Nitta et al., 1995	Japan	*Panax ginseng* extract	8 g/kg	12 days 33 days	Oral (n.s.)	Rat	Fisher	25 27	M	12 9	12 9	Cognition (*in vivo*)
Ohta et al., 1994	Japan	Paeoniflorin	0.01;0.1 mg/kg	n.s.	Oral (n.s.)	Rat	Fisher	25	M	9;4	9	Cognition (*in vivo*)
Jaenicke et al., 1991	Germany	*Panax ginseng* extract	30 mg/kg	13 days	Drinking water	Rat	Wistar	27	F	10	10	Cognition (*in vivo*); Physical performance
Amagaya et al., 1990	Japan	Shosaikoto (herbal mix)	120 mg/kg	9.5 months	Diet	Rat	Fisher	27	M	9-12	9-12	Cognition (*in vivo- ex vivo*)
Kessler et al., 1986	Israel	A plant lipid preparation	3%	6 months	Diet	Rat	Sprague Dawley	24	M	9	9	Physical performance

Note: *Age at the time of testing is expressed in months; § semicolons separate the number of subjects when different doses are administered (number of subjects for each dose); #same animals (or subgroup of animals) receiving extended treatment. *Abbreviations*: i.p.= intraperitoneal injection; w/w= weight/weight; n.s.= not specified.

neuronal plasticity (e.g. *Ngf*, *Bdnf*, PSD-95, SNAP-25, CREB, PKA), assessment of neurogenesis and neuronal proliferation by immunohistochemistry (i.e. DCX cells +, BrdU cell +, Ki67 cell +), as well as by the evaluation of neurotransmitters levels (i.e. DA, NE, 5-HT). Fourteen studies assessed stress resistance, principally focussing on oxidative stress. Most measured levels of antioxidant enzymes (e.g. SOD, CAT, GSH-Px) or amount of mRNA of specific genes regulating the expression of antioxidant proteins (i.e. *Nrf2*). Other papers assessed directly markers of oxidative stress (e.g. ROS, MDA, TBARS). In addition to oxidative stress, two studies evaluated inflammatory parameters, and only one investigated the activation of the HPA axis. Metabolic homeostasis was characterised in two studies by ex vivo analyses on liver and blood; while physical performance, which includes the assessment of grip strength and locomotion, was evaluated in 10 studies through in vivo specific behavioural tests such as the open field, the rotarod test, the grip strength test and measurement of spontaneous activity.

3.3. Risk of bias

The quality study assessment was carried-out by means of the SYRCLE's Risk of Bias tool. Overall, reporting was quite poor; thus, the methodological quality of many studies was unclear. In particular, while almost half of the studies (49 %, n = 19) described methods to generate a random allocation sequence, only 8 % of the studies (n = 3) report details on the baseline characteristics of the animals, 2.5 % (n = 1) on allocation concealment, 5 % (n = 2) on random outcome assessment and 2.5 % (n = 1) on blinding of outcome assessment; none of the studies reported methodological details on random housing (unclear risk of bias = 100 %). Interestingly, 92 % of the studies (n = 36) were free of selective outcome reporting. A high risk of bias was present in only three domains. More specifically, 8 % of the studies (n = 3) did not address adequately incomplete outcomes, 5 % (n = 2) showed selective outcome reporting, while 20.5 % (n = 8) had a conflict of interest (other bias domain) mainly because they reported support by private funding (e.g., pharmaceutical companies who provided the testing product) (see Fig. 2).

3.4. Effect of natural compounds on cognition

The effects of natural compounds on cognition on all reported

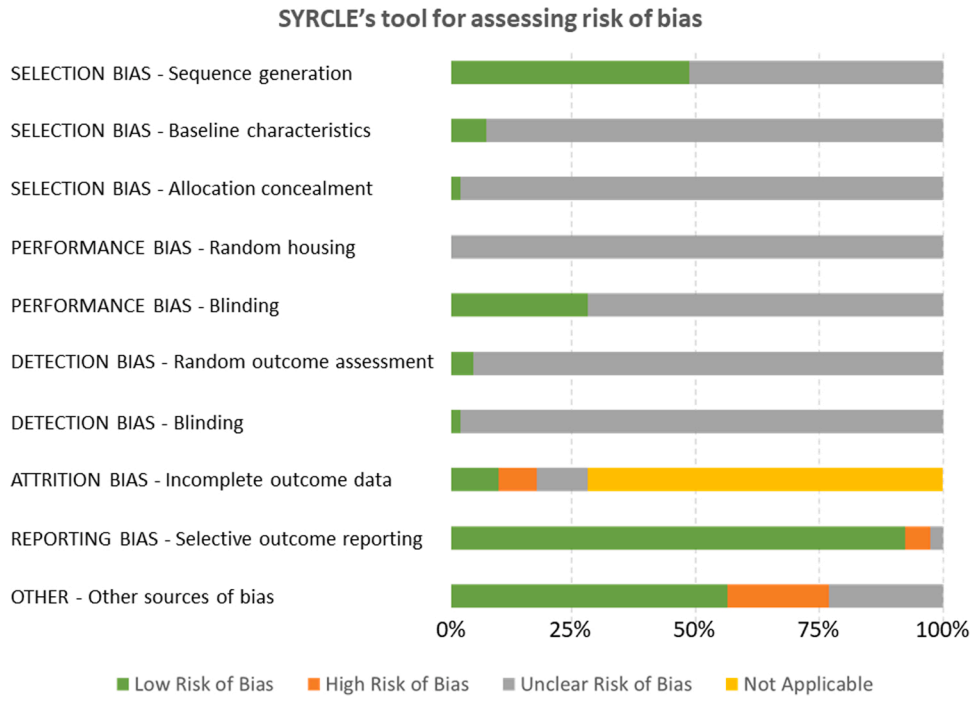

Fig. 2. Risk of bias and quality assessment by means of the SYRCLE's tool. The score (%) represents risk of bias for each component of the tool.

Table 2a
Cognition (in vivo measures): study-wise list of dependent variables and results.

Study	Compound	Dose(s) (Treatment duration[#])	Outcome: **Learning**			Outcome: **Memory**		
			Test	Parameter	Results	Test	Parameter	Results
Zhao et al., 2019	Guilingji	37.5 mg/kg	MWM	Escape latency (s)	=	MWM	Time in target (s)	↑
		70 mg/kg	MWM	Escape latency (s)	↑	MWM	Time in target (s)	↑
		150 mg/kg	MWM	Escape latency (s)	↑	MWM	Time in target (s)	↑
Bensalem et al., 2018	Polyphenol-rich extract from grape and blueberry	500 mg/kg	*n.a.*			MWM	Time in target (%)	=
Asseburg et al., 2016	Grape Skin Extract	200 mg/kg	*n.a.*			Y-maze	Spontaneous alternation (%)	=
Beracochea et al., 2016	Wild blueberry full spectrum powder	1000 mg/kg	*n.a.*			MWM	Time in target (%)	↑
						T-maze	Correct alternation (%)	↑
						CSD	Correct response (%)	↑
	Wild blueberry extract	50 mg/kg	*n.a.*			MWM	Time in target (%)	=
						T-maze	Correct alternation (%)	=
						CSD	Correct response (%)	↑
Gray et al., 2016	*Centella asiatica*	2 mg/ml	MWM	Escape latency (s)	↑	MWM	Time in target (%)	↑
Hosseini-Sharifabad et al., 2016	*Boswellia serrata* gum resin	100 mg/kg	MWM	Escape latency (s)	↑	*n.a.*		
Lee & Oh 2016	Red ginseng	200 mg/kg	MWM	Escape latency (s)	↑	MWM	Time in target (%)	↑
						Y-maze	Spontaneous alternation (%)	↑
Saoji et al., 2016	*Centella* extract	300 mg/kg	MWM	Escape latency (s)	↑	MWM	Time in target (s)	=
Zhang, et al., 2015	Icariin	0.02%	MWM	Escape latency (s)	=	MWM	Time in target (s)	↑
	Epimedium flavonoids	0.06%	MWM	Escape latency (s)	=	MWM	Time in target (s)	=
Liu et al., 2014	Multi-herbal formula CMT	50 mg/kg	MWM	Escape latency (s)	=	MWM	Time in target (%)	=
		100 mg/kg	MWM	Escape latency (s)	=	MWM	Time in target (%)	=
		200 mg/kg	MWM	Escape latency (s)	↑	MWM	Time in target (%)	↑
Taridi et al., 2014	Tocotrienol-rich Fraction	200 mg/kg	MWM	Escape latency (s)	↑	MWM	Time in target (%)	=
Yang et al., 2014	Ginsenoside Rg1	6 mg/kg	MWM	Escape latency (s)	↑	MWM	Time in target (s)	↑
						Y-maze	Spontaneous alternation (%)	↑
Paula-Freire et al., 2013	Root of *Heteropterys tomentosa* A. Juss	50 mg/kg	T-maze	Time to learn (s)	=	*n.a.*		
	Branches of *Heteropterys tomentosa* A. Juss	50 mg/kg	T-maze	Time to learn (s)	=	*n.a.*		
Rastogi et al., 2012	Bacosides (steroidal saponins from *Bacopa monnieri*)	200 mg/kg	*n.a.*			PA	Latency to step-through (s)	↑
Papandreou et al., 2011	Saffron	60 mg/kg	*n.a.*			PA	Latency to step-through (s)	↑
Pyrzanowska et al., 2010	*Curcuma longa* extract	10 mg/kg	MWM	Escape latency (s)	↑	MWM	Time in target (%)	↑
		50 mg/kg	MWM	Escape latency (s)	↑	MWM	Time in target (%)	=
Li et al., 2009	Green tea catechins	40 mg/kg	*n.a.*			MWM	Time in target (s)	=
		80 mg/kg	*n.a.*			MWM	Time in target (s)	↑
		160 mg/kg	*n.a.*			MWM	Time in target (s)	↑
Song et al., 2009	*Scutellaria baicalensis* Georgi	35 mg/kg	MWM	Escape latency (s)	↑	MWM	Time in target (s)	=
		70 mg/kg	MWM	Escape latency (s)	↑	MWM	Time in target (s)	↑
		140 mg/kg	MWM	Escape latency (s)	↑	MWM	Time in target (s)	↑
Li et al., 2008	Fuzhisan (mix)	1.2 g/kg[§]	MWM	Escape latency (s)	↑	*n.a.*		
Zhang et al., 2008	Tenuifolin (extracted from *Radix Polygalae*)	0.02 g/kg	*n.a.*			PA	Latency to step-through (s)	=
						Y-maze*	Latency to safe zone (s)	=
		0.04 g/kg	*n.a.*			PA	Latency to step-through (s)	=
						Y-maze*	Latency to safe zone (s)	↑
		0.08 g/kg	*n.a.*			PA	Latency to step-through (s)	↑
						Y-maze*	Latency to safe zone (s)	↑
Sarkaki et al., 2007	Grape seed extract	100 mg/kg	MWM	Escape latency (s)	↑	MWM	Time in target (%)	↑
Wang et al., 2007	Tetrahydroxystilbene glucoside	30 mg/kg	PWM	Errors (number)	=	*n.a.*		
		60 mg/kg	PWM	Errors (number)	↑	*n.a.*		
Zhang et al., 2007	Essential oil from *Acorus gramineus*	0.02 g/kg	*n.a.*			PA	Latency to step-through (s)	=
						Y-maze*	Latency to safe zone (s)	↑
		0.04 g/kg	*n.a.*			PA	Latency to step-through (s)	↑
						Y-maze*	Latency to safe zone (s)	↑
		0.08 g/kg	*n.a.*			PA	Latency to step-through (s)	↑

(*continued on next page*)

Table 2a (*continued*)

Study	Compound	Dose(s) (Treatment duration#)	Outcome: **Learning**			Outcome: **Memory**		
			Test	Parameter	Results	Test	Parameter	Results
						Y-maze*	Latency to safe zone (s)	↑
Balu et al., 2005	Grape seed extract	100 mg/kg	*n.a.*			T-maze	Correct trials (%)	↑
		125 mg/kg	*n.a.*			PA	Latency to step-through (s)	=
Kou et al., 2005	Danggui-Shaoyao-San (mix)	250 mg/kg	*n.a.*			PA	Latency to step-through (s)	=
		500 mg/kg	*n.a.*			PA	Latency to step-through (s)	↑
Topic et al., 2002	Zingicomb	1 mg/kg	MWM	Escape latency (s)	↑	*n.a.*		
		10 mg/kg	MWM	Escape latency (s)	=	*n.a.*		
Ward et al., 2002	*Ginkgo biloba* extract	100 mg/kg (28 days)	MWM	Escape latency (s)	=	MWM	Time in target (%)	=
		100 mg/kg (70 days)	MWM	Escape latency (s)	=	MWM	Time in target (%)	=
Nitta et al., 1995	*Panax ginseng* extract	8 g/kg (12 days)	OBDT	Incorrect responses (%)	=	*n.a.*		
		8 g/kg (33 days)	RAM	Errors (number)	↑	*n.a.*		
Ohta et al., 1994	Paeoniflorin	0.01 mg/kg	OBDT	Incorrect responses (%)	↑	*n.a.*		
		0.1 mg/kg	OBDT	Incorrect responses (%)	=	*n.a.*		
Jaenicke et al., 1991	*Panax ginseng* extract	30 mg/kg	PA	Latency to step-through (s)	↑	*n.a.*		
Amagaya et al., 1990	Shosaikoto (herbal mix)	120 mg/kg	PA	Acquisition time (s)	↑	*n.a.*		

Note: # treatment duration was indicated only for those studies reporting multiple treatment durations; *with electric shock; § data relative to 0.3 and 0.6 g/kg doses are not shown in the manuscript. Abbreviations: ↑: statistically significant improvement; ↓:statistically significant worsening; =: no changes; CSD: contextual serial discrimination; MWM: Morris water maze; n.a.: not available; OBDT: Operant brightness discrimination task; PA: passive avoidance; PWM: passageway water maze; RAM: radial arm maze.

parameters (within each test) are illustrated in Table 2a (in vivo measures, learning and memory) and in Table 2b (ex vivo measures). Significant effects ($p < 0.05$) are indicated as arrows. As for the assessment of learning, most of the studies (n = 15) used the MWM, two studies the PA, two studies the OBDT, one the T-maze, one the PWM and one the RAM. Regardless of the specific behavioural test used, all the natural compounds administered improved learning abilities compared to controls, except for three papers that did not show any improvement (Paula-Freire et al., 2013; Ward et al., 2002; Zhang et al., 2015). All the studies testing multiple doses revealed that the highest dose was the most effective, except for two studies where only the lowest dose was effective (without any toxicity effect by the high dose) (Ohta et al., 1994; Topic et al., 2002) (Table 2a).

As for memory retention, the most used test was the MWM (n = 15), followed by the PA (n = 5), the Y-maze (n = 3); the modified Y-maze with electric shock (n = 2); the T-maze (n = 2), and the CSD (n = 1).

Twenty-two studies measured the efficacy of natural compounds on memory retention (Table 2a). Of these, 21 (23 independent comparisons) were included in the meta-analysis. Overall, administration of natural compounds (highest dose) led to a significant improvement of memory retention in animal models of physiological ageing (239 treated animals, 211 control animals; SMD = 1.23; CI95 %: 0.88–1.58, Z = 6.83, $p < 0.001$; Fig. 3). Between-study heterogeneity (I^2) was substantial (63 %).

Thirteen studies (15 comparisons) assessed the effect of natural compounds in mouse models of ageing, eight studies in rat models of ageing. Improvement in memory retention was observed both in mouse (161 treated animals, 134 control animals; SMD = 1.09; CI95 %: 0.66–1.53; Z = 4.95; $p < 0.001$, $I^2 = 68$ %) and in rat models (78 treated animals, 77 control animals; SMD = 1.48; CI95 %: 0.88–2.08; Z = 4.86, $p < 0.001$, $I^2 = 47$ %) (**Supplementaryitem 2,Fig.S1**).

Sensitivity analyses - performed by including measures taken from the groups receiving the lowest dose and by means of non-selected tests - confirmed the beneficial effect of natural compounds on memory retention (lowest dose: SMD = 1.02; CI95 %: 0.68–1.35; Z = 5.90, $p < 0.001$, $I^2 = 62$ %; other tests: SMD = 1.12; CI95 %: 0.80–1.44; Z = 6.84, $p < 0.001$, $I^2 = 59$ %).

Inspection of the funnel plot of the study effect sizes (SMDs) against standard errors (Fig. 4) suggested asymmetry. Specifically, the funnel plot shows larger studies (smaller SE, appearing towards the top of the graph) dispersed across a wider range of values, while smaller studies (higher SE, appearing towards the bottom of the graph) are more concentrated on the right side of the mean effect size (i.e., larger effects). Egger's test confirmed asymmetry that was consistent with publication bias ($p = 0.001$).

Many of these in vivo observations are corroborated by ex vivo analyses aimed at assessing possible changes in the structure and/or the functionality of the brain (Table 2b). Interestingly, most of the natural compounds administered affected neurotransmitter systems, neuronal plasticity, neurogenesis and neuronal proliferation in cerebral areas such as prefrontal cortex, cortex, hippocampus, striatum and hypothalamus. Specifically, investigations on neurotransmitter systems (cholinergic, dopaminergic, noradrenergic, serotoninergic) were carried-out in 12 studies. Three studies used immunohistochemistry techniques to assess neurogenesis and neuronal differentiation in the hippocampus, two of these (Liu et al., 2014; Osman et al., 2016) indicated increased levels of neurogenesis and proliferation through specific markers (such as DCX, BrdU-, Ki67-cells +) in natural compounds-treated subjects; while one of these showed no changes in these parameters (Bensalem et al., 2018). Eight papers focused on neuronal plasticity, measuring the expression of mRNA (i.e. *Ngf*, *Bdnf*) and proteins (i.e. NGF, BDNF, GAP-43, SNAP-25, PSD95, synaptophysin, CaMKII-α, mTOR, pCREB, pPKA) involved in the remodelling and functionality of synapses, mainly in the hippocampus. Data from these studies showed beneficial effects of natural compounds in preventing age-related decreases in neuronal plasticity.

3.5. Effect of natural compounds on stress resistance

In two studies (Guo et al., 2014; Lee and Oh, 2015) markers of peripheral and central inflammation were measured, suggesting beneficial effects of natural compounds on the immune system. Only one study (Pyrzanowska et al., 2010) evaluated activation of the HPA axis, finding decreased levels of plasma corticosterone in subjects treated with

Table 2b
Cognition (ex vivo measures): study-wise list of dependent variables and results.

Study	Compound	Dose(s)	Parameter	Brain Region	Results
Zhao et al., 2019	Guilingji	37.5 mg/kg	AChE	Serum	=
			ACh	Serum	↑
		70 mg/kg	AChE; ACh	Serum	↑
		150 mg/kg	AChE; ACh	Serum	↑
Bensalem et al., 2018	Polyphenol-rich extract from grape and blueberry	500 mg/kg	mRNA Ngf	Hippocampus	↑
			mRNA Bdnf	Hippocampus	=
			DCX cells +	Hippocampus	=
Lee et al., 2017	Red ginseng extract	200 mg/kg	AChE, ChAT activity	Cortex	=
			ChAT; VAChT; GAP-43; SNAP-25; NFG; BDNF	Hippocampus	↑
	Black ginseng extract	200 mg/kg	AChE activity	Cortex	=
			ChAT activity	Cortex	↑
			ChAT; VAChT; GAP-43; SNAP-25; NFG; BDNF	Hippocampus	↑
Asseburg et al., 2016	Grape Skin Extract	200 mg/kg	CREB	Whole brain	=
			PSD95; synaptophysin	Hippocampus	↑
Gray et al., 2016	*Centella asiatica*	2 mg/ml	PSD95; synaptophysin	Prefrontal cortex	↑
			PSD95; synaptophysin	Cerebellum	=
Osman et al., 2016	*Ginko biloba* leaf extract	100 mg/kg	Ki67 cell +	Hippocampus (DG)	↑
			DCX cell +	Hippocampus (DG)	↑
			DCX protein	Hippocampus (DG)	↑
Liu et al., 2014	Multi-herbal formula CMT	50 mg/kg	BrdU cell +	Hippocampus (DG)	=
		100 mg/kg	BrdU cell +	Hippocampus (DG)	=
		200 mg/kg	BrdU cell +	Hippocampus (DG)	↑
Yang et al., 2014	Ginsenoside Rg1	6 mg/kg	Synaptophysin; GluN1; PSD-95; CaMKII- α; mTOR	Hippocampus	↑
Rastogi et al., 2012	Bacosides (steroidal saponins from *Bacopa monnieri*)	200 mg/kg	Ach; AChE; 5-HT; DA	Cortex	↑
			NE	Cortex	=
Papandreou et al., 2011	Saffron	60 mg/kg	AChE activity	Whole brain	=
Mizoguchi et al., 2010	Yokukansan	3% (w/w)	5-HT; DA	Prefrontal cortex	↑
			5-HT; DA; NA	Prefrontal cortex	=
		10 mg/kg	5-HT; DA; NA	Hippocampus	=
			5-HT; DA; NA	Striatum	=
			5-HT; DA; NA	Hypothalamus	=
Pyrzanowska et al., 2010	*Curcuma longa* extract	50 mg/kg	5-HT	Prefrontal cortex	↑
			DA, NA	Prefrontal cortex	=
			5-HT	Hippocampus	↑
			DA, NA	Hippocampus	=
			5-HT; DA; NA	Striatum	=
			5-HT	Hypothalamus	↑
			DA; NA	Hypothalamus	=
Li et al., 2009	Green tea catechins	40 mg/kg	pCREB; pPKA; PSD-95; CaMKII-α; BDNF	Hippocampus	=
		80 mg/kg	pCREB; pPKA; PSD-95; CaMKII- α; BDNF	Hippocampus	↑
		160 mg/kg	pCREB; pPKA; PSD-95; CaMKII- α; BDNF	Hippocampus	↑
Li et al., 2008	Fuzhisan (mix)	0.3 g/kg	ChAT, Ach	Hippocampus	↑
		0.6 g/kg	ChAT, Ach	Hippocampus	↑
		1.2 g/kg	ChAT, Ach	Hippocampus	↑
Zhang et al., 2008	Tenuifolin (extracted from *Radix Polygalae*)	0.02 g/kg	5-HT: NE; DA	Hippocampus	=
			AChE activity	Cortex	↑
		0.04 g/kg	5-HT	Hippocampus	=
			NE; DA	Hippocampus	↑
			AChE activity	Cortex	↑
		0.08 g/kg	5-HT	Hippocampus	=
			NE; DA	Hippocampus	↑
			AChE activity	Cortex	↑
Sarkaki et al., 2007	Grape seed extract	100 mg/kg	5-TH	Prefrontal cortex	=
Wang et al., 2007	Tetrahydroxystilbene glucoside	30 mg/kg	Number of synapsis; SYP	Hippocampus	=
		60 mg/kg	Number of synapsis; SYP	Hippocampus	↑
Zhang et al., 2007	Essential oil from Acorus gramiineus	0.02 g/kg	5-HT; DA	Whole brain	=
			NE; AChE	Whole brain	↑
		0.04 g/kg	5-HT	Whole brain	=
			NE; DA; AChE	Whole brain	↑
		0.08 g/kg	5-HT; NE; DA; AChE	Whole brain	↑
Kou et al., 2005	Danggui-Shaoyao-San (mix)	125 mg/kg	NE; DA; 5-HT	Cortex	=
		250 mg/kg	NE	Cortex	=
			DA; 5-HT	Cortex	↑
		500 mg/kg	NE; DA; 5-HT	Cortex	↑
Ward et al., 2002	*Ginkgo biloba* extract	100 mg/kg	CREB	Hippocampus	=
Amagaya et al., 1990	Shosaikoto (herbal mix)	120 mg/kg	NE; DA	Whole brain	↑
			5-HT	Whole brain	=

Abbreviations: ↑: statistically significant improvement; ↓=statistically significant worsening; =: no changes; 5-HT: 5-hydroxytryptamine or serotonin; Ach: Acetylcholine; AChE: Acetylcholinesterase; BDNF: Brain-derived neurotrophic factor; BrdU: Bromodeoxyuridine; ChAT: Choline acetyltransferase; CaMKII-α: Calcium/calmodulin dependent protein kinase II α; CREB: cyclic adenosine monophosphate-responsive element-binding; DA: Dopamine; DCX: Doublecortin; DG: dentate gyrus; GAP-43: Growth-associated protein-43; mTOR: mammalian target of rapamycin; NA: Noradrenaline; NE: Norepinephrine; NGF: Nerve growth factor; pCREB:phosphorylated cyclic adenosine monophosphate-responsive element-binding; pPKA: phosphorylated protein kinase A; PSD95: Postsynaptic density protein 95; SNAP 25: Synaptosomal-associated protein 25; SYP: Synaptophysin; VAChT: Vesicular acetylcholine transporter.

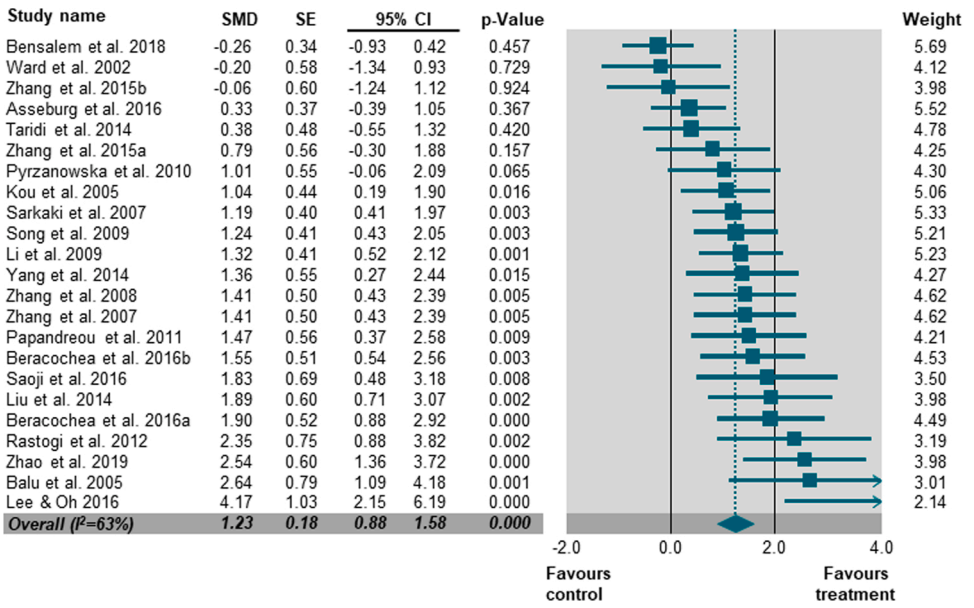

Fig. 3. Forest plot (effect size and 95 % CI) of individual comparisons of animals receiving natural compounds (n = 239) vs. control animals (n = 211) on memory retention (overall effect). Notes. Horizontal lines represent 95 % CIs. The area of each square is proportional to the study weight in the analysis. The diamond represents pooled estimates from random-effects meta-analysis. Abbreviations. SMD: standardized mean difference, SE: standard error, CI: confidence interval.

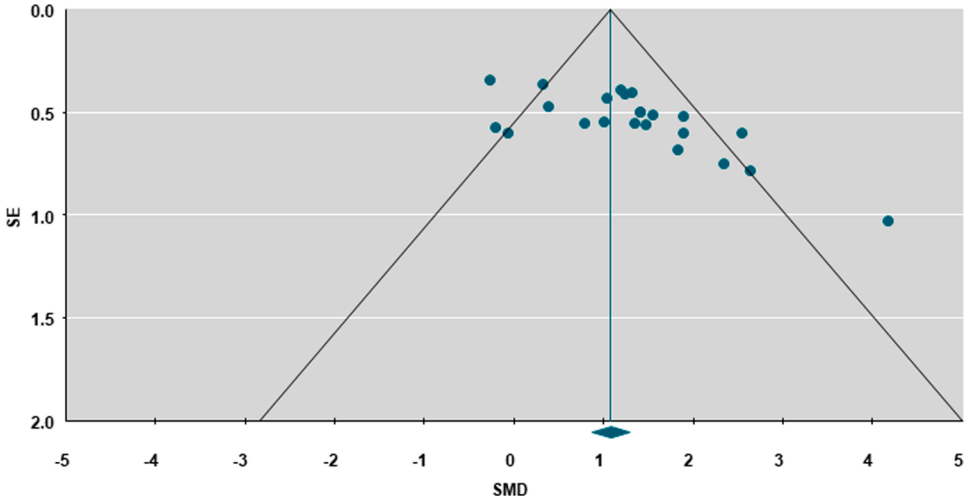

Fig. 4. Funnel plot of standardized mean differences (SMDs) from 21 studies/23 independent comparisons (filled circles) on the effects of receiving natural compounds on memory retention. Abbreviations. SMD: standardized mean difference, SE: standard error.

natural compounds. Twelve studies assessed the effects of a natural compound on oxidative stress, analysing the balance between antioxidant defences and products of oxidation processes (see Table 3 for the effect of natural compounds on oxidative stress on all reported parameters and body regions).

Of these, 11 studies (12 independent comparisons) were included in the meta-analysis. Overall, administration of natural compounds (highest dose) led to a significant improvement in parameters indicative of oxidative stress in animal models of physiological ageing (103 treated animals, 91 control animals; SMD = 1.40; CI95 %: 0.92–1.89, Z = 5.63, $p < 0.001$; Fig. 5). Between-study heterogeneity (I^2) was moderate/substantial (54 %).

Five studies (six comparisons) assessed the effect of natural compounds in mouse models of ageing, six studies in rat models of ageing. Improvement in parameters indicative of stress resistance was observed both in mouse (52 treated animals, 41 control animals; SMD = 1.43; CI95 %: 0.70–2.17; Z = 3.81; $p < 0.001$, $I^2 = 65$ %) and in rat models (51 treated animals, 50 control animals; SMD = 1.41; CI95 %: 0.69–2.13; Z = 3.81, $p < 0.001$, $I^2 = 49$ %) (**Supplementaryitem 2,Fig.S2**).

Seven studies assessed the effects of natural compounds in central measures, four studies (five comparisons) in peripheral measures. Improvement in stress resistance was observed both in central (56 treated animals, 55 control animals; SMD = 1.75; CI95 %: 1.09–2.42; Z = 5.15, $p < 0.001$, $I^2 = 69$ %) and in peripheral measures (47 treated animals, 36 control animals; SMD = 0.99; CI95 %: 0.27–1.72; Z = 2.68; $p = 0.007$, $I^2 = 0$%) (**Supplementaryitem 2,Fig.S3**).

A sensitivity analysis, performed including measures taken after the administration of the lowest dose, confirmed the beneficial effect of natural compounds on parameters indicative of stress resistance (SMD = 1.51; CI95 %: 0.97–2.05; Z = 5.45, $p < 0.001$, $I^2 = 62$ %).

Inspection of the funnel plot of study effect sizes (SMDs) against standard errors (Fig. 6) suggested asymmetry. Also, in this case the graph shows a lack of small studies with small effect sizes: larger studies (top of the graph) are clustered near and on the left side of the mean effect size (i.e., smaller effects or no effects), while smaller studies (bottom of the graph) are more concentrated on the right side of the

Table 3
Stress resistance (oxidative stress measures): study-wise list of dependent variables and results.

Study	Compound	Dose(s)	Parameter	Body Region	Results
Chen et al., 2019	Liuwei Dihuang	0.432 g/kg	SOD	Serum	=
			MDA	Serum	↑
		37.5 mg/kg	SOD; CAT; GSH-Px; MDA	Serum	=
Zhao et al., 2019	Guilingji	70 mg/kg	CAT; GSH-Px; MDA	Serum	=
			SOD	Serum	↑
		150 mg/kg	SOD; CAT; GSH-Px; MDA	Serum	↑
Asseburg et al., 2016	Grape Skin Extract	200 mg/kg	SOD2; CAT; GPx	Whole brain	=
			mRNA of *Nfr2*	Hippocampus	↑
Gray et al., 2016	Centella asiatica	2 mg/mL	mRNA of *Nfr2*	Frontal cortex	↑
			mRNA of *Nfr2*	Cerebellum	↑
			mRNA of *Nfr2*	Liver	↑
Lee & Oh, 2016	Red ginseng	200 mg/kg	NRF2; HO-1	Hippocampus	↑
Zhang et al., 2015	Icariin	0.02 %	SOD; MDA	Liver	↑
	Epimedium flavonoids	0.06 %	SOD; MDA	Liver	↑
Taridi et al., 2014	Tocotrienol-rich Fraction	200 mg/kg	MDA	Plasma	↑
			SOD; GPx; CAT	Erythrocytes	↑
Rastogi et al., 2012	Bacosides (steroidal saponins from Bacopa monnieri)	200 mg/kg	GR; TBARS; LHP; GSH; GPx; catalase	Whole brain	↑
			SOD; protein carbonyls	Whole brain	=
Papandreou et al., 2011	Saffron	60 mg/kg	Ascorbic acid; MDA; GSH	Whole brain	↑
		35 mg/kg	SOD; MDA	Hippocampus	=
			SOD; MDA	Cortex	=
			SOD; MDA	Hippocampus	↑
Song et al., 2009	Scutellaria baicalensis Georgi	70 mg/kg	SOD	Cortex	=
			MDA	Cortex	↑
		140 mg/kg	SOD; MDA	Hippocampus	↑
			SOD; MDA	Cortex	↑
Balu et al., 2005	Grape seed extract	100 mg/kg	ROS; protein carbonyl; total thiol	Spinal cord	↑
			ROS; protein carbonyl; total thiol	Cortex	↑
			ROS; protein carbonyl; total thiol	Striatum	↑
			ROS; protein carbonyl; total thiol	Hippocampus	↑
Topic et al., 2002	Zingicomb	10 mg/kg	protein carbonyl, lipid peroxidation	Whole brain	↑

↑: statistically significant improvement; ↓:statistically significant worsening; =: no changes; CAT: Catalase; GR: Glutathione reductase; GSH-Px or GPx: Glutathione peroxidase; HO-1: Heme oxygenase 1; LHP: Lipid hydroperoxides; MDA: Malondialdehyde; Nfr2: Nuclear factor erythroid 2-related factor 2; ROS: Reactive oxygen species; SOD: Superoxide dismutase; TBARS: Thiobarbituric acid reactive substances.

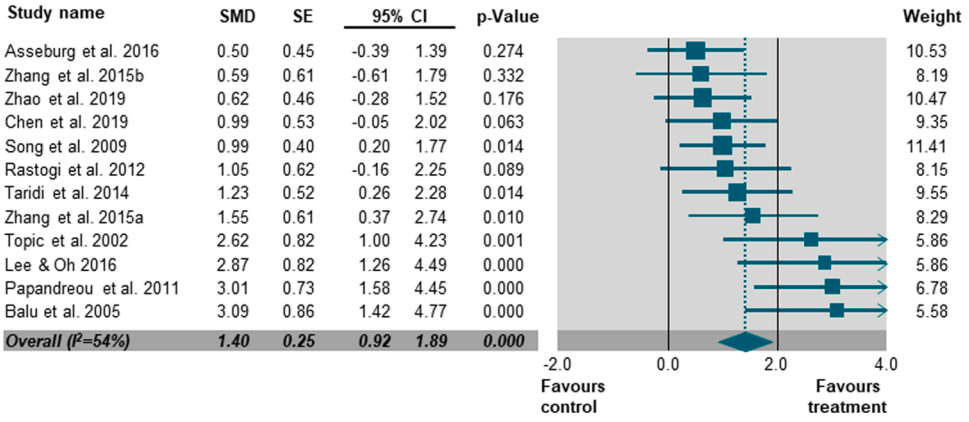

Fig. 5. Forest plot (effect size and 95 % CI) of individual comparisons of animals receiving natural compounds (n = 103) vs. control animals (n = 91 animals) on oxidative stress (overall effect). Notes. Horizontal lines represent 95 % CIs. The area of each square is proportional to the study weight in the analysis. The diamond represents pooled estimates from random-effects meta-analyses. Abbreviations. SMD: standardized mean difference, SE: standard error, CI: confidence interval.

mean effect size (larger effect). Egger's test confirmed asymmetry that was consistent with publication bias (p = 0.001).

3.6. Effect of natural compounds on metabolic homeostasis

Two studies investigated metabolic homeostasis (Table 4). The first (Velavan and Hazeena Begum, 2007) focused on glucose homeostasis, measuring plasma concentration of glucose and insulin, and evaluating the activity of specific enzymes involved in glucose metabolism; the target of the second study was the lipid profile (Ninomiya et al., 2001). While results from the former suggest that natural compounds were able to improve glucose metabolism, the latter shows no effect.

3.7. Effect of natural compounds on physical performance

A characterisation of the physical performance was carried-out in 10 papers through in vivo specific behavioural tests (Table 5). Specifically, five studies observed the spontaneous activity of the experimental subjects. Results were not homogeneous, and only in some cases the results indicated a positive effect of natural compounds, preventing the reduction in spontaneous activity occurring during the ageing process. Four studies used the open field to assess locomotor activity, showing that administration of natural compounds did not affect this parameter, except for one study that reported an improvement in locomotion (Rastogi et al., 2012). Two studies (Guo et al., 2014; Ninomiya et al., 2001) used the grip strength test or the inclined screen test showing that

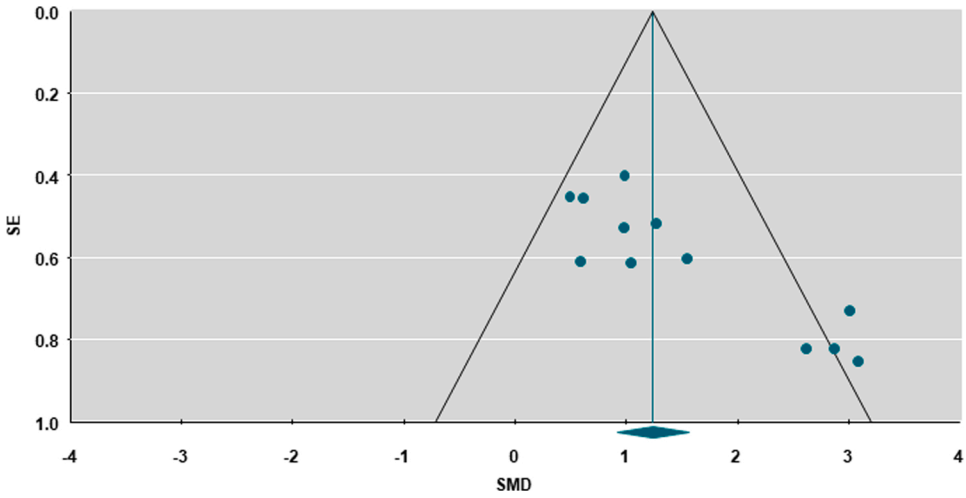

Fig. 6. Funnel plot of standardized mean differences (SMDs) from 11 studies/12 independent comparisons (filled circles) on the effects of treatment with natural compounds on stress resistance. Abbreviations. SMD: standardized mean difference, SE: standard error.

Table 4
Metabolic homeostasis: study-wise list of dependent variables and results.

Study	Compound	Dose	Parameter	Tissue	Results
Velavan & Begum 2007	Asparagus racemosus	500 mg/kg	Glucose (mg/dl); insulin (microU/mL); insulin resistance index	Plasma	↑
			Hepatic hexokinase activity; glucose-6-phosphatase activity; fructose-1,6-bisphosphatase activity	Liver	↑
Ninomiya et al., 2001	Hachimi-Jio-Gan	7 %	Triglycerides; cholesterol	Blood	=

Abbreviations. ↑: statistically significant improvement; ↓:statistically significant worsening; =: no changes.

Table 5
Physical performance (locomotion and grip strength measures): study-wise list of dependent variables and results.

Study	Compound	Dose(s)	Test	Parameter	Results
Beracochea et al., 2016	Wild blueberry full spectrum powder	1000 mg/kg	Automated recording	Runs (number)	=
	Wild blueberry extract	50 mg/kg	Automated recording	Runs (number)	↑
Zhang et al., 2015	Icariin	0.02 %	Rotarod	Latency to fall (s)	↑
	Epimedium flavonoids	0.06 %	Rotarod	Latency to fall (s)	↑
Guo et al., 2014	Coffee	n.s.	Spontaneous activity	n.s.	=
			Grip strength	n.s.	↑
Taridi et al., 2014	Tocotrienol-rich Fraction	200 mg/kg	Open field	Crossings (number)	=
Rastogi et al., 2012	Bacosides (steroidal saponins from Bacopa monnieri)	200 mg/kg	Open field	Activity (s)	↑
Mizoguchi et al., 2010	Yokukansan	3% (w/w)	Spontaneous activity	n.s.	=
Li et al., 2009	Green tea catechins	40 mg/kg	Open field	Crossings (number)	=
		80 mg/kg	Open field	Crossings (number)	=
		160 mg/kg	Open field	Crossings (number)	=
Ninomiya et al., 2001	Hachimi-Jio-Gan	7 %	Spontaneous wheel running	m/day	↑
			Inclined screen test	Slide angle	↑
Jaenicke et al., 1991	Panax ginseng extract	30 mg/kg	Open field	Activity (n.s.)	=
Kessler et al., 1986	A plant lipid preparation	3 %	Spontaneous activity	Activity (n.s.)	↑

Abbreviations. ↑: statistically significant improvement; ↓ :statistically significant worsening; =: no changes; n.s. = not specified; w/w = weight/weight.

natural compounds improved physical strength in aged subjects. Only one study used the rotarod test, showing that natural compound - treated subjects had improved motor performance with increased latency to fall from the apparatus (Zhang et al., 2015).

4. Discussion

In this study, we performed a systematic review and meta-analyses aimed at identifying the efficacy of natural compounds to affect specific features of healthspan (cognition, stress resistance, metabolic homeostasis, or physical performance) in mouse and rat models of physiological ageing. Compared to previous searches, the originality of this study is the focus on healthspan, rather than lifespan.

Overall, results of this study indicate that natural products administered in aged healthy rodents show a number of effects on stress resistance and cognitive abilities, which offer promise for translation to human ageing. Indeed, for our search we chose specifically to focus on rodent models (rat and mouse) of physiological ageing, rather than models of specific diseases of ageing, as the relatively mild action of natural compounds may not be sufficiently strong to reverse a disease process (Lee et al., 2014). Furthermore, based upon the hypothesised mechanism of action, prolonged use of natural compounds at moderate dosages, for a relatively long period, should be able to prevent, rather than cure, disorders of ageing (see below).

We have previously operationalised health through a series of terms that have been used in this investigation to search through the literature (as previously indicated by Fuellen et al., 2019). Based upon these search terms overall 39 studies were included in the systematic review. On the final selected papers, targeted meta-analyses were subsequently performed to quantitatively assess the effect of these compounds on memory retention and oxidative stress.

As far as the basic characteristics of the studies included in the

systematic review, most of these were conducted in China (or more generally in Asia), and only a few in Europe or in other non-European countries. This geographical distribution is in line with the fact that oriental traditional medicine has been using mixtures of herbs and other natural compounds to promote health for thousands of years. The compounds administered varied greatly among the studies selected for final analysis, as did the doses and the duration of the treatment, as well as the administration route. Studies were equally distributed between the two species selected (rats and mice). Most of the studies used males, so sex differences in the effects cannot be appreciated by our analyses. The majority of the selected studies found significant positive effects of natural compounds on the dependent measures studied, ranging from an improvement in oxidative stress parameters, greater neural plasticity, improved physical performance and better memory in a number of behavioural tests.

Stress resistance is considered as the key to a long and healthy life (Mattson and Cheng, 2006; Trewavas and Stewart, 2003). Papers in our systematic review mostly indicated generalized effects of natural compounds on stress resistance: two studies (Guo et al., 2014; Lee and Oh, 2015) measured markers of peripheral and central inflammation, suggesting beneficial effects of natural products on the immune system, while one study (Pyrzanowska et al., 2010) evaluated activation of the HPA axis, finding decreased levels of plasma corticosterone, suggesting that natural compounds might hold potential for decreasing neuroendocrine responses to stressors. Twelve studies assessed the effect of natural compounds specifically on oxidative stress parameters, analysing the balance between antioxidant defences and products of oxidation processes. Of these, the 11 studies included in the meta-analysis indicate that administration of the highest doses led to a significant improvement of oxidative stress in animal models of physiological ageing. Five studies measured levels of antioxidant enzymes (i.e. SOD, CAT, GHP-Px) and mRNA of specific genes regulating the expression of antioxidant proteins (i.e. Nrf2) in peripheral tissues such as blood and liver (Chen et al., 2019; Gray et al., 2016; Taridi et al., 2014; Zhang et al., 2015; Zhao et al., 2019). Six studies evaluated the same parameters in addition to levels of the enzyme glutathione reductase, total thiols and ascorbic acid, in different brain areas such as hippocampus, prefrontal cortex, cortex and striatum (Asseburg et al., 2016; Gray et al., 2016; Lee and Oh, 2015; Papandreou et al., 2011; Rastogi et al., 2012; Song et al., 2009). All these data indicate a positive effect of nutraceuticals in boosting the antioxidant defences of an aged organism. In four studies, levels of peripheral MDA (liver and blood), a marker of oxidative stress, were found decreased in treated subjects (Chen et al., 2019; Taridi et al., 2014; Zhang et al., 2015; Zhao et al., 2019). Likewise, five studies indicated reduced levels of MDA, TBARS, ROS, LHP and protein carbonyl in brain areas of treated subjects (Balu et al., 2005; Papandreou et al., 2011; Rastogi et al., 2012; Song et al., 2009; Topic et al., 2002). While improvement in stress resistance was observed both in peripheral tissues and in the central nervous system, it is important to underline that the central nervous system is particularly vulnerable to oxidative damage, thus such effects could bear a specific advantage for the ageing brain.

4.1. Stress resistance as a key mechanism in healthspan

Comparable effects across species suggest basic common mechanisms and pathways. Many plant extracts are naturally rich in polyphenols, which are well recognized for their antioxidant activities. However, the amount present in the diet is hardly sufficient to achieve the concentrations required to act as free radical scavengers, as also indicated by the ineffectiveness of antioxidants such as vitamin E in clinical trials (Williams and Fisher, 2005). Indeed, most clinical trials of antioxidants administered supra-physiological doses, and perhaps for this reason some showed detrimental effects.

There is evidence that combining different anti-ageing compounds may have additive or even synergistic effects (Admasu et al., 2018; Castillo-Quan et al., 2019; Dakik et al., 2019). This suggests multiple mechanisms and pathways (mutations, ROS, protein damage, autophagy, mitochondria, telomeres, immunity, etc.). Some compounds may affect only one or several aspects of ageing, but compounds that affect the core ageing process should have a beneficial effect on many organ systems and functions. These multiple pathways may then impinge upon common mechanisms that enable the organism to thrive in the face of stressors and age.

Low amounts of toxic substances or stressors may enable stress-sensitive pathways to be engaged at sub-threshold level, rendering the organisms more resistant to subsequent stressors or challenges (Mattson and Cheng, 2006; Ristow and Schmeisser, 2014; Zimmermann et al., 2014). According to the “hormesis hypothesis” cells (throughout the body and the brain) may recognize phytochemicals as potentially dangerous, and respond adaptively by engaging stress signalling pathways that enhance the resistance of the organism to stressors that can cause disease(s). This may drive an adaptive cellular program that is central to healthspan, as in *C. elegans* (Maglioni et al., 2014; Möller et al., 2018). The relevance of hormesis is now starting to get more and more recognized (Calabrese et al., 2010; Lee et al., 2014; Mattson and Cheng, 2006; Ristow and Schmeisser, 2014; Zimmermann et al., 2014). Hormesis can engage multiple pathways, from activation of proteostasis to the induction of autophagy (Zimmermann et al., 2014). It is of interest that different conditions/activities that have been shown to promote health (physical exercise, dietary energy restriction and cognitive stimulation) all induce adaptive cellular stress responses in neurons, resulting in enhanced neurogenesis and synaptic plasticity, and resistance to injury and disease (Lee et al., 2014). Even a mild dietary stress, that is calorie restriction without malnutrition, might exert its beneficial effects through hormesis, similar to moderate exercise regimens (Gómez-Pinilla, 2008). One specific pathway involves the transcription factor NRF-2, which binds the antioxidant response element (ARE) upstream of genes encoding cytoprotective antioxidant enzymes and phase-2 proteins. The latter pathway is activated by curcumin, sulforaphane (present in broccoli) and allicin (present in garlic) (Balogun et al., 2003; Chen et al., 2004; Dinkova-Kostova et al., 2002; Zhang et al., 2007a, 2007b). Other phytochemicals may activate the sirtuin – FOXO pathway resulting in increased expression of antioxidant enzymes and cell survival-promoting proteins; resveratrol has been shown to activate this pathway (Frescas et al., 2005). Ingestion of other phytochemicals may activate the hormetic transcription factors NF-κB and CREB, resulting in the induction of genes encoding growth factors and anti-apoptotic proteins (Mabuchi et al., 2001; Mattson and Meffert, 2006). In our sample of studies, both NRF2 and the CREB signalling pathways were found to be activated following administration of natural compounds.

4.2. Motor function

Senescence is a biological process that relies upon physio-morphological changes leading to functional decay of tissues, organs and systems, with a deep impact on physical performance. In our systematic review, we found that only 10 papers out of 39 investigated physical performance in response to administration of natural compounds as a relevant trait of healthspan. The main aspects of physical performance that were investigated comprised: locomotor activity (automated recording, spontaneous activity, open field, running wheel), physical strength (grip strength, inclined screen test) and motor balance/motor coordination (rotarod test). Of these, six found a specific improvement in at least one of the physical performance parameters investigated upon administration of the natural product, while four reported no difference between treated and control subjects. Motor decline greatly affects the quality of life during ageing, and seems to be the end result of multi-factorial causes involving changes in the musculoskeletal system, hormones and oxidative stress in the peripheral and central nervous system (Tudoraşcu et al., 2014). As for the

mechanisms involved in this age-related decline, hormonal changes, oxidative stress and mitochondrial dysfunctions may well play a role (Jang and Van Remmen, 2011; Rygiel et al., 2014; Thompson, 2009). We have recently provided evidence for trehalose (a natural compound that can be found in algae, fungi, insects and yeast) to prevent the time-dependent decrease in motor function in senescent mice tested on beam walking, and to improve motor learning as assessed in the rotarod test; these effects were associated to a sex-dependent increase in antioxidant defences in the brain, particularly NRF2 (Berry et al., 2020). This piece of data suggests that trehalose might act by improving not only purely physical performance but also motor learning abilities that rely upon central coordination, possibly through mechanisms involving central modulation of oxidative stress status. Such hypothesis is here confirmed and enlarged to a range of other natural compounds by the above-mentioned effects observed on stress resistance.

4.3. Cognition and healthspan

A large body of evidence leads to a general consensus that the majority of old humans showing cognitive decline are characterised by deficits in learning and retention of novel acquired information (episodic memory impairment). This issue becomes apparent in everyday life when retention over a delay is needed (e.g. remembering conversations and appointments or a shopping list or a news event) (Albert et al., 2011). In the laboratory, episodic memory can be assessed in rodents by a range of tasks. In this regard, one of the most widely used and reliable tests is the MWM (Brandeis et al., 1989; Morris, 1984; Vorhees and Williams, 2014). In this systematic review, we found that 20 studies assessed the effectiveness of natural products on learning abilities. Of these, all but three found an improvement in this parameter assessed mainly - although not exclusively - by testing spatial abilities. As for the remaining three studies, no difference in learning abilities was reported between treated and control subjects. As for memory retention, we found that 22 studies investigated this specific aspect of healthspan, and 21 were included in our meta-analysis. Indeed, we confirm that the MWM was the most-used test to assess memory retention (68 % of the studies included) followed by PA, Y-maze, modified Y-maze with electric shock, T-maze and CSD (in order of abundance).

Overall, taking into account both the results from the systematic review and the meta-analysis, natural compounds appear effective in counteracting age-related cognitive decline, resulting in the absence of overt pathology or memory impairment. This result is corroborated by ex vivo analyses on different brain areas. Interestingly, most of the different compounds administered were able to affect neurotransmitters systems, neuronal plasticity, or neurogenesis and neuronal proliferation in brain areas such as the prefrontal cortex, hippocampus, striatum and hypothalamus. In detail, an investigation on the functionality of major neurotransmitter systems (cholinergic, dopaminergic, noradrenergic, serotoninergic) was carried-out in 12 studies. Three studies used immunohistochemistry techniques to assess neurogenesis and neuronal differentiation in the hippocampus. Two of these (Liu et al., 2014; Osman et al., 2016) indicated increased levels of neurogenesis and proliferation through specific markers, such as DCX-, BrdU-, Ki67-cells +, in nutraceutical-treated subjects; while one of these did not show any changes (Bensalem et al., 2018). Eight papers focused on neuronal plasticity, measuring the expression of mRNA (i.e. *Ngf*, *Bdnf*) and proteins (i.e. NGF, BDNF, GAP-43, SNAP-25, PSD95, synaptophysin, CaMKII-α, mTOR, pCREB, pPKA) involved in the remodelling and functionality of synapses, mainly in the hippocampus. Data from these studies show beneficial effects of natural compounds in preventing the age-related reduction of neuronal plasticity.

Interestingly, the vast majority of animal studies indicate beneficial effects of natural compounds in a dose-dependent manner with the highest dosages generally being more effective compared to the lowest dosages (e.g. Reeta et al., 2009; Sun et al., 2013; Tiwari et al., 2019; Tiwari and Chopra, 2013; Wang et al., 2014; Zhang et al., 2015). However, sensitivity analyses demonstrated a significant effect of natural products on memory retention and oxidative stress also at the lowest dose.

4.4. Limitations

There are some limitations of this study that need to be acknowledged. Studies brought together in our systematic review were heterogeneous with respect to many factors, as is common in animal studies (Hooijmans, IntHout et al., 2014; Hooijmans, Rovers et al., 2014; Vesterinen et al., 2014). The extent of heterogeneity (differences between treatment effects from individual studies in the meta-analysis) was considerable, both in the main analyses and within each subgroup. The presence of heterogeneity affects the extent to which generalizable conclusions can be arrived at, and limits our ability to draw firm conclusions about treatment effect within the subgroups, in particular in mouse and rat models. Future studies with larger sample sizes should further explore heterogeneity in other factors (e.g., strain, procedures, etc.). A specific interest on how methodological aspects of studies relate to their results is recommended. Another limitation has to do with the quality of the studies which we found hard to assess for the lack of information on the methodology used, especially regarding randomization procedures. Moreover, it is important to note that conclusions exclusively based on published studies can be misleading. In our study a potential source of type I error (increase of false positive results) in both meta-analyses (cognition and stress resistance) was evidenced by asymmetry in the funnel plots which occurs when statistically significant "positive" results have a better chance of being published. This calls for an effort on the part of researchers and editors to publish studies which are non-significant or have an effect in the opposite direction from that expected, in order to have a clearer picture of the effects of nutraceuticals on the investigated outcomes.

5. Conclusions

There is an increasing interest in natural compounds; the lack of effective pharmacotherapy has led researchers to seek alternative approaches to deal with conditions such as neuroinflammation, oxidative stress, mitochondrial dysfunction, or autophagy, which have been linked to disorders of ageing (Amor et al., 2014, 2010; Guo et al., 2018; Kim et al., 2015). The emerging evidence that natural compounds can activate hormetic responses in central and peripheral tissues, can be exploited for basic and applied research in the field of ageing (Mattson, 2008).

More information is clearly needed to establish the right dose and the frequency of administration. At the same time, it might be more appropriate to target multiple molecular targets/pathways. Indeed, most of the studies that were selected in this search indicate that traditional medicines use mostly mixtures of phytochemicals, rather than individual compounds: plant extracts are composed of many substances that may act on multiple molecular targets in an additive or even synergistic manner (Long et al., 2015; Tewari et al., 2018). In the future, research focusing on identifying active plant ingredients and investigating their mechanisms of action is needed to ensure safety and to maximize their therapeutic potential (Tewari et al., 2018).

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and WL. All authors approved the final version of the manuscript.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.neubiorev.2020.12.001.

References

Admasu, T.D., Chaithanya Batchu, K., Barardo, D., Ng, L.F., Lam, V.Y.M., Xiao, L., Cazenave-Gassiot, A., Wenk, M.R., Tolwinski, N.S., Gruber, J., 2018. Drug synergy slows aging and improves healthspan through IGF and SREBP lipid signaling. Dev. Cell 47, 67–79. https://doi.org/10.1016/j.devcel.2018.09.001 e5.

Albert, M.S., Dekosky, S.T., Dickson, D., Dubois, B., Feldman, H.H., Fox, N.C., Gamst, A., Holtzman, D.M., Jagust, W.J., Petersen, R.C., Snyder, P.J., Carrillo, M.C., Thies, B., Phelps, C.H., 2011. The diagnosis of mild cognitive impairment due to Alzheimer’s disease: recommendations from the National Institute on Aging-Alzheimer’s Association workgroups on diagnostic guidelines for Alzheimer’s disease. Alzheimers Dement. 7, 270–279. https://doi.org/10.1016/j.jalz.2011.03.008.

Amor, S., Puentes, F., Baker, D., Van Der Valk, P., 2010. Inflammation in neurodegenerative diseases. Immunology. https://doi.org/10.1111/j.1365-2567.2009.03225.x.

Amor, S., Peferoen, L.A.N., Vogel, D.Y.S., Breur, M., van der Valk, P., Baker, D., Van Noort, J.M., 2014. Inflammation in neurodegenerative diseases - an update. Immunology. https://doi.org/10.1111/imm.12233.

Asseburg, H., Schäfer, C., Müller, M., Hagl, S., Pohland, M., Berressem, D., Borchiellini, M., Plank, C., Eckert, G.P., 2016. Effects of grape skin extract on age-related mitochondrial dysfunction, memory and life span in C57BL/6J mice. Neuromolecular Med. 18, 378–395. https://doi.org/10.1007/s12017-016-8428-4.

Balogun, E., Hoque, M., Gong, P., Killeen, E., Green, C.J., Foresti, R., Alam, J., Motterlini, R., 2003. Curcumin activates the haem oxygenase-1 gene via regulation of Nrf2 and the antioxidant-responsive element. Biochem. J. 371, 887–895. https://doi.org/10.1042/BJ20021619.

Balu, M., Sangeetha, P., Murali, G., Panneerselvam, C., 2005. Age-related oxidative protein damages in central nervous system of rats: modulatory role of grape seed extract. Int. J. Dev. Neurosci. 23, 501–507. https://doi.org/10.1016/j.ijdevneu.2005.06.001.

Beracochea, D., Krazem, A., Henkouss, N., Haccard, G., Roller, M., Fromentin, E., 2016. Intake of Wild Blueberry Powder Improves Episodic-Like and Working Memory during Normal Aging in Mice. Planta Med. 82 (13), 1163–1168. https://doi.org/10.1055/s-0042-104419. Epub 2016 Apr 19.

Bensalem, J., Dudonné, S., Gaudout, D., Servant, L., Calon, F., Desjardins, Y., Layé, S., Lafenetre, P., Pallet, V., 2018. Polyphenol-rich extract from grape and blueberry attenuates cognitive decline and improves neuronal function in aged mice. J. Nutr. Sci. 7, e19. https://doi.org/10.1017/jns.2018.10.

Berry, A., Cirulli, F., 2013. The p66(Shc) gene paves the way for healthspan: evolutionary and mechanistic perspectives. Neurosci. Biobehav. Rev. 37, 790–802. https://doi.org/10.1016/j.neubiorev.2013.03.005.

Berry, A., Greco, A., Giorgio, M., Pelicci, P.G., de Kloet, R., Alleva, E., Minghetti, L., Cirulli, F., 2008. Deletion of the lifespan determinant p66(Shc) improves performance in a spatial memory task, decreases levels of oxidative stress markers in the hippocampus and increases levels of the neurotrophin BDNF in adult mice. Exp. Gerontol. 43, 200–208. https://doi.org/10.1016/j.exger.2007.10.016.

Berry, A., Marconi, M., Musillo, C., Chiarotti, F., Bellisario, V., Matarrese, P., Gambardella, L., Vona, R., Lombardi, M., Foglieni, C., Cirulli, F., 2020. Trehalose administration in C57BL/6N old mice affects healthspan improving motor learning and brain anti-oxidant defences in a sex-dependent fashion: a pilot study. Exp. Gerontol. 129 https://doi.org/10.1016/j.exger.2019.110755.

Brandeis, R., Brandys, Y., Yehuda, S., 1989. The use of the Morris water maze in the study of memory and learning. Int. J. Neurosci. 48, 29–69. https://doi.org/10.3109/00207458909002151.

Budovsky, A., Muradian, K.K., Fraifeld, V.E., 2006. From disease-oriented to aging/longevity-oriented studies, in: rejuvenation Research. Rejuvenation Res. 207–210. https://doi.org/10.1089/rej.2006.9.207.

Budovsky, A., Shteinberg, A., Maor, H., Duman, O., Yanai, H., Wolfson, M., Fraifeld, V.E., 2014. Uncovering the geroprotective potential of medicinal plants from the Judea region of Israel. Rejuvenation Res. 17, 134–139. https://doi.org/10.1089/rej.2013.1509.

Calabrese, V., Cornelius, C., Dinkova-Kostova, A.T., Calabrese, E.J., Mattson, M.P., 2010. Cellular stress responses, the hormesis paradigm, and vitagenes: novel targets for therapeutic intervention in neurodegenerative disorders. Antioxidants Redox Signal. https://doi.org/10.1089/ars.2009.3074.

Campisi, J., Kapahi, P., Lithgow, G.J., Melov, S., Newman, J.C., Verdin, E., 2019. From discoveries in ageing research to therapeutics for healthy ageing. Nature. https://doi.org/10.1038/s41586-019-1365-2.

Castillo-Quan, J.I., Tain, L.S., Kinghorn, K.J., Li, L., Grönke, S., Hinze, Y., Blackwell, T.K., Bjedov, I., Partridge, L., 2019. A triple drug combination targeting components of the nutrient-sensing network maximizes longevity. Proc. Natl. Acad. Sci. U. S. A. 116, 20817–20819. https://doi.org/10.1073/pnas.1913212116.

Chen, C., Pung, D., Leong, V., Hebbar, V., Shen, G., Nair, S., Li, W., Tony Kong, A.N., 2004. Induction of detoxifying enzymes by garlic organosulfur compounds through transcription factor Nrf2: effect of chemical structure and stress signals. Free Radic. Biol. Med. 37, 1578–1590. https://doi.org/10.1016/j.freeradbiomed.2004.07.021.

Chen, W., Wang, J., Shi, J., Yang, X., Yang, P., Wang, N., Yang, S., Xie, T., Yang, H., Zhang, M., Wang, H., Fei, J., 2019. Longevity effect of Liuwei Dihuang in both Caenorhabditis elegans and aged mice. Aging Dis. 10, 578. https://doi.org/10.14336/ad.2018.0604.

Dakik, P., McAuley, M., Chancharoen, M., Mitrofanova, D., Rodriguez, M.E.L., Junio, J.A. B., Lutchman, V., Cortes, B., Simard, É., Titorenko, V.I., 2019. Pairwise combinations of chemical compounds that delay yeast chronological aging through different signaling pathways display synergistic effects on the extent of aging delay. Oncotarget 10, 313–338. https://doi.org/10.18632/oncotarget.26553.

de Vries, R.B.M., Hooijmans, C.R., Langendam, M.W., van Luijk, J., Leenaars, M., Ritskes-Hoitinga, M., Wever, K.E., 2015. A protocol format for the preparation, registration and publication of systematic reviews of animal intervention studies. Evidence-based Preclin. Med. 2, e00007 https://doi.org/10.1002/ebm2.7.

Dinkova-Kostova, A.T., Holtzclaw, W.D., Cole, R.N., Itoh, K., Wakabayashi, N., Katoh, Y., Yamamoto, M., Talalay, P., 2002. Direct evidence that sulfhydryl groups of Keap1 are the sensors regulating induction of phase 2 enzymes that protect against carcinogens and oxidants. Proc. Natl. Acad. Sci. U. S. A. 99, 11908–11913. https://doi.org/10.1073/pnas.172398899.

Egger, M., Smith, G.D., Schneider, M., Minder, C., 1997. Bias in meta-analysis detected by a simple, graphical test measures of funnel plot asymmetry. Bmj 315, 629–634. https://doi.org/10.1136/bmj.315.7109.629.

Finkel, T., 2015. The metabolic regulation of aging. Nat. Med. https://doi.org/10.1038/nm.3998.

Frescas, D., Valenti, L., Accili, D., 2005. Nuclear trapping of the forkhead transcription factor FoxO1 via sirt-dependent deacetylation promotes expression of glucogenetic genes. J. Biol. Chem. 280, 20589–20595. https://doi.org/10.1074/jbc.M412357200.

Fuellen, G., Jansen, L., Cohen, A.A., Luyten, W., Gogol, M., Simm, A., Saul, N., Cirulli, F., Berry, A., Antal, P., Köhling, R., Wouters, B., Möller, S., 2019. Health and aging: unifying concepts, scores, biomarkers and pathways. Aging Dis. https://doi.org/10.14336/AD.2018.1030.

Gómez-Pinilla, F., 2008. Brain foods: the effects of nutrients on brain function. Nat. Rev. Neurosci. https://doi.org/10.1038/nrn2421.

Gray, N.E., Harris, C.J., Quinn, J.F., Soumyanath, A., 2016. Centella asiatica modulates antioxidant and mitochondrial pathways and improves cognitive function in mice. J. Ethnopharmacol. 180, 78–86. https://doi.org/10.1016/j.jep.2016.01.013.

Guo, Y., Niu, K., Okazaki, T., Wu, H., Yoshikawa, T., Ohrui, T., Furukawa, K., Ichinose, M., Yanai, K., Arai, H., Huang, G., Nagatomi, R., 2014. Coffee treatment prevents the progression of sarcopenia in aged mice in vivo and in vitro. Exp. Gerontol. 50, 1–8. https://doi.org/10.1016/j.exger.2013.11.005.

Guo, F., Liu, X., Cai, H., Le, W., 2018. Autophagy in neurodegenerative diseases: pathogenesis and therapy. Brain Pathol. 28, 3–13. https://doi.org/10.1111/bpa.12545.

Higgins, J.P.T., Thompson, S.G., 2002. Quantifying heterogeneity in a meta-analysis. Stat. Med. 21, 1539–1558. https://doi.org/10.1002/sim.1186.

Higgins, J.P.T., Thompson, S.G., Deeks, J.J., Altman, D.G., 2003. Measuring inconsistency in meta-analyses. Br. Med. J. https://doi.org/10.1136/bmj.327.7414.557.

Higgins, J., Thomas, J., Chandler, J., Cumpston, M., Li, T., Page, M., Welch, V. (Eds.), 2019. Cochrane Handbook for Systematic Reviews of Interventions, 2nd ed. John Wiley & Sons, Chichester (UK).

Hooijmans, Carlijn R., IntHout, J., Ritskes-Hoitinga, M., Rovers, M.M., 2014. Meta-analyses of animal studies: an introduction of a valuable instrument to further improve healthcare. ILAR J. 55 https://doi.org/10.1093/ILAR/ILU042.

Hooijmans, C.R., Rovers, M.M., de Vries, R.B., Leenaars, M., Ritskes-Hoitinga, M., Langendam, M.W., 2014. SYRCLE’s risk of bias tool for animal studies. BMC Med. Res. Methodol. 14, 43. https://doi.org/10.1186/1471-2288-14-43.

Jaenicke, B., Kim, E.J., Ahn, J.W., Lee, H.S., 1991. Effect of Panax ginseng extract on passive avoidance retention in old rats. Arch. Pharm. Res. 14 (1), 25–29. https://doi.org/10.1007/BF02857809.

Jang, Y.C., Van Remmen, H., 2011. Age-associated alterations of the neuromuscular junction. Exp. Gerontol. https://doi.org/10.1016/j.exger.2010.08.029.

Kaeberlein, M., 2018. How healthy is the healthspan concept? GeroScience. https://doi.org/10.1007/s11357-018-0036-9.

Kessler, A.R., Kessler, B., Yehuda, S., 1986. In vivo modulation of brain cholesterol level and learning performance by a novel plant lipid: indications for interactions between hippocampal-cortical cholesterol and learning. Comparative Study Life Sci. 38 (13), 1185–1192. https://doi.org/10.1016/0024-3205(86)90173-6.

Kim, G.H., Kim, J.E., Rhie, S.J., Yoon, S., 2015. The role of oxidative stress in neurodegenerative diseases. Exp. Neurobiol. 24, 325–340. https://doi.org/10.5607/en.2015.24.4.325.

Lee, Y., Oh, S., 2015. Administration of red ginseng ameliorates memory decline in aged mice. J. Ginseng Res. 39, 250–256. https://doi.org/10.1016/j.jgr.2015.01.003.

Lee, J., Jo, D.G., Park, D., Chung, H.Y., Mattson, M.P., 2014. Adaptive cellular stress pathways as therapeutic targets of dietary phytochemicals: focus on the nervous system. Pharmacol. Rev. 66, 815–868. https://doi.org/10.1124/pr.113.007757.

Li, X.L., Wang, D.S., Zhao, B.Q., Li, Q., Qu, H.Y., Zhang, T., Zhou, J.P., Sun, M.J., 2008. Effects of Chinese herbal medicine fuzhisan on aged rats. Exp Gerontol. 43 (9), 853–858. https://doi.org/10.1016/j.exger.2008.05.018. Epub 2008 Jun 19.

Li, Q., Zhao, H.F., Zhang, Z.F., Liu, Z.G., Pei, X.R., Wang, J.B., Cai, M.Y., Li, Y., 2009. Long-term administration of green tea catechins prevents age-related spatial learning and memory decline in C57BL/6 J mice by regulating hippocampal cyclic amp-response element binding protein signaling cascade. Neuroscience. 159 (4), 1208–1215. https://doi.org/10.1016/j.neuroscience.2009.02.008. Epub 2009 Feb 11.

Liberati, A., Altman, D.G., Tetzlaff, J., Mulrow, C., Gøtzsche, P.C., Ioannidis, J.P.A., Clarke, M., Devereaux, P.J., Kleijnen, J., Moher, D., 2009. The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate health care interventions: explanation and elaboration. PLoS Med. 6, e1000100 https://doi.org/10.1371/journal.pmed.1000100.

Liu, L., Zhang, M., Zhang, R., Lee, M., Wang, Z., Hou, J., Sung, C.K., 2014. The multi-herbal formula Chong-Myung-Tang improves spatial memory and increases cell genesis in the dentate gyrus of aged mice. Biosci. Biotechnol. Biochem. 78, 1710–1715. https://doi.org/10.1080/09168451.2014.930319.

Long, F., Yang, H., Xu, Y., Hao, H., Li, P., 2015. A strategy for the identification of combinatorial bioactive compounds contributing to the holistic effect of herbal medicines. Sci. Rep. 5 https://doi.org/10.1038/srep12361.

Luyten, W., Antal, P., Braeckman, B.P., Bundy, J., Cirulli, F., Fang-Yen, C., Fuellen, G., Leroi, A., Liu, Q., Martorell, P., Metspalu, A., Perola, M., Ristow, M., Saul, N., Schoofs, L., Siems, K., Temmerman, L., Smets, T., Wolk, A., Rattan, S.I.S., 2016. Ageing with elegans: a research proposal to map healthspan pathways. Biogerontology 17, 771–782. https://doi.org/10.1007/s10522-016-9644-x.

Mabuchi, T., Kitagawa, K., Kuwabara, K., Takasawa, K., Ohtsuki, T., Xia, Z., Storm, D., Yanagihara, T., Hori, M., Matsumoto, M., 2001. Phosphorylation of cAMP response element-binding protein in hippocampal neurons as a protective response after exposure to glutamate in vitro and ischemia in vivo. J. Neurosci. 21, 9204–9213. https://doi.org/10.1523/JNEUROSCI.21-23-09204.2001.

Maglioni, S., Schiavi, A., Runci, A., Shaik, A., Ventura, N., 2014. Mitochondrial stress extends lifespan in C. Elegans through neuronal hormesis. Exp. Gerontol. 56, 89–98. https://doi.org/10.1016/j.exger.2014.03.026.

Marx, W., Moseley, G., Berk, M., Jacka, F., 2017. Nutritional psychiatry: the present state of the evidence. Proc. Nutr. Soc. 76, 427–436. https://doi.org/10.1017/S0029665117002026.

Mattson, M.P., 2008. Dietary factors, hormesis and health. Ageing Res. Rev. https://doi.org/10.1016/j.arr.2007.08.004.

Mattson, M.P., Cheng, A., 2006. Neurohormetic phytochemicals: low-dose toxins that induce adaptive neuronal stress responses. Trends Neurosci. https://doi.org/10.1016/j.tins.2006.09.001.

Mattson, M.P., Meffert, M.K., 2006. Roles for NF-κB in nerve cell survival, plasticity, and disease. Cell Death Differ. https://doi.org/10.1038/sj.cdd.4401837.

Mizoguchi, K., Tanaka, Y., Tabira, T., 2010. Anxiolytic effect of a herbal medicine, yokukansan, in aged rats: involvement of serotonergic and dopaminergic transmissions in the prefrontal cortex. Comparative Study J Ethnopharmacol. 127 (1), 70–76. https://doi.org/10.1016/j.jep.2009.09.048. Epub 2009 Sep 30.

Moher, D., Liberati, A., Tetzlaff, J., Altman, D.G., 2009. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. PLoS Med. 6, e1000097 https://doi.org/10.1371/journal.pmed.1000097.

Möller, S., Saul, N., Cohen, A.A., Köhling, R., Sender, S., Escobar, H.M., Junghanss, C., Cirulli, F., Berry, A., Antal, P., Adler, P., Vilo, J., Boiani, M., Jansen, L., Repsilber, D., Grabe, H.J., Struckmann, S., Barrantes, I., Hamed, M., Wouters, B., Schoofs, L., Luyten, W., Fuellen, G., 2018. Healthspan pathway maps in C. Elegans and humans highlight transcription, proliferation/biosynthesis and lipids. bioRxiv 355131. https://doi.org/10.1101/355131.

Morris, R., 1984. Developments of a water-maze procedure for studying spatial learning in the rat. J. Neurosci. Methods 11, 47–60.

Ninomiya, H., Kato, S., Okuda, H., 2001. Effects of Hachimi-jio-gan in aged rats. J. Altern. Complement. Med. 7, 355–359. https://doi.org/10.1089/107555301750463233.

Nitta, H., Matsumoto, K., Shimizu, M., Watanabe, H., Ni, X.H., 1995. Panax ginseng extract improves the performance of aged fischer 344 rats in radial maze task but not in operant brightness discrimination task. Biol. Pharm. Bull. 18, 1286–1288. https://doi.org/10.1248/bpb.18.1286.

Ohta, H., Matsumoto, K., Shimizu, M., Watanabe, H., 1994. Paeoniflorin attenuates learning impairment of aged rats in operant brightness discrimination task. Pharmacol. Biochem. Behav. 49, 213–217. https://doi.org/10.1016/0091-3057(94)90478-2.

Osman, N.M.S., Amer, A.S., Abdelwahab, S., 2016. Effects of Ginko biloba leaf extract on the neurogenesis of the hippocampal dentate gyrus in the elderly mice. Anat. Sci. Int. 91, 280–289. https://doi.org/10.1007/s12565-015-0297-7.

Papandreou, M.A., Tsachaki, M., Efthimiopoulos, S., Cordopatis, P., Lamari, F.N., Margarity, M., 2011. Memory enhancing effects of saffron in aged mice are correlated with antioxidant protection. Behav. Brain Res. 219, 197–204. https://doi.org/10.1016/j.bbr.2011.01.007.

Paula-Freire, L.I.G., Mendes, F.R., Molska, G.R., Duarte-Almeida, J.M., Carlini, E.A., 2013. Comparison of the chemical composition and biological effects of the roots, branches and leaves of Heteropterys tomentosa A. Juss. J. Ethnopharmacol. 145, 647–652. https://doi.org/10.1016/j.jep.2012.12.004.

Pyrzanowska, J., Piechal, A., Blecharz-Klin, K., Lehner, M., Skórzewska, A., Turzyńska, D., Sobolewska, A., Plaznik, A., Widy-Tyszkiewicz, E., 2010. The influence of the long-term administration of Curcuma longa extract on learning and spatial memory as well as the concentration of brain neurotransmitters and level of plasma corticosterone in aged rats. Pharmacol. Biochem. Behav. 95, 351–358. https://doi.org/10.1016/j.pbb.2010.02.013.

Rastogi, M., Ojha, R.P., Prabu, P.C., Devi, B.P., Agrawal, A., Dubey, G.P., 2012. Prevention of age-associated neurodegeneration and promotion of healthy brain ageing in female wistar rats by long term use of bacosides. Biogerontology 13, 183–195. https://doi.org/10.1007/s10522-011-9367-y.

Reeta, K.H., Mehla, J., Kumar Gupta, Y., 2009. Curcumin Is Protective Against Phenytoin-induced Cognitive Impairment and Oxidative Stress in Rats. https://doi.org/10.1016/j.brainres.2009.09.027.

Ristow, M., Schmeisser, K., 2014. Mitohormesis: promoting health and lifespan by increased levels of reactive oxygen species (ROS). DoseResponse 12, 288–341. https://doi.org/10.2203/dose-response.13-035.Ristow.

Rubio-Aparicio, M., Sánchez-Meca, J., López-López, J.A., Botella, J., Marín-Martínez, F., 2017. Analysis of categorical moderators in mixed-effects meta-analysis: consequences of using pooled versus separate estimates of the residual between-studies variances. Br. J. Math. Stat. Psychol. 70, 439–456. https://doi.org/10.1111/bmsp.12092.

Rygiel, K.A., Grady, J.P., Turnbull, D.M., 2014. Respiratory chain deficiency in aged spinal motor neurons. Neurobiol. Aging 35, 2230–2238. https://doi.org/10.1016/j.neurobiolaging.2014.02.027.

Song, H.R., Cheng, J.J., Miao, H., Shang, Y.Z., 2009. Scutellaria flavonoid supplementation reverses ageing-related cognitive impairment and neuronal changes in aged rats. Brain Inj. 23, 146–153. https://doi.org/10.1080/02699050802649670.

Sun, C.Y., Qi, S.S., Zhou, P., Cui, H.R., Chen, S.X., Dai, K.Y., Tang, M.L., 2013. Neurobiological and pharmacological validity of curcumin in ameliorating memory performance of senescence-accelerated mice. Pharmacol. Biochem. Behav. 105, 76–82. https://doi.org/10.1016/j.pbb.2013.02.002.

Taridi, N.M., Abd Rani, N., Abd Latiff, A., Ngah, W.Z.W., Mazlan, M., 2014. Tocotrienol rich fraction reverses age-related deficits in spatial learning and memory in aged rats. Lipids 49, 855–869. https://doi.org/10.1007/s11745-014-3919-2.

Tewari, D., Stankiewicz, A.M., Mocan, A., Sah, A.N., Tzvetkov, N.T., Huminiecki, L., Horbanczuk, J.O., Atanasov, A.G., 2018. Ethnopharmacological approaches for dementia therapy and significance of natural products and herbal drugs. Front. Aging Neurosci. 10, 3. https://doi.org/10.3389/fnagi.2018.00003.

Thompson, L.D.V., 2009. Age-related muscle dysfunction. Exp. Gerontol. 44, 106–111. https://doi.org/10.1016/j.exger.2008.05.003.

Tiwari, V., Chopra, K., 2013. Resveratrol abrogates alcohol-induced cognitive deficits by attenuating oxidative-nitrosative stress and inflammatory cascade in the adult rat brain. Neurochem. Int. 62, 861–869. https://doi.org/10.1016/j.neuint.2013.02.012.

Tiwari, S.K., Agarwal, S., Seth, B., Yadav, A., Nair, S., Bhatnagar, P., Karmakar, M., Kumari, M., Chauhan, L.K.S., Patel, D.K., Srivastava, V., Singh, D., Gupta, S.K., Tripathi, A., Chaturvedi, R.K., Gupta, K.C., 2019. Correction: curcumin-loaded nanoparticles potently induce adult neurogenesis and reverse cognitive deficits in alzheimers disease model via canonical wnt/-Catenin pathway (ACS nano (2014) 8:1 (76-103) DOI: 10.1021/nn405077y). ACS Nano. https://doi.org/10.1021/acsnano.9b03830.

Topic, B., Tani, E., Tsiakitzis, K., Kourounakis, P.N., Dere, E., Hasenöhrl, R.U., Häcker, R., Mattern, C.M., Huston, J.P., 2002. Enhanced maze performance and reduced oxidative stress by combined extracts of zingiber officinale and ginkgo biloba in the aged rat. Neurobiol. Aging 23, 135–143. https://doi.org/10.1016/s0197-4580(01)00241-x.

Trewavas, A., Stewart, D., 2003. Paradoxical effects of chemicals in the diet on health. Curr. Opin. Plant Biol. https://doi.org/10.1016/S1369-5266(03)00011-6.

Tudoraşcu, I., Sfredel, V., Riza, A.L., Dănciulescu Miulescu, R., Ianoşi, S.L., Dănoiu, S., 2014. Motor unit changes in normal aging: a brief review. Rom. J. Morphol. Embryol. 55, 1295–1301.

Velavan, S., Hazeena Begum, V.M., 2007. Modulatory role of Asparagus racemosus on glucose homeostasis in aged rats. Int. J. Pharmacol. 3, 149–154. https://doi.org/10.3923/ijp.2007.149.154.

Vesterinen, H.M., Sena, E.S., Egan, K.J., Hirst, T.C., Churolov, L., Currie, G.L., Antonic, A., Howells, D.W., Macleod, M.R., 2014. Meta-analysis of data from animal studies: a practical guide. J. Neurosci. Methods. https://doi.org/10.1016/j.jneumeth.2013.09.010.

Vorhees, C.V., Williams, M.T., 2014. Assessing spatial learning and memory in rodents. ILAR J. 55, 310–332. https://doi.org/10.1093/ilar/ilu013.

Wang, C., Zhang, X., Teng, Z., Zhang, T., Li, Y., 2014. Downregulation of PI3K/Akt/mTOR signaling pathway in curcumin-induced autophagy in APP/PS1 double transgenic mice. Eur. J. Pharmacol. 740, 312–320. https://doi.org/10.1016/j.ejphar.2014.06.051.

Ward, C.P., Redd, K., Williams, B.M., Caler, J.R., Luo, Y., McCoy, J.G., 2002. Ginkgo biloba extract: cognitive enhancer or antistress buffer. Pharmacol. Biochem. Behav. 72, 913–922. https://doi.org/10.1016/S0091-3057(02)00768-2.

Williams, K.J., Fisher, E.A., 2005. Oxidation, lipoproteins, and atherosclerosis: which is wrong, the antioxidants or the theory? Curr. Opin. Clin. Nutr. Metab. Care. https://doi.org/10.1097/00075197-200503000-00006.

World health statistics, 2019. Monitoring Health for the SDGs, Sustainable Development Goals. World Health Organization, Geneva, 2019. Licence: CC BY-NC-SA 3.0 IGO.

Zhang, X.L., Jiang, B., Li, Z.B., Hao, S., An, L.J., 2007a. Catalpol ameliorates cognition deficits and attenuates oxidative damage in the brain of senescent mice induced by D-galactose. Pharmacol. Biochem. Behav. 88, 64–72. https://doi.org/10.1016/j.pbb.2007.07.004.

Zhang, H., Han, T., Yu, C.-H., Rahman, K., Qin, L.-P., Peng, C., 2007b. Ameliorating effects of essential oil from Acori graminei rhizoma on learning and memory in aged rats and mice. J. Pharm. Pharmacol. 59, 301–309. https://doi.org/10.1211/jpp.59.2.0016.

Zhang, S.Q., Cai, W.J., Huang, J.H., Wu, B., Xia, S.J., Chen, X.L., Zhang, X.M., Shen, Z.Y., 2015. Icariin, a natural flavonol glycoside, extends healthspan in mice. Exp. Gerontol. 69, 226–235. https://doi.org/10.1016/j.exger.2015.06.020.

Zhao, S.-J., Liu, X.-J., Tian, J.-S., Gao, X.-X., Liu, H.-L., Du, G.-H., Qin, X.-M., 2019. Effects of Guilingji on aging rats and its underlying mechanisms. Rejuvenation Res. https://doi.org/10.1089/rej.2018.2118.

Zimmermann, A., Bauer, M.A., Kroemer, G., Madeo, F., Carmona-Gutierrez, D., 2014. When less is more: hormesis against stress and disease. Microb. Cell. https://doi.org/10.15698/mic2014.05.148.