

Systematic review of the antidepressant activity and associated antioxidant and anti-inflammatory effects of flavonoids in rodents

Revisão sistemática da atividade antidepressiva e dos efeitos antioxidantes e anti-inflamatórios dos flavonóides em roedores

Revisión sistemática de la actividad antidepresiva y los efectos antioxidantes y antiinflamatorios asociados de los flavonoides en roedores

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Abstract

Introduction: Flavonoids have received an increasing attention from the scientific community in the last decade due to its antioxidant and anti-inflammatory effects, showing benefits in various conditions, including major depression in animal models. The aim of this study was to review the evidence produced in the last 10 years regarding the antidepressant, antioxidant and anti-inflammatory effect of flavonoids in rodent models of depression. **Material and methods:** It was performed a systematic review to gather articles published between 2009 and 2019 that evaluate those effects of flavonoids in rodent models of depression. **Results:** 43 studies were included in the review. The most frequently studied flavonoids were hesperidin (14%) and baicalin (9%). The major natural source of flavonoids were citrus fruits (19%) and *Scutellaria baicalensis* Georgi (9%). Mice were used in the majority of the studies (86%). The majority of the studies did not use a specific model of depression (40%), and the most frequently used one was Chronic Unpredictable Mild Stress (21%). The most frequently used behavioral tests were forced swim test (81%), tail suspension test (56%) and open field test (51%). **Discussion:** Considering total tests, 93% of them presented an antidepressant activity, and all the studies that evaluated oxidative stress (37%) and inflammation (39%) found a significant antioxidant and anti-inflammatory result, respectively. **Conclusions:** Those findings demonstrate that the antidepressant, antioxidant and anti-inflammatory effects of flavonoids that were already evidenced in the study of other pathological conditions are also present in rodent depression models.

Keywords: Depression; Antidepressant; Flavonoids; Animal model.

Resumo

Introdução: Os flavonóides têm recebido cada vez mais atenção da comunidade científica na última década devido aos seus efeitos antioxidantes e anti-inflamatórios, apresentando benefícios em diversas condições, incluindo depressão maior em modelos animais. O objetivo deste estudo foi revisar as evidências produzidas nos últimos 10 anos sobre o efeito antidepressivo, antioxidante e anti-inflamatório dos flavonóides em modelos de depressão em roedores. **Material e métodos:** foi realizada uma revisão sistemática para reunir artigos publicados entre 2009 e 2019 que avaliam os efeitos dos flavonóides em modelos de depressão em roedores. **Resultados:** 43 estudos foram incluídos na revisão. Os flavonóides mais frequentemente estudados foram hesperidina (14%) e baicalina (9%). A principal fonte natural de

flavonóides foram frutas cítricas (19%) e *Scutellaria baicalensis* Georgi (9%). Camundongos foram usados na maioria dos estudos (86%). A maioria dos estudos não usou um modelo específico de depressão (40%), e o mais usado foi o Estresse Leve Imprevisível Crônico (21%). Os testes comportamentais mais usados foram o teste de natação forçada (81%), o teste de suspensão da cauda (56%) e o teste de campo aberto (51%). Discussão: Considerando o total de testes, 93% deles apresentaram atividade antidepressiva, e todos os estudos que avaliaram estresse oxidativo (37%) e inflamação (39%) encontraram resultado antioxidante e anti-inflamatório significativo, respectivamente. Conclusões: Esses achados demonstram que os efeitos antidepressivos, antioxidantes e anti-inflamatórios dos flavonóides já evidenciados no estudo de outras patologias também estão presentes em modelos de depressão em roedores.

Palavras-chave: Depressão; Antidepressivo; Flavonoides; Modelo animal.

Resumen

Introducción: Los flavonoides han recibido una atención creciente por parte de la comunidad científica en la última década debido a sus efectos antioxidantes y antiinflamatorios, mostrando beneficios en diversas afecciones, incluida la depresión mayor en modelos animales. El objetivo de este estudio fue revisar la evidencia producida en los últimos 10 años sobre el efecto antidepressivo, antioxidante y antiinflamatorio de los flavonoides en modelos de depresión en roedores. Material y métodos: Se realizó una revisión sistemática para recopilar artículos publicados entre 2009 y 2019 que evalúan los efectos de los flavonoides en modelos de depresión en roedores. Resultados: Se incluyeron 43 estudios en la revisión. Los flavonoides más estudiados fueron la hesperidina (14%) y la baicalina (9%). La principal fuente natural de flavonoides fueron los cítricos (19%) y *Scutellaria baicalensis* Georgi (9%). Se utilizaron ratones en la mayoría de los estudios (86%). La mayoría de los estudios no utilizaron un modelo específico de depresión (40%), y el más utilizado fue el estrés leve crónico impredecible (21%). Las pruebas de comportamiento más utilizadas fueron la prueba de natación forzada (81%), la prueba de suspensión de la cola (56%) y la prueba de campo abierto (51%). Discusión: Considerando el total de pruebas, el 93% de ellas presentó actividad antidepressiva, y todos los estudios que evaluaron estrés oxidativo (37%) e inflamación (39%) encontraron un resultado antioxidante y antiinflamatorio significativo, respectivamente. Conclusiones: Estos hallazgos demuestran que los efectos antidepressivos, antioxidantes y antiinflamatorios de los flavonoides que ya se evidenciaron en el estudio de otras condiciones patológicas también están presentes en modelos de depresión en roedores.

Palabras clave: Depresión; Antidepressivo; Flavonoides; Modelo animal.

1. Introduction

Depression is recognized as a public health problem and is evidenced by the impairment of the individual's daily activities, mainly in social relationships. It is considered a chronic, recurrent and potentially fatal disorder that affects about 20% of the population (Du, Tang, Liu, Zhang, Zhao, Ren & Leng, 2014; Comasseto, Pinto, Prestes, Lopes, Júnior & Antunes, 2018). Antidepressant drugs generally have undesirable side effects, such as cholinergic symptoms, withdrawal problems, sexual dysfunction, and worsening of insomnia, which are often the most common causes for medication discontinuation (Barboza & Silva, 2015; Morley, 2017). Since antiquity, mood disorders have been treated with medicinal plants and some studies have shown that there are plants that have antidepressant activity, either by their extract or by the substance that can be isolated from them (Sarris, Panossian, Schweitzer, Stough & Scholey, 2011). Psychotropic plants that have multiple bioactivities and few side effects have gained significant attention as complementary and alternative drugs (Huerta-Reyes, Herrera-Ruiz, González-Cortazar, Zamilpa, León, Reyes-Chilpa, Aguilar-Rojas & Tortoriello, 2013).

The flavonoids have many functions: elimination of free radicals; antioxidant, anti-aging and anti-stress properties; regulation of the immune and endocrine systems; beneficial effects on the central nervous system (CNS), protecting neurons against lesions induced by stress, suppressing activation of microglia and astrocytes and improving synaptic plasticity, memory and cognitive function (Jäger & Saaby, 2011; Shuo & Mingsan, 2014).

In the last two decades, from 1998 to 2019, the number of publications with the keyword “flavonoid” had a sevenfold increase, from 1811 to 7971 articles on the PubMed platform. This data reflects the growing attention that this group of substances has received in recent years due to its beneficial properties. To study the effect of these substances in human populations is difficult due to ethical limitations and the lack of control over the individual. Therefore, animal models emerge as a great tool to achieve this goal. Rodents have genetic similarities with humans and the general biological and metabolic processes are similar.

Based on those facts, studies with flavonoids have been of great interest to researchers due to their variability in nature and because they have antidepressant and antioxidant activity (de la Garza, Garza-Cuellar, Silva-Hernandez, Cardenas-Perez, Reyes-Castro, Zambrano, Gonzalez-Hernandez, Garza-Ocañas, Fuentes-Mera & Camacho, 2019; Herrera-Ruiz, Santillán-Urquiza, Romero-Cerecero, Zamilpa, Jiménez-Ferrer & Tortoriello, 2020; Zhang, He, Wang, Zhang, Li, Yang & Wu, 2019). Thus, the objective of this study is to analyze the most recent studies conducted on the effect of flavonoids in the model of depression in mice and rats.

2. Methodology

The systematic review of the literature was conducted from August to October 2019, including the following databases: PubMed, ScienceDirect and Cochrane Library. The choice of those databases occurred due to the fact that, in preliminary searches, the other databases seemed poor in laboratory animal studies in the field of depression using flavonoids, returning few or mostly any results. In order to generate an up-to-date review, only articles published in the last 10 years (2009 to 2019) in English were analyzed. First, the keywords "depression", "depression-like", "antidepressant", "flavonoid", "mice" and "rat" were used, which returned 3776 articles in the three databases altogether. It was observed that most studies consisted of human studies and reviews, so the search tool was refined so that only studies performed on animal models of depression with rats or mice using flavonoids were filtered.

Thus, only articles with the words "depression", "depression-like" or "antidepressant" and "rat" or "mice" in the title were selected, and with "flavonoid" in the body of the article. After this process, the result was a total of 54 articles, which were fully reviewed by the authors to assess whether they met the following inclusion or exclusion criteria, aiming to adequate the articles selected to the purpose of the review. This process resulted in the exclusion of 11 articles. Seven were duplicates, two analyzed substances that were not flavonoids (gallic acid and ellagic acid), one was a rat ovarian morphology study (it did not evaluate antidepressant effect of the flavonoid used) and the last one was an article that consisted in the presentation of a chromatography method. By the end, 43 studies were included in the review (Figure 1).

Study selection

Inclusion criteria

To be included in the review, the article was required to be an original laboratory study in an animal model of depression induced by any method in rodents, mainly rats or mice. In addition, at least one of the tested substances necessarily had to be a flavonoid. Only articles published after 2009 were included in the analyses.

Exclusion criteria

We excluded studies that used other animals rather than rodents or that evaluated other pathological conditions that were not classic depression. Experiments that did not include any flavonoids on the tests were not included.

Data screening

Three authors reviewed screened titles, abstracts and full texts to identify the adequation to the inclusion or exclusion criteria. In a second stage, full-text articles were independently evaluated by two other researchers to select studies for inclusion; differences were resolved by consensus by all the authors in scheduled presentational meetings.

Data extraction

Four authors extracted into Excel spreadsheets data from the selected studies on: (I) author and year of publication, (II)

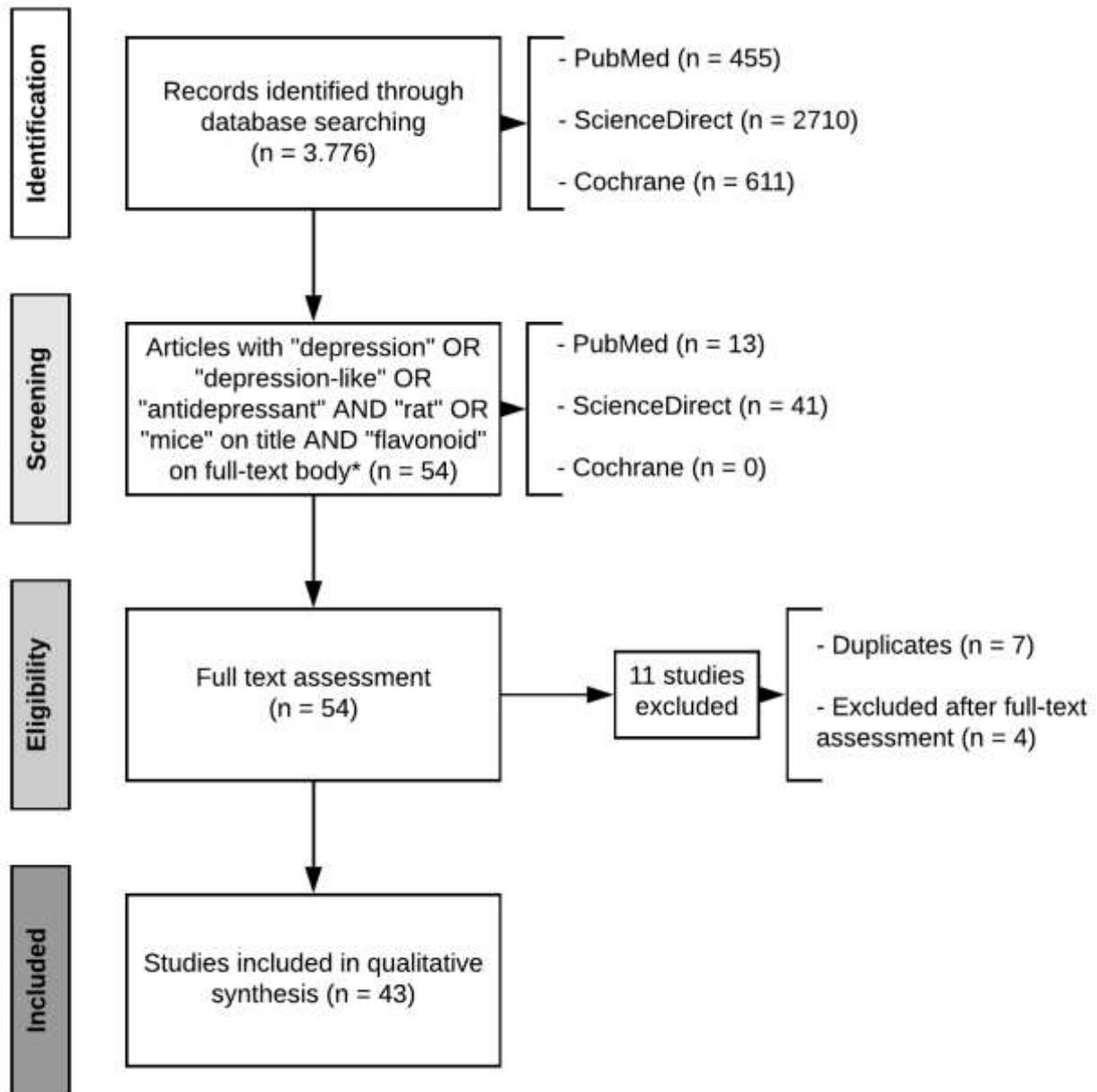
substance tested (flavonoid), (III) origin of the flavonoid (e.g. specific plant), (IV) animal used (mice and/or rat), (V) model of depression induction, (VI) behavioral effects (positive or negative results in behavioral tests), (VII) oxidative stress effect (based on the dosage of oxidants and antioxidants substances in brain tissue) and (VIII) neuroinflammation effect (changes in TNF- α , interleukins or other inflammatory mediators). The results of the behavioral tests were considered positive (+) when the substance was able to significantly change the behavior of the animal of the depression model towards a similar behavior to the control (healthy) group. A result was considered negative (-) when it represented an effect that drives the behavior towards the behavior of the animal that belongs to the depression model group that did not receive treatment. A neutral (0) result was obtained when there was no significant difference between the treated group and the depression model group with no treatment.

Analyses

The study characteristics and results obtained after data extraction were compiled in Table 1. Then, quantitative analyzes were made using Excel and were reported in the “Results” section. Regarding the analyses of the behavioral tests, the results were classified as positive (+) when there was a significant improvement of the parameters of the model of depression group treated with flavonoids when compared to the model of depression group (negative control) or to the control group, only when there was not a model group present; as neutral (0) when the results did not obtain a statistically significant difference between the groups; and negative (-) when the tested flavonoid worsened the performance of the animal in the behavioral test, with statistical significance, when compared to the animal model of depression group or to the control group, only when there was not a model group present.

The antioxidant and anti-inflammatory effect of the substance was evaluated by comparing the model of depression group treated with the studied flavonoid with the animal model of depression group that did not receive a therapeutic substance (negative control). The substance was considered to have an antioxidant or anti-inflammatory if in at least one parameter (e.g. nitrite or malondialdehyde levels for oxidative stress measure or TNF- α or IL-6 for inflammatory activity measure) the treated group showed a statistically significant improvement (reduction in the oxidant or inflammatory substances levels when compared to the negative control group).

Figure 1 - Flowchart of study selection.



*Written code used to search in Pubmed: (((("depression"[Title] OR "depression-like"[Title] OR "antidepressant"[Title] AND "flavonoid") AND ("rat"[Title] OR "mice"[Title]))))

Source: Barroso, Esmeraldo, de Oliveira, Rodrigues Junior e Silva (2021).

3. Results

A total of 43 articles were included in this review, according to the methods described previously. The flavonoids tested were widely variable, composing 32 varieties of categories, being most of them a single substance but some are a mixture of flavonoids, for example, XBXT-2 (total flavonoids extracted from Xiaobuxin-Tang) (An, Li, Yu, Xue, Yu, Chen, Zhang, Zhao, Li & Zhang, 2015). The most frequently studied flavonoids were hesperidin (6 articles, 14% of total), baicalin (4 articles, 9% of total), naringenin (3 articles, 7% of total), quercetin (3 articles, 7% of total), apigenin (2 articles, 5% of total) and chrysin (2 articles, 5% of total). The rest of the isolated flavonoids or mixtures appeared only once each one, which compose the 47% remaining studies.

Regarding the origin of the flavonoids, the most frequent source was citrus fruits (8 articles, 19% of total), being *Citrus sinensis* the most frequent fruit studied (4 articles, 9% of total). *Scutellaria baicalensis* Georgi, a plant that belongs to the *Lamiaceae* family, was used in 4 studies (9% of total), which makes it the most frequently studied plant in this review. *Solanum lycopersicum* was a relatively common source of flavonoids, studied in 3 articles (7% of total). *Passiflora caerulea* and honey appeared in 2 articles (5% of total). The rest of the origins of the flavonoids were varied, being studied only in one article each, composing altogether 30 of the remaining articles (70% of the total).

There were found only studies that used rodents during the literature review process, either with mice or rats. Mice were used in the majority of the studies (37 articles, 86% of total), while rats were used in only 6 articles (14% of total).

The models of depression were a variable parameter too. In total, 15 different models were used in these studies. 17 articles (40% of total) did not describe in the methodology a specific depression model, generally because the antidepressant activity was assessed comparing control healthy animals with the tested ones, not having a pathological parameter. The most frequently used model was Chronic Unpredictable Mild Stress (CUMS), accounting for 21% (9 articles) of the studies included in the review. The second most frequent were depression induction with Lipopolysaccharide (LPS) and with para-chlorophenylalanine methyl ester hydrochloride (PCPA), present in 3 articles each (7% of total each). Chronic corticosterone treatment and alpha-methyl-p-tyrosine (AMPT) were used in 2 studies each (5% of total each). The rest of the 9 models appeared only once in the review.

In the 43 articles included in this review, 16 different behavioral tests were performed. Even so, a few tests were commonly present in most studies. The most popular test was the Forced Swim Test (FST), present in 35 articles (81% of total); the second one was the Tail Suspension Test (TST), present in 24 articles (56% of total); and the third one was the Open Field Test (OFT), present in 22 articles (51% of total). There were no articles in the review that did not contain at least one of these three tests. The fourth most frequently used to test assess the animal's behavior was the sucrose preference test, found in 8 articles (19% of total). Following, the elevated plus maze and rotarod tests were used in 4 (9% of total) and 2 (5% of total) articles, respectively. All the other 10 tests were used only once in one article.

In general, the vast majority of the behavioral tests analyzed obtained positive (+) results. From a total of 105 behavioral tests analyzed, 98 presented positive results (93% of total tests), and the remnant, a total of 7 behavioral tests results (7% of total tests), were classified as neutral (0). The neutral results were obtained in 1 FST, 5 OFT and 1 rotarod test only. No negative (-) results were found in this review.

The majority of articles did not evaluate oxidative stress (27 articles, 63% of the total) nor inflammation (28 articles, 65% of the total). Even though every article that evaluated one of those biological processes obtained a favorable result according to the methodology described previously at least in one parameter, which is 16 articles for oxidative stress (37% of the total) and 15 articles for inflammation (35% of the total).

Table 1 - Summary of systematic review findings.

Author/Year	Flavonoid	Plant	Animal	Model of Depression	Behavioral Antidepressant Effects	Oxidative Stress Effect	Neuroinflammatory Effect
Abbasi-Maleki et al, 2017	Crude extract of Mentha piperita	Mentha piperita	Mice	No specific depression model	Forced Swim Test (+), Open Field Test (0)	N/A	N/A
Abbasi-Maleki et al, 2019	Origanum majorana essential oil	Origanum majorana	Mice	No specific depression model	Forced Swim Test (+), Open Field Test (0)	N/A	N/A
Aggarwal et al, 2010	Naringenin	Solanum lycopersicum, citrus fruits	Mice	Cerebral ischemia/reperfusion (Post-stroke depression)	Inclined Beam-Walking Test (+), Forced Swim Test (+), Assessment of Gross Behavioral Activity (+)	Antioxidant	N/A
An et al., 2015	XBXT-2 (total flavonoids extracted from Xiaobuxin-Tang)	Xiaobuxin-Tang	Mice	Lipoplysaccharide (LPS)	Forced Swim Test (+), Tail Suspension Test (+)	Antioxidant	Anti-inflammatory
Ayuob et al, 2017	Apigenin	Ocimum basilicum	Mice	Chronic unpredictable mild stress (CUMS)	Forced Swim Test (+), Open Field Test (+)	N/A	Anti-inflammatory
Bansal et al., 2018	Naringenin	Solanum lycopersicum, citrus fruits	Mice	Bilateral olfactory bulbectomy	Forced Swim Test (+), Open Field Test (+)	Antioxidant	Anti-inflammatory
Borges Filho et al., 2016	Chrysin	Passiflora caerulea, honey	Mice	Chronic unpredictable mild stress (CUMS)	Forced Swim Test (+), Sucrose Preference Test (+)	Antioxidant	Anti-inflammatory
Cárdenas et al, 2017	Tanacetum parthenium L. Schultz-Bip extract flavonoids	Tanacetum parthenium L	Mice	No specific depression model	Burying Behavior Test (+), Elevated Plus Maze Test (+), Forced Swim Test (+), Open Field Test (+)	N/A	N/A
Cassani et al., 2015	Aqueous extract	Chrysactinia mexicana	Mice	No specific depression model	Forced Swim Test (+), Tail Suspension Test (+)	N/A	N/A
Colla et al. 2012	Quercetin	Eugenia brasiliensis	Mice	Several pharmacological agonists and antagonists	Tail Suspension Test (+), Open Field Test (+)	N/A	N/A
De La Garza et al., 2019	Astragalín and Narirutín	Phytolacca americana (Astragalín) and Orange Juice (Narirutín)	Rat	No specific depression model	Forced Swim Test (+)	Antioxidant	Anti-inflammatory
Donato et al., 2014	Hesperidin	Citrus sinensis, citrus fruits	Mice	No specific depression model	Tail Suspension Test (+), Open Field Test (+)	Antioxidant	Anti-inflammatory
Donato et al., 2015	Hesperidin	Citrus sinensis, citrus fruits	Mice	Intracerebroventricular injection of K ⁺ channel blockers/openers	Tail Suspension Test (+), Open Field Test (+)	Antioxidant	N/A
Gapski et al., 2019	Catechin, epicatechin, anthocyanis	Blueberry extract	Mice	No specific depression model	Tail Suspension Test (+)	Antioxidant	N/A
Gladino et al., 2009	Lafloensia pacari A. St.-Hil. ethanolic extract flavonoids	Lafloensia pacari	Mice	No specific depression model	Forced Swim Test (+), Tail Suspension Test (+), Open Field Test (0)	N/A	N/A
Gorka et al. 2019	Hyperoside	Impatiens glandulifera	Mice	Para-chlorophenylalanine methyl ester hydrochloride (PCPA) or Alpha-methyl-p-tyrosine (AMPT)	Forced Swim Test (+), Tail Suspension Test (+)	N/A	N/A
Herrera-Ruiz et al., 2019	Rutin	Bauhinia blakeana Dunn	Mice	Lipoplysaccharide (LPS)	Forced Swim Test (+), Open Field Test (+)	N/A	Anti-inflammatory
Ibarrola et al. 2016	Quercetin	Kyllinga brevifolia rhizomes	Mice	No specific depression model	Forced Swim Test (+)	N/A	N/A
Ishola et al. 2012	Amentoflavone	Cnestis ferruginea	Mice	Metergoline	Forced Swim Test (+), Tail Suspension Test (+)	N/A	N/A
Ishola et al. 2014	Quercetin	Alchornea cordifolia	Mice	No specific depression model	Forced Swim Test (+), Open Field Test (+)	N/A	N/A
Jesse et al., 2015	Chrysin	Passiflora caerulea, honey	Mice	Chronic unpredictable mild stress (CUMS)	Forced Swim Test (+)	Antioxidant	Anti-inflammatory
Kikko et al., 2009	Extrato das folhas e flores	Baccharis ilimita	Mice	No specific depression model	Forced Swim Test (+)	N/A	N/A
Kosari-Nasab et al., 2018	Hesperidin	Citrus sinensis, citrus fruits	Mice	Mild Traumatic Brain Injury	Forced Swim Test (+), Tail Suspension Test (+), Sucrose Preference Test (+), Novelty-Suppressed Feeding Test (+)	Antioxidant	Anti-inflammatory
Liu et al. 2012	Ethanol extract from Suanzaorenhehuan (Flavonoids)	Suanzaorenhehuan Formula (SHF)	Mice	Chronic unpredictable mild stress (CUMS)	Forced Swim Test (+), Tail Suspension Test (+)	N/A	N/A
Lu et al, 2019	Baicalin	Scutellaria baicalensis Georgi	Mice	Chronic unpredictable mild stress (CUMS)	Sucrose Preference Test (+), Tail Suspension Test (+), Open Field Test (+)	N/A	N/A
Machado et al. 2009	Hesperidin	Rosmarinus officinalis L	Mice	Para-chlorophenylalanine methyl ester hydrochloride (PCPA)	Forced Swim Test (+), Tail Suspension Test (+)	N/A	N/A
Meyer et al, 2017	Myricitrin	Myrica cerifera	Mice	No specific depression model	Tail Suspension Test (+)	Antioxidant	N/A
Miao et al, 2016	Cynomorium total flavone	Cynomorium songaricum	Rat	Depression model of perimenopausal rat	Forced Swim Test (+), Open Field Test (+), Sucrose Preference Test (+)	N/A	N/A
Muller et al. 2012	Hesperidin	Valeriana glechomifolia	Mice	No specific depression model	Forced Swim Test (+), Tail Suspension Test (+), Open Field Test (0), Rotarod (0)	N/A	N/A
Pawar et al., 2011	Methabolic extracts of rhizomes	Acorus calamus	Mice	No specific depression model	Forced Swim Test (+), Tail Suspension Test (+)	N/A	N/A
Souza et al. 2013	Hesperidin	Citrus sinensis, citrus fruits	Mice	Alpha-methyl-p-tyrosine (AMPT)	Forced Swim Test (+), Tail Suspension Test (+)	N/A	N/A
Tao et al, 2016	Liquiritigenin	Glycyrrhiza	Mice	Chronic unpredictable mild stress (CUMS)	Forced Swim Test (+), Tail Suspension Test (+), Sucrose Preference Test (+), Open Field Test (+)	Antioxidant	Anti-inflammatory
Wang et al., 2017	Fisetin	Strawberries	Mice	Spatial restraint stress-induction	Forced Swim Test (+), Tail Suspension Test (+)	Antioxidant	Anti-inflammatory
Weng et al, 2016	Apigenin	Parsley, celery, celeriac, and chamomile tea	Mice	Chronic corticosterone treatment	Sucrose Preference Test (+), Forced Swim Test (+)	N/A	N/A
Wu et al, 2019	Malva sylvestris extract	Malva sylvestris	Mice	Lipoplysaccharide (LPS)	Elevated Plus Maze (+), Open Field Test (+), Morris Water Maze (+), Rotarod (+)	N/A	Anti-inflammatory
Xu et al. 2010	Proanthocyanidin	Malus domestica	Mice	No specific depression model	Forced Swim Test (+), Tail Suspension Test (+)	N/A	N/A
Yi et al., 2010	Naringenin	Solanum lycopersicum, citrus fruits	Rat	Para-chlorophenylalanine methyl ester hydrochloride (PCPA) or Alpha-methyl-p-tyrosine (AMPT)	Tail Suspension Test (+), Forced Swim Test (0), Open Field Test (0)	N/A	N/A
Yue Suet al, 2014	Xiaochaihutang	Radix bupleuri	Rat	Chronic unpredictable mild stress (CUMS)	Open Field Test (+), Food Consumption Test (+), Sucrose Preference Test (+)	N/A	N/A
Zhang et al, 2012	Fructus Aurantii	Citrus aurantium L.	Rat	No specific depression model	Forced Swim Test (+), Open Field Test (+)	N/A	N/A
Zhang et al. 2018	Baicalin	Scutellaria baicalensis Georgi	Rat	Chronic unpredictable mild stress (CUMS)	Forced Swim Test (+), Open Field Test (+)	Antioxidant	Anti-inflammatory
Zhang et al., 2019	Baicalin	Scutellaria baicalensis Georgi	Mice	Chronic corticosterone treatment	Dexamethasone Suppression Test (+), Forced Swim Test (+), Open Field Test (+), Tail Suspension Test (+), Elevated Plus Maze (+)	Antioxidant	Anti-inflammatory
Zhong, J. et al., 2019	Baicalin	Scutellaria baicalensis Georgi	Mice	Chronic unpredictable mild stress (CUMS)	Sucrose Preference Test (+), Forced Swim Test (+), Open Field Test (+), Tail Suspension Test (+)	Antioxidant	Anti-inflammatory
Zhou et al, 2018	Polygala japonica Hoult Extract	Polygala japonica Hoult	Mice	No specific depression model	Forced Swim Test (+), Tail Suspension Test (+), Elevated Plus Maze (+), Novel Object Recognition Test (+), Object Location Test (+),	N/A	N/A

Subtitles: (+) Positive result; (0) Neutral result; (-) Negative result; N/A Not Applicable. Source: Barroso et al (2021).

4. Discussion

This systematic review about the antidepressant, antioxidant and anti-inflammatory activity of the flavonoids revealed that those substances have been broadly studied in the last decade at labs in diverse countries, including Brazil. Despite that fact, the absolute number of articles found with our inclusion criteria that were published in the last decade can be considered small (43) in view of the promising results that were obtained by those studies.

It is notable that, even though the spectrum of flavonoids studied by the authors is considerably diversified, including 32 different substances, there are still thousands of flavonoids that were not characterized in its possible antidepressant, antioxidant or anti-inflammatory activity, since there are approximately 6000 natural flavonoids currently known (Panche, Diwan & Chandra, 2016). The most studied flavonoid regarding its antidepressant activity according to this review was hesperidin, and indeed it is a notable example of a substance that has vast potential benefit in neurological diseases such as Parkinson's disease, Alzheimer's disease, Huntington's disease, multiple sclerosis, brain ischemia and traumatic brain injury (Kim, Wie, Ahn, Tanaka, Matsuda & Shin, 2019). The benefit of hesperidin administration to those conditions is almost always linked to an antioxidant and anti-inflammatory effect, common in flavonoids, but regarding its antidepressant activity, there seems to be influence in some other pivotal mechanisms in depression physiopathology such as involvement of serotonergic 5-HT_{1A} receptors (Donato, Borges Filhos, Giacomeli, Alvater, Del Fabbro, Antunes, de Gomes, Goes, Souza, Boeira & Jesse, 2015), l-arginine-NO-cGMP pathway and BDNF levels (Donato, de Gomes, Goes, Filho, Del Fabbro, Antunes, Souza, Boeira & Jesse, 2014) and potassium channel inhibition (Donato, et al., 2015).

Taking those evidences into consideration, it is possible to affirm that probably the antidepressant effect observed after the administration of some flavonoids, mainly hesperidin, is not only a result of the already well-established antioxidant and anti-inflammatory effects attributed to flavonoids: it has multiple mechanisms of action that can influence the symptoms and probably the course of the disease. Citrus fruits such as *Citrus sinensis* were the most frequent source of flavonoids according to the review. Most of them are flavanones, a class that is generally present in all citrus fruits such as oranges, lemons and grapes (Panche, et al., 2013).

Some recent studies show evidence that citrus flavanones such as hesperidin, hesperetin and naringenin can traverse the blood-brain barrier and may play an effective role in the intervention for neurochemical disorders such as depression or neurodegenerative diseases (Panche, et al., 2013). That fact may explain why hesperidin seems to have so many different mechanisms of action against depression psychopathology, as cited before. *Scutellaria baicalensis* Georgi was the most frequent plant as a flavonoid source, mainly flavones such as baicalin, wogonoside and their aglycones baicalein wogonin are the major bioactive compounds extracted from the root of *S. baicalensis*. These flavonoids have been reported to have various pharmacological functions, including anti-cancer, hepatoprotection, antibacterial and antiviral, antioxidant, anticonvulsant and neuroprotective effects (Zhao, Chen & Martin, 2016) and, more recently, antidepressant activity, as confirmed by this present review.

Even though 37 of 43 (86% of the total) of the studies in the review used mice and only the 6 left (14% of the total) used rats, the literature shows that rats are easier to handle, present less stress when manipulated by researchers and have more similarity to humans regarding metabolic and neurologic aspects, especially in cognitive processes such as behavior, memory and emotions, thus being the best animal for studying depression among rodents (Iannaccone & Jacob, 2009). A factor that may justify the most frequent use of mice is the reduced cost, since rats are usually more expensive.

The most used model according to this review, the CUMS, which is a robust animal model of depression and is strongly associated with anhedonic behavior in rodents (Antoniuk, Bijata, Ponimaskin & Włodarczyk, 2019). The consequences of CUMS include cortical and limbic brain region atrophy, decreases in hippocampal neurogenesis, sensitization of the serotonergic system,

excessive activation of the noradrenergic system, increases in hippocampal inflammatory proteins, microglial proliferation and activation, and hypothalamic-pituitary-adrenal (HPA) axis disturbances (Antoniuk, et al., 2019). It is notable that the antidepressant mechanisms of most of the flavonoids studied by the articles included in this review act on one or more of those CUMS consequences, which justifies the positive results obtained in those studies (Liu, Lan, Ren, Wu, Wang, Huang & Yu, 2015).

The most frequently used test to assess the depression-like behavior used by the studies in this review, the FST, and it is indeed one of the most traditional tests for used for evaluation of antidepressant drugs, antidepressant efficacy of new compounds, and experimental manipulations that are aimed at rendering or preventing depressive-like states (Can, Dao, Arad, Terrillion, Piantadosi & Gould, 2012). Due to its popularity, there is a vast amount of data regarding various antidepressants, which is a great advantage when testing a new class of drugs. When using rats, the test is even capable of differentiating between serotonin and noradrenaline acting compounds depending on the observed behavior (swimming and climbing, respectively) (Detke & Lucki, 1995). Considering that 34 of 35 (97%) of the tests analyzed in this review obtained positive results in favor of the antidepressant activity of flavonoids, the FST can be considered an indispensable experiment when regarding the current evidence of the antidepressant activity of flavonoids.

The other two behavioral tests that appeared several times at the analyzed studies, TST and the OFT, are well-established tests in the study of animal models of depression. The TST, which achieved 100% of positive results in favor of flavonoids as antidepressant agents (24 articles), is closely related to the FST but presents key differences that can be useful for differentiating drugs mechanisms of action and for avoiding confounders. The TST avoids problems related to the hypothermic exposure of animals in the FST and to a lack of coordination required to swim in genetically modified rodents. In addition, there is an apparent increased sensitivity in the TST when compared to the FST (Cryan, Mombereau & Vassout, 2005). Regarding the OFT, the results in this review are not as homogenous as the previously mentioned tests (77% positive results and 23% neutral results from a total of 22 articles), finding that can be partially explained by the wide variety of protocol setups or static variables such as time, lighting conditions, etc (Seibenhener & Wooten, 2015). The possibility of the test to measure both anxiety-like behavior or locomotor activity depending on the protocol used is another important factor to explain the more heterogeneous results in this review.

5. Conclusion

In conclusion, those findings demonstrate that the antioxidant and anti-inflammatory effects of flavonoids that were already evidenced in the study of other pathological conditions are also present in rodent depression models. More detailed animal and human studies are now necessary to detail the antidepressant, antioxidant and anti-inflammatory action of flavonoids and to analyze if those effects can translate to clinical benefits to patients affected by major depression.

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