

Anxiolytic Potential of Natural Flavonoids

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GABA

Abstract

Anxiety is the major mental health related problem; it affects the life style of millions of people around the world. Flavonoids are polyphenols found in many plant families and obtained from various plant parts. Consumption of plant parts known to be rich in flavonoids has been reported to result in anti-anxiety effect. In addition to the anxiolytic effect, flavonoids were shown to prevent neuro-degeneration. In this work, we review the potential anxiolytic properties of naturally occurring flavonoids.

Introduction

Millions of peoples are suffering from mental disorders worldwide. Anxiety is the major problems related to mental health and has become a significant research area in the field of psychopharmacology. The prevalence of anxiety is 16.6% worldwide, in the United States 55 million people are suffering from anxiety and depression. The American psychiatric association defines anxiety as “panic disorder with and without agoraphobia; Obsessive-Compulsive Disorder (OCD); social phobia; Generalized Anxiety Disorder (GAD); specific phobia; and Post Traumatic Stress Disorder (PTSD). Anxiety is associated with significant disability resulting in negative impacts on the patient’s quality of life [1]. Anxiety is characterized by persistent felling of worries that make an individual unable to relax [2]. These include anxiety episodes such as before surgery or menstrual cycle to general anxiety disorders such as social phobia, panic disorders, obsessive-compulsive disorder and generalized anxiety disorders [3]. The common symptoms of anxiety are muscle tension and aches, sweating, trembling and headache.

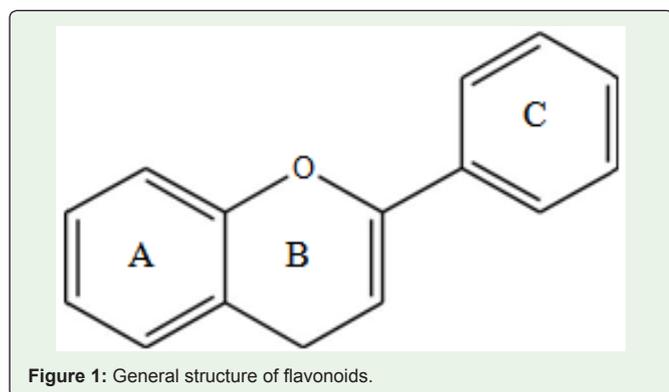
Anxiety is believed to be due to the dys-regulation of certain CNS neurotransmitter such as dopamine, serotonin, and Gamma Amino Butyric Acid (GABA) [4]. The current measures to control anxiety include psychotherapy, pharmacological therapy and behavioral interventions [5]. Cognitive behavior therapy has been proved very effective for treating anxiety disorder that requires long term treatment [6]. Selective Serotonin Reuptake Inhibitors (SSRIs) and benzodiazepines proved to be the most successful agents to control anxiety and are most widely used in clinical practice [7]. However the two classes showed several adverse effects such as sexual dysfunction, decrease alertness, increased suicidal attempts, dependency and high cost [7].

Plant derived constituents provide a large source of available pharmaceuticals in modern medicine which are directly or indirectly derived from natural sources. In drug discovery process, the natural products are of great interest due to their natural diversity and lower side effects [8-10]. Although several classes of natural compound have been shown to have anxiolytic potential [11].

Flavonoids have proven to be very selective anxiolytic agents that lack additional CNS effects. They are one of the most important natural products used commonly across the globe. Flavonoids are low molecular weight compounds present as secondary metabolites in higher plants. The wide range of biological activities exhibiting by flavonoids is attributed to the diversity in their chemical structure. Flavonoids have several advantages such as low cost, availability, low side effects [2]. Flavonoids found to be effective in animal and human models [12]. This review will explore flavonoids found in various plants and examine their potential to be used in relieving anxiety.

Flavonoids

Flavonoids are naturally occurring compounds found in plants where they help plants regulate growth and combat oxidative stress. Flavonoids consist of a large group of polyphenolic compounds having benzo-pyrone structure. Plants use phenylpropanoid pathway for flavonoid bio-synthesis. They have been reported to possess several beneficial pharmacological actions such as anti-bacterial,



anticancer, beneficial in cardiovascular disorders, age related diseases and mental disorders [13]. In 1930 a substance was isolated from orange and was given the name of Vitamin P, later it was found that it was the flavonoids rutin. More than four thousands flavonoids have been identified ever since. Chemically flavonoids consists of fifteen carbon skeleton consisting of two benzene rings 'A' and 'B' rings and one heterocyclic pyrone ring 'C' (Figure 1). There are different classes of flavonoids depending on the pattern of substitution and level of oxidation of C ring. They include the flavonoles (fistin, kaempferol, quercetin, myricetin) and the flavones (naringenin, flavon and herperetin). Individual compound within a class varies from each other on the basis of level of substitution of the two benzene ring [14]. Flavonoids occur as glycoside, aglycones and methylated derivatives. The basic structure of flavonoids is aglycon. In case of flavonones and flavonols six member ring of benzene is condensed with α -pyrone or its dihydroxy derivatives. Flavonols differ from flavonones by hydroxyl group at 3 position and C_2-C_3 double bond (Figure 2). Different carbohydrates have been found to bind with flavonoids at 3 and 7 positions such as D-glucose, galatose, glucorhamnose, arabinose or L rhamnose [15].

Spectral Characteristics of Flavonoids

Flavonoids exhibit two major absorption bands between 320nm to 385nm corresponding to ring 'B' and the other is a 250nm to 285nm band that represents ring 'A' absorption. Various functional groups attached to flavonoid skeleton causes shift of absorption such as 371 nm in quercetin (3,5,7,3',4'- hydroxyl groups), 367 nm kaempferol (3,5,7,4'-hydroxyl groups), to 374 nm in myricetin (3,5,7,3',4',5-hydroxyl groups), and band II absorption includes toxifolin (285), naringenin (288).

Metabolism of Flavonoids

Absorption of flavonoids in the intestine from food depends on their physicochemical properties such as liphophilicity, pKa, solubility, configuration and molecular size. Major portion of flavonoid is absorbed from small intestine while very little quantity is absorbed from colon. Flavonoids with sugar parts need conversion into aglycon before absorption [16]. Quercetin is hydrophilic in nature and is transported across the small intestine by intestinal Na^+ -glucose transporter. Another mechanism suggests that outside the brush border cells lactase phloridzin hydrolase liberate aglycon part from glycosidic flavonoid. After absorption flavonoids are conjugated in the liver to various groups by sulfation, glucuronidation or methylation or are metabolized to small phenolic compounds [12]. This is the reason no free flavonoids are found in urine or blood except for catechins [17]. Bioavailability of flavonoids varies depending on its source such as quercetin absorption from onion is larger than from tea or apple. Those flavonoids that are not absorbed in the small intestine and those that are secreted in the bile are degraded by microflora present in the colon that also breakdown flavonoid ring structure. Oligomeric flavonoids are converted into dimeric and monomeric flavonoids in the acidic environment of the stomach. Isoflavones shows highest bioavailability among all classes of flavonoids [12]. Table 1 summarizes various flavonoids, their sources and structures and Table 2 summarizes various flavonoids containing plants, their possible mechanisms and the animal models used for the screening of anxiolytic activity.

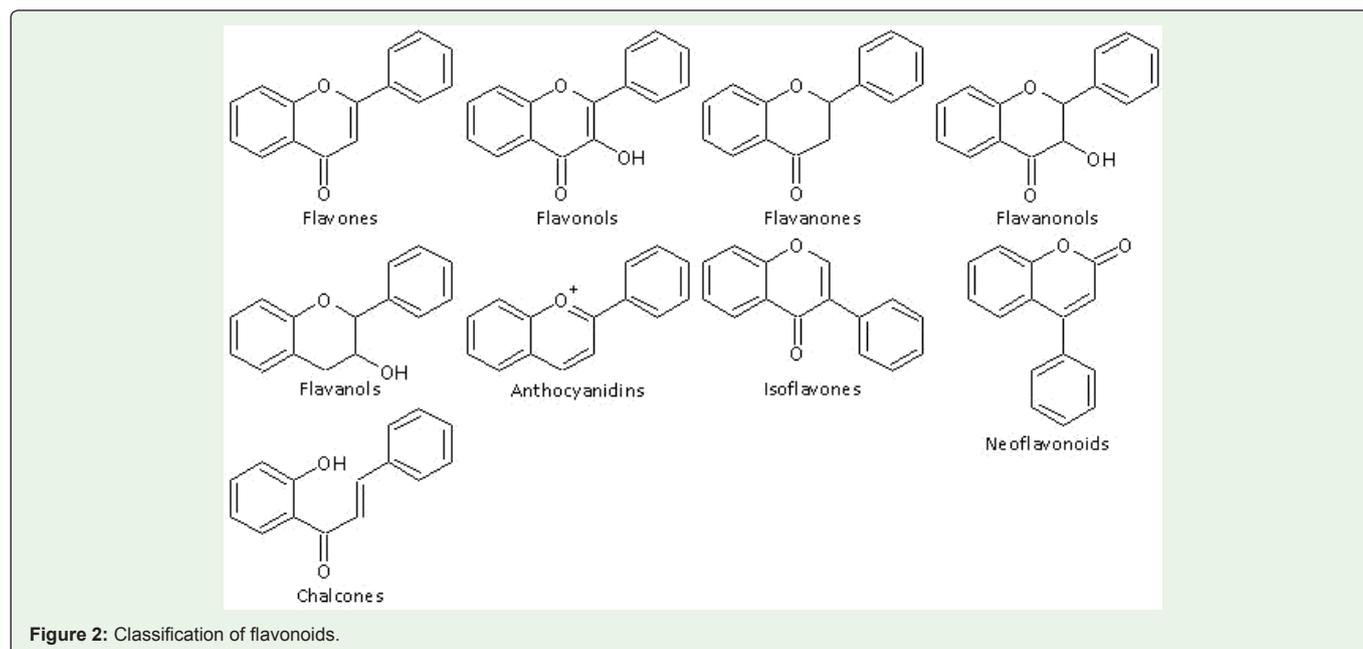


Table 1: Flavonoids and their natural sources.

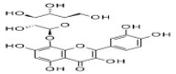
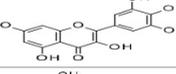
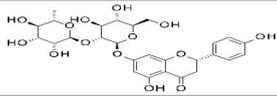
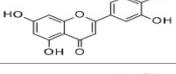
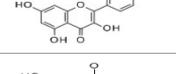
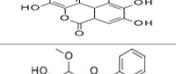
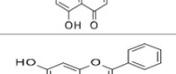
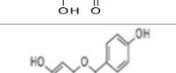
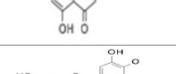
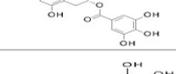
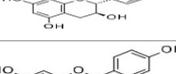
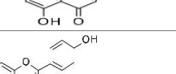
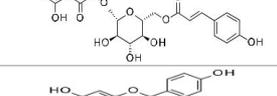
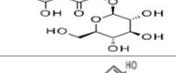
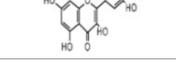
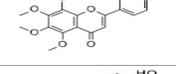
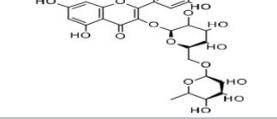
S/No	Flavonoid	Chemical Structure	Plant Sources	Reference
1	Gossypin		Gossypium indicum, Vitifolius and Hibiscus esculentus, Hibiscus Vitifolius	[59,60]
2	Myricitin		Physalis peruviana Linn.	[61]
3	Naringin		Citrus grandis	[62]
4	Luteolin		Elsholtzia rugulosa	[63]
5	Kaempferol		Apocynum venetum, Telia Spp	[64]
6	Ellagic Acid		Jatropha dioica, Euphorbia antisyphylitica, Turnera diffusa	[65]
7	Wogonin		Scutellaria baicalensis	[66]
8	Chrysin		Jatropha cillata	[40]
9	Apigenin		Chamomile tea	[40]
10	(-)-epigallocate chin gallate		Chamomile tea	[67,68]
11	(+)-Catechin		Camellia sinensis	[69,70]
12	Apigenin		Matricaria Chamomilla Linn. Passiflora quadrangularis, Turnera diffusa	[71]
13	Tiliroside		Tilia argentea	[32,72]
14	Astragalin		Morus alba L.	[73]
15	Quercetin		Poacynum hendersonii, Telai spp	[74]
16	Tangeretin		Citrus reticulata, Citrus sinensis, Citrus paradisi, Citrus aurantium	[75]
17	Rutin		Physalis peruviana Linn	[76]

Table 2: Plants containing anxiolytic flavonoids.

S/No	Botanical Name	Common Name	Mechanism	Animal Model
1	<i>Albies pindrow</i>	Western Himalayan Fir	NA	EPM, Hole board test [77]
2	<i>Achillea millefolium</i>	Yarrow	NA	EPM [78]
3	<i>Albizia julibrissin</i>	Mimosa	5-HT1A	EPM [51]
4	<i>Apocynum venetum</i>	Luobuma	GABA, 5-HT	EPM [79]
5	<i>Cecropia glazioui</i>	Yarumo	5-HT, NE, DA	EPM [80]
6	<i>Coriandrum Sativum</i>	Coriander	Unknown	EPM [81]
7	<i>Davilla rugosa</i>	Davilla	Unknown	EPM [82]
8	<i>Equisetum arvense</i>	Horsetail Silica	Unknown	EPM [83]
9	<i>Sphaerathus Indicus</i>	East Indian globe thistle	Unknown	EPM [64]
10	<i>Stachys Lavandulifolia</i>	Wood betony	NA	EPM [84]
11	<i>Tilia Americana</i>	Lime blossom Tiliroside	NA	EPM, open-field and hole-board tests [30]
12	<i>Turnera aphrodisiaca</i>	Damiana	GABA	EPM [85]
13	<i>Rubus brasiliensis</i>	Brazilian raspberry	GABA	EPM [86]
14	<i>Sphaerathus Indicus</i>	East Indian globe thistle	NA	EPM [64]
15	<i>Stachys Lavandulifolia</i>	Wood betony	NA	EPM [84]
19	<i>Helianthus annuus</i>	Sunflower	GABA	Light Dark box test, EPM [87]
20	<i>Aronia melanocarpa</i>	Black chokeberry	NA	EPM [35]

Anxiolytic Flavonoids

Gossypin, Myricitrin, and Naringin

Naringin, gossypin and myricitin (Table 1) are found in many plants. All three flavonoids relieve anxiety at the dose level below 3mg/kg when given intra-peritoneally. At high doses they have been reported to cause sedation but no muscle relaxant property [12]. The anxiolytic effect of these flavonoids have been studied using mice 6-8 weeks old and 30g to 35g weight and the anxiolytic effect was confirmed using elevated plus maze and locomotor activity. Diazepam 2 and 10 mg/kg dose were used which increased the number of open arm entries and percent of time spent in open arm as compared to vehicle control. Myricitin at dose 1mg/kg significantly increased number of open arm entries and also time spent in open arm. However at high doses of myricitin this exploratory behavior decreased and no significant difference was noted in number of open arm entries and time spent in open arm as compared to vehicle control. Similar results have been shown for naringin at dose 1 and 3 mg/kg and for gossypin at dose 1, 3 and 10 mg/kg. It is clear from their study that myricitin show anxiolytic activity however higher dose up to 30mg/kg where 27% mice show myorelaxant action as shown from impairing grasping the wire. Maximal anxiolytic effect was noted at dose level of 10mg/kg. This is also evident from study of Marder et al [18], which has shown that flavones at the level of dose 3 to 10 mg/kg to have excellent anxiolytic potential with no myorelaxation, sedation, or significant reduction in locomotor activity. Naringin and gossypin both have shown similar profile. At high doses these flavonoids show increase open arm exploration and decrease locomotor activity as shown from reduction in close arm entries. Naringin and gossypin have anxiolytic and sedative potential at high doses. Naringin at dose 30 mg/kg show a slight myorelaxant effect in the horizontal wire test [12]. Flavones and its derivatives

are mediators of GABA receptors and are helpful in relieving anxiety. These flavonoids also have some other targets such as Human Ether-a-go-go-Related Gene (hERG) voltage-dependent potassium channels and Inwardly Rectifying Potassium Channels (GIRK). These flavonoids have positive modulating effect on GIRK channels [19].

Chalcones

Chalcones are flavonoids having two benzene rings linked through α , β unsaturated carbonyl group. They are important component of human diet and are found in many plants. They possess many properties such as antioxidant, anti-inflammatory, anticancer, antifungal and antibacterial [20]. They are extensively studied and are found in many plants such as citrus fruits and apples. Jamal and coworkers in 2008 studied different chalcones for their anxiolytic activities [21]. They used Swiss male albino rats (200-250 g) and used different animals' models such as Grip test, elevated plus maze, rota rod and open field behavior test. Different chalcones were used such as ISL, isoliquiritigenin; BUT, butein; DHC, 2 ϵ , 2-dihydroxychalcone; HDMC, 2 ϵ -hydroxy-3, 4-dimethoxychalcone; DCC, 4 ϵ , 4-dichlorochalcone; CCP, 1, 3-bis (4-chlorophenyl)-3-(carboxymethylthio) propan-1-one; CMC, 4 ϵ -chloro, 4-methoxychalcone; CMCP, 1-(4-chlorophenyl)-3 (4-methoxyphenyl)-3-(carboxymethylthio) propan-1-one and diazepam (1 and 2 mg/kg) was used as positive control. Reduce grip strength of chalcones 15mg/kg [F(4,45) = 12.250, P < 0.001] and 25mg/kg [F(5,54) = 12.478, P < 0.001]. Chalcones were used at dose level of 15 and 25 mg/kg. Their experiment showed that chalcones show excellent anxiolytic activities. Diazepam also significantly increased open arm entries and decrease close arm entries along with increasing time spend in open arm entries in elevated plus maze. ISL and BUT also showed significant anxiolytic activity. In rotating rod test, most of the chalcones showed no alteration in the amount of time of permanence on the rotating rod. Intraperitoneal administration

of chloro substituted chalcones (DCC, CCP and CMC) showed a decrease in the time of permanence [F(3,36) = 106.527, P < 0.001, 15mg/kg; F(3,36) = 114.554, P < 0.001, 25mg/kg] on the rotating rod as compared with the control group [21].

Kaempferol

Kaempferol is 3,5,7-trihydroxy-2-(4-hydroxyphenyl)-4H-1-benzopyran-4-one (Table 1). Kaempferol is an important flavonoid found in many plants such as *Acaena splendens*, *Aconitum spp*, *Bauhinia malabarica*, *Brassica campestris* and *Cannabis sativa*. Studies have shown that kaempferol have many pharmacological activities such as antioxidant, neuroprotective, analgesic, anticancer and anxiolytic. People who consume food that contain kaempferol suffer less from anxiety. Different sugars are bound to kaempferol such as rutinose, galactose and rhaminose. Asragalin is a kaempferol most commonly found in plants which is kaempferol-3-o-glycoside. Several other studies show that kaempferol has anxiolytic potential [22-25].

Quercetin

Quercetin (3,5,7,3',4'-pentahydroxyflavone) is a polyphenolic flavo-nol molecule that occurs in many fruits and vegetables, such as onions, apples, berries, peanuts, soybeans, potatoes, broccoli, grapes, citrus fruits and tea [26]. Since it is largely present in the human diet, up to 1g/day average intake of quercetin has been reported [27], representing 60 to 75 % of the overall polyphenols ingestion [28]. Quercetin efficiently scavenges free radicals, inhibits ROS-generating enzymes and pre-vents oxidative stress-induced neuronal injuries [29]. Along with this potent antioxidant property, quercetin has been found to exert an anxiolytic-like effect. Several evidence shows that there is strong relation between ant-anxiety and antioxidant activities of quercetin. This dietary antioxidant potential of quercetin improve cognitive functions and prevent stress induce neurobehavioral disorder [30,31].

Quercetin is also found in *Telia maxicana*, *Telia rubra*, and is found to attached with glucose unit. Quercetin has been shown to have anxiolytic potential. Various flavonoid have been isolated from Talia species [32] such as rutin (quercetin-3-O-rutinoside), hyperoside (quercetin-3-O-galactoside), isoquercitrin (quercetin-3-O-glucoside), quercitrin (quercetin-3-O-rhamnoside), kaempferol-3-O-rhamnoside, astragaloside (kaempferol-3-O-glucoside), tiliroside (kaempferol-3-O-(6-p-coumaryl)-glucoside), quercetin-3,7-dirhamnoside, kaempferol-3,7-dirhamnoside, quercetin-3-O-glucoside-7-O-rhamnoside, and kaempferol-3-O-glucoside-7-O-rhamnoside. The authors showed that these flavonoids have anxiolytic potential. They used different animal model for evaluation pharmacological activities of these compounds such as elevated plus maze, open field test and hole bored test. Phenobarbital was used to induce sleep and hypnosis in the animals. Male Swiss albino mice having weight 25g to 30g were used for their study. Diazepam 2mg/kg, buspiron 30mg/kg and the methanolic extract 10-300 mg/kg i.p were used. The methanolic extract of *Telia inflorescence* at dose level of 100mg/kg lengthened the duration of sodium phenobarbital induced hypnosis. The extract from the given plant at 30mg/kg significantly reduced mice activity in open field and hole board test as indicated from anxiolytic response from their experiment. Anxiolytic effect was evident from the increase time spent in open arm and decrease time

spent in close arm. At higher doses sedation was more pronounced [30]. Other studies also show that quercetin produced sedation and anxiolytic action [23,33]. Anxiolytic like response of these flavonoids are similar to diazepam and buspiron. In another study by Priprem and coworkers [34] also shown that quercetin at dose 300mg/kg displayed anxiolytic effect. Attachment of glycosidic moiety into the aglycon part of these flavonoids increases their lipophilicity and has great influence on its pharmacokinetic properties. Glycosidic linkage of quercetin is not essential for its anxiolytic activity rather it influences its pharmacokinetic behaviour [30].

Abnormal activation of Hypothalamic Pituitary Adrenal axis (HPA) during stressful condition is the major risk factor for depression and anxiety. HPA activation during stressful condition is important for survival. HPA axis is activated by CRF (Corticotropin Releasing Factor) and then secretion of adrenocorticotropic hormones from anterior pituitary and glucocorticoid from adrenal cortex are the major endocrine responses to stress. CRF regulate stress responses on endocrinological, neuronal and at immune level and coordinate autonomic, behavioural and hormonal responses to stress [35]. CRF system is active during stress and depression [36]. Quercetin reverses anxiety and depression induced by CRF. Quercetin was shown to reverse the effect of CRF. They used different animal model for studying effects of quercetin such as social interaction test, forced swim test and locomotor activity test. Quercetin (10, 20 and 40 mg/kg), CRF (0.01, 0.1, 0.3 nmol/mouse), antalarmin (1, 2 and 40 mg/kg), diazepam (0.5, 1, 2 mg/kg), fluoxetine (5, 10 and 20 mg/kg) were used along with carboxy methyl cellulose and tween 80 as vehicle control were used. After required time animal were subjected to various behavioural tests. All drugs had positive effect on social interaction, CRF reduce social interaction time while other agent including diazepam, quercetin and antalarmin increased social interaction time. Their experiment also showed that quercetin reduced anxiety and CRF induce it [35]. CRF impairs the memory and learning [36,37], whereas quercetin improve memory and learning [38].

Luteolin

Luteolin (Table 1) is an important flavonoid belongs to flavones group. Many other flavones such as apigenin, chrysin, wagonin has affinity for benzodiazepine receptor and are anxiolytic. Luteolin has been isolated from many plants such as *Passiflora coerulea*, *Matricaria recutita*, *Tilia tomentosa*, *Jatropha ciliata*, *Salvia guaranitica*, *Matricaria chamomilla*, *Ziziphus jujuba* and also many other plants such as peppers, carrots, olive oil, celery, peppermint, thyme, oregano and rosemary [39]. Recently, these authors have reported the isolation of luteolin-7-O-(2-rhamnosylglucoside) from *Passiflora edulis*. In another study luteolin has been shown to have anxiolytic potential, using various animal models such as elevated plus maze, male albino mice having weight 20 to 25 g were used. Diazepam 1mg/kg was used as positive control and luteolin at doses 0.1 to 50 mg/kg were used for anxiolytic activities. Their result showed that 5mg/kg dose of luteolin significantly increased the percent of open arm entries and also increased its exploratory behavior in open area indicating anxiolytic effect of luteolin [40].

Ellagic Acid

Ellagic acid (Table 1) is a flavonoid found in many naturally occurring plants such as nut, eucalyptus species and raspberries. It has

several pharmacological effects such as anticancer, cardioprotective, antioxidant, anti-inflammatory and antifibrotic [41]. Ellagic acid also has antidepressant activity by interacting with serotonergic and adrenergic system [41]. Many of the anxiolytic drugs have effect on 5-Hydroxytryptamine 1A (5-HT_{1A}) acting as partial agonist of this receptor such as buspirone, ipsapirone and gepirone [41]. Flavonoid showing affinity for 5-HT_{1A}, which are located at post synaptic and presynaptic sites have been shown to possess anxiolytic effects. This is the most selective receptor for anxiety activity because drugs which has effect on this receptor is associated with very less side effects including decrease spontaneous locomotor activity and motor impairment as opposed to benzodiazepines [42]. Girish and coworkers have also shown that ellagic acid has anxiolytic potential by using adult male Swiss mice. All animals were provided required favorable conditions. Elevated plus maze was used to study the anxiolytic activity of ellagic acid. Their experiment showed that ellagic acid at dose level of 25, 50 and 100 mg/kg and diazepam 1mg/kg significantly increased open arm entries and per cent time spent in open arm. Total arm entries (open plus close) were not affected at all doses. Picrotoxin at dose 1mg/kg reversed the action of ellagic acid. The action of diazepam and ellagic acid was also blocked by flumazenil. Mice when treated with PCPA and pindolol (5-HT_{1A}/IB antagonist) did not reverse the action of diazepam and ellagic acid indicating non involvement of serotonergic system in relieving anxiety. GABA system is the most probable system involve in relieving anxiety. Prunus domestica contain chlorogenic acid and certain polyphenol compounds such as (-) epigallocatechin gallate that has also anxiolytic action affecting GABAergic system [43].

Apigenin-glycoside

Apigenin-7-glycoside (Table 1) is found in many plants such as *Stachys tibetica*. It is a flavonoid having anxiolytic activity affecting GABA receptor function. Kumar and co worker [11] have shown that apigenin 7-glycoside have anxiolytic action. They isolated apigenin 7-glycoside from *S. tibetica*. They used albino rates of both sexes 150-200 g in weight. Acute toxicity of the compound was checked and 2.5mg/kg dose of the compound was selected for studying anxiolytic activity. Diazepam was used as a positive control. Apigenin 7-glycoside was suspended in sodium carboxy methyl cellulose and control group received aqueous sodium carboxy methyl cellulose (1%). Elevated plus maze test showed anxiolytic activity of apigenin 7-glycoside as evident from an increase in number of open arm entries and decrease in number of close arm entries [11].

Naringin

Naringenin (4',5,7-trihydroxyflavanone) (Table 1), a dietary flavonoid abundant in peels of citrus fruits, has gained increasing interest because of its positive health effects in biology and medicine. It has neuroprotective [44] and monoamine oxidase inhibitory [45] activity and also has benzodiazepine binding affinity [46]. Neuroprotective and monoamine oxidase inhibitory activities may provide protection against oxidative neurodegeneration and alleviate central nervous system disorders such as depression and anxiety [47,48]. Naringin is a flavonoid found in much citrus food provides them bitter taste. It is also found in much other plant such as grapefruit and mandarin. It has many biological activities such as antioxidant, anti-inflammatory, anti-mutagenic, anti cancer and neuroprotective. Mani et al [46] have shown that naringin have anxiolytic activity.

They use male albino rats (200-240 g) for their experiment using various animal models and have showed that naringin have anxiolytic activity in addition to many other CNS activities [46]. It has binding affinity with benzodiazepine receptor. Anderson and his colleagues [49] have studied naringin for its anxiolytic activity in using male rats (200-250 g) evaluated in elevated plus maze test. Control group received ethanol (40%) and DMSO, group 2 receive naringin and DMSO, group 3 receive midazolam and DMSO and group 4 received naringin and midazolam. There was no significant difference between control and all other group in open arm entries and time spend in open arm in elevated plus maze suggest that naringin does not produce anxiolysis by modulating the GABA-A benzodiazepine receptor site. When rats were injected midazolam, rats went to the close arms and decrease their total movement was decreased. Midazolam, midazolam + naringin, and naringin + flumazenil showed significant decrease in movement compared with control group. The midazolam + naringin group had the least amount of movement in both basic and fine categories as compared to all other groups. This was followed by increase amount of both fine and basic movement in midazolam alone, naringin alone and flumazenil and naringin. The decreased in fine and basic movements are indication of anxiolytic actions. Naringin produce anxiolysis by not modulating GABA but rather some other mechanism. Extracts from mentha species have been shown to possess dose dependent activity mediated via GABA receptor. Other studies have shown that *Mentha aquatica* extract has inhibitory effect on MAO-A [49].

Chrysin

Chrysin (5,7-dihydroxyflavone) (Table 1) is found in many naturally occurring plants and has anxiolytic activity. Benzodiazepines are used for relieving anxiety, but are associated with several side effects such as amnesia. Some naturally occurring flavonoids have anxiolytic action but are not associated with some effects associated with benzodiazepines such as sedation, myorelaxation and anticonvulsant effects therefore have shown selectivity in treating anxiety. These flavonoids are considered partial agonist of benzodiazepine receptor. Salguero et al [50] studied the effect of chrysin on rats 70-90 days old having weight 240-280 g for anxiolytic activity. Elevated plus maze showed anxiolytic potential of chrysin. They also studied other effects of chrysin along with anxiolytic activity such as amnesia and hypnosis. Diazepam 2mg/kg was given to animals for avoidance test. Diazepam increased inhibitory avoidance, sleep down latency, and decrease locomotor activity and decrease retention of habituation to the open field. Tail flick test showed analgesic action of diazepam but had no effect on memory enhancing effect of chrysin. They concluded that chrysin has anxiolytic activity with no side effects associated with diazepam [50].

Wogonin

Wogonin (Table 1), a flavone found in *Scutellaria baicalensis georgi*, an important medicinal herb used in traditional Chinese medicine, has been reported to elicit anxiolysis in mice when administered orally (7.5 to 30 mg•kg⁻¹) [51] and significantly block convulsions induced by pentylenetetrazole and electroshock but not strychnine when administered i.p. (10mg/kg) [52]. No sedative or myorelaxation effects were observed, and both the anxiolytic and anticonvulsant actions could be blocked by co-administration of flumazenil. Another flavonoid isolated from *S. baicalensis georgi*,

oxylin A, identified as an antagonist at the benzodiazepine site, and selectively abolished the anxiolytic, myorelaxant and motor in-coordination, but not the sedative and anticonvulsant effects of diazepam [53]. These reports suggest that both compounds act with some subtype selectivity on GABA receptor [54].

Rutin

Rutin (Table 1) is an important flavonoid found in many plants. It has been shown to have excellent anxiolytic potential. *Hypericum*

perforatum (St John's Wart) has been used for centuries for many CNS problems such as anxiety, sleep disorder or depression. It has been shown that SJW is rich source of flavonoids having prominent action on GABA-A, GABA-B and glutamine receptors, inhibition of monoamine oxidase A and B and also inhibition of reuptake of serotonin noradrenaline and dopamine [2]. This plant contains many classes of flavonoids such as amentoflavon, quercetin and rutin. Gobbi and Mennini [55] have reviewed flavonoids where they have shown molecular mechanisms of flavonoid binding to its receptors for relieving anxiety [55].

Table 3: Affinity of natural flavonoids for the benzodiazepine receptors.

Name	Structure	Ki (mM)
	Monoflavonoids	
Flavone	2-Phenylchromone	1
Norwogonin	5,7,8-Trihydroxyflavone	0.88
Dinatin	5,7,40-Trihydroxy-6-methoxyflavone	3-Jan
Chrysin	5,7-Dihydroxyflavone	3
Apigenin	5,7,40-Trihydroxyflavone	3
Tangeretin	5,6,7,8,40-Pentamethoxyflavone	2-9.
Baicalein	5,6,7-Trihydroxyflavone	4
Isoquercitrin	5,7,30,40-Tetrahydroxyflavone-3-O-Glc	10
Skrofullein	5,40-Dihydroxy-6,7-dimethoxyflavone	23
Eriodictyol	5,7,30,40-Tetrahydroxyflavanone	>25
Naringenin	5,7,40-Trihydroxyflavanone	>25
Genistein	5,7,40-Trihydroxyisoflavone	>25
Phloretin	4,20,40,60-Tetrahydroxydihydrochalcone	>25
Flavanone	7,30-Dihydroxyisoflavan	>40
Tectochrysin	5-Hydroxy-7-methoxyflavone	50
Equol	7,40-Dihydroxyisoflavan	80
Kaempferol	3,5,7,40-Tetrahydroxyflavone	93
Galangin	3,5,7-Trihydroxyflavone	>100.
Luteolin	5,7,30,40-Tetrahydroxyflavone	>100
Fisetin	3,7,30,40-Tetrahydroxyflavone	. >100
Quercetin	3,5,7,30,40-Pentahydroxyflavone	>100
Ruti	5,7,30,40-Tetrahydroxyflavone-3-O-Glc-Rha	>100
Rhoifolin	5,40-Dihydroxyflavone-7-O-Glc-Rha	>100
Myricetin	3,5,7,30,40,50-Hexahydroxyflavone	>100
Morin	3,5,7,20,40-Pentahydroxyflavone	>100
Cirsiliol	5,30,40-Trihydroxy-6,7-dimethoxyflavone	>100
Acacetin	5,7-Dihydroxy-40-methoxyflavone	>100
	Biflavonoids	
Amentoflavone	I-40, II-40, I-5, II-5, I-7, II-7-Hexahydroxy [I-30, II-8] biflavone	0.007
Ginkgetin	II-40, I-5, II-5, II-7-Tetrahydroxy-I-40, I-7-dimethoxy [I-30, II-8] biflavone	2.-6
Isoginkge	I-5, II-5, I-7, II-7-Tetrahydroxy-I-40, II-40-dimethoxy [I-30, II-8] biflavone	2.-6
Bilobetin	II-40I-5, II-5, I-7, II-7-Pentahydroxy-I-40-methoxy [I-30, II-8] biflavone	>5
Sciadopitysin	I-5, II-5, II-7-Trihydroxy-I-40, I-7, II-40-trimethoxy [I-30, II-8] biflavone	>5

The K_i values estimate the inhibition of [3H] diazepam or [3H] flunitrazepam binding to rat cerebral cortical synaptosomal membranes [92].

Trigonella foenum-graecum L. belongs to family fabacea. It was used by Egyptians (1500 BC) for several curative purposes. This plant has been widely studied and it has been shown that it has anxiolytic potential. Assadi and Alam [56] studied seeds of *T. foenum graecum L* for their anxiolytic potential and found that this plant has excellent anxiolytic potential using. Using dark transition model and hole bored tests. After 15 days of feeding methanolic extract of the plant to the mice, they noted anxiolytic potential of the plant. They studied methanolic extract of the seeds (50, 100, 200 mg/kg) in comparison to standard dose of diazepam (1mg/kg) [56].

Isoorientin

Isoorientin (Table 1) has been isolated from *Passiflora edulis*. It is an important plant in the family passifloraceae. It is grown in tropical and subtropical regions for juice and fresh fruit. It is also used traditionally for anxiety, alcoholism, insomnia, migraine and nervousness [57]. Various flavonoids have been isolated from this plant including luteolin-6-C-chinovoside, isoorientin, lucenin-2, luteolin-6-C-fucoside and vicenin-2, isovitexin monitored at various spectral ranges. Several studies have showed that *P. edulis* has anxiolytic activity. A study conducted by Li et al 2011 [58], has showed that *P. edulis* has potential anxiolytic effects due to the presence of several flavonoids as given above. They use RP-HPLC with diode array detection and chromatogram for identification and separation of different flavonoids in the plant. The Swiss albino rats of both sexes were used weighing 20-25 g each and at 5 weeks age. The animal received different concentration of the extract such as 100, 200 and 400 mg/kg suspended in carboxymethyl cellulose (1% w/w). Diazepam was used as reference drug for anxiolytic activity at 2mg/kg dose. From their experiments they concluded that the given plant have sufficient concentration of flavonoids that have potential for relieving anxiety [58].

Flavonoids Affinity for GABA Receptor

GABA plays an important role in anxiety. Benzodiazepine binds with GABA receptor mediating anxiety. Table 3, shows the compounds having affinity for the GABA receptor and believed to play an important role in anxiety [12]. Experiment in transgenic animals (mice) both knock-in and knock out showed that GABA has various receptors subtypes and each of them plays different physiological roles [88,89]. Comparing mutant and wild types mice have shown different responses to diazepam indicated from their behavior studies; GABA receptors containing $\alpha 1$ -subunits mediate sedation and serve as targets for hypnotic and sedative actions, while $\alpha 2$ - and $\alpha 3$ -containing receptors mediate anxiolysis while $\alpha 5$ -containing receptors are associated with memory [90,91]. Other studies have also shown that 6-hydroxyflavone act as a subtype selective partial positive allosteric modulator at the flumazenil-sensitive benzodiazepine site. 6-Hydroxyflavone exhibit significant preference for $\alpha 2$ - and $\alpha 3$ - compared to $\alpha 1$ - and $\alpha 5$ -containing receptors expressed in HEK 293T cells. *In vivo*, 6-hydroxyflavone displayed anxiolytic effects in the elevated plus-maze test, with no sedation, myorelaxation, cognitive impairment, anticonvulsant, or motor in-coordination effects at anxiolytic doses [54].

Concluding Remarks

In short, the current anti-anxiety and antidepressants are challenged by many limitations in terms of overall bioavailability/

pharmacokinetic, generalized sensitivity, efficacy, safety etc. and therefore patient compliance is a crucial issue causing millions of peoples attempt suicide every year. As a result, new effective and safe agents most warrant meeting the challenges. In this regard, flavonoids could be ideal therapeutic agents for the treatment of such disorders. Most of the flavonoids are isolated from dietary components and therefore, possessed intrinsic safety.

The different flavonoids mentioned in the review illustrated marked anti-anxiety effects, significant affinity for the benzodiazepine receptor and pharmacokinetic data are also available. Using modern sophisticated techniques, the pharmacokinetic parameters could be modifying as per requirement through synthesis of derivatives as well as through different dosage/drug delivery systems. To know the real picture of these reported flavonoids, clinical trials are strongly recommends for their clinical fate.

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