

The anxiolytic effect of some plant extracts in clinical trials and animal models

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Abstract

The phenomenon of anxiety remains poorly understood and there is much to be learnt about its effective treatment. Here we discuss the data of available scientific literature concerning the use of plant derived extracts in the treatment of anxiety. Study of the treating effects of natural alternatives to prescription drugs is very important in regard to limitations of current drug therapies. Significance of the study of treating efficacy of endemic plant species, endemic to Georgia in particular, and the use of animal models, such as rat grooming and rat behavior in maze, for evaluation of the anxiolytic efficacy of phytomedicines is discussed as well. We conclude, that medical as well as experimental trials in animal models of anxiety provide strong argument for the use of plant extracts as an alternative to the current drug therapies for anxiety.

Keywords: Anxiety; Treatment; Plant extracts; Animal models

1. Introduction

Stress-induced anxiety, as a consequence of the impact of stressogenic environment on living things, remains poorly understood and there is much to be learnt about its effective treatment. Nowadays, treatment of anxiety gets special significance with respect to the negative influence of COVID pandemic on the emotional status of people [1]. Antidepressant and anxiolytic drugs are most commonly prescribed in order to treat anxiety. However current drug therapies have limitations such as high financial expense and side effects [2-4] as well as drug dependence and stigma associated with consuming and depending on pharmaceuticals [5,6]. Therefore, further research is necessary to find new anxiolytics with less adverse effects and higher availability for wider community [7]. Herbal alternatives are distinguished with their lower costs and consequent availability for wider community as compared to drugs, produced by pharmaceutical industry. Many of these natural alternatives are available in some form at local pharmacies, or are easily obtainable from online websites, making them more accessible than prescription drugs. According to World Health Organization, about three-quarters of the world population relies upon traditional remedies for the health care [8].

Natural alternatives are derived from plants and have demonstrated anxiolytic effects in both humans and animals. They have been used as traditional food and medicines for a range of illnesses for centuries [9]. A systematic review of the published literature revealed trial data for several plant species (*Ginkgo biloba*, *Lavandula angustifolia*, *Hypericum perforatum*, *Valeriana officinalis*, *Crataegus oxyacantha* and *Eschscholzia californica* as well as *Matricaria recutita*, *Melissa officinalis*, *Passiflora incarnate* and *Piper methysticum*) demonstrating high level of evidence for the treatment of anxiety disorders [10]. Several human clinical trials provide preliminary positive evidence of anxiolytic activity of

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extracts of *Matricaria recutita*, *Ginkgo biloba*, *Passiflora incanata*, *Echium. amoenum* and *Scutellaria lateriflora* [9]. Other systematic reviews of the anxiolytic effect of essential oils in translational medicine models suggest the extracts of plant species *Achillea wilhemsii*, *Angelica sinensis*, *Alpinia zerumbet*, *Celastarus paniculatus*, *Chamaecyparis obtuse* and *Citrus aurantium* subsp. *bergamia*, as well as *Citrus janus*, *Citrus latifolia*, *Citrus reticulate*, *Citrus sinensis*, *Cymbopogon citratus* and *Copaifera reticulate*, *Ducrosia anethifolia*, *Foeniculum vulgare*, *Lavendula angustifolia*, *Lippia alba*, *Spiranthera odoratissima* and *Stachys tibetica* to exert anxiolytic effect [11]. *Salix aegyptiaca* is shown to decrease the anxiety in animals as well [12]. Various plant species have been used from immemorial in Asia by local tribes as food and medicine [13]. The search for endemic plants species, as a candidates for medicinal use, has special importance with this respect. Endemism is the ecological state of a species being unique to a defined geographic location, such as an island, nation, country or other defined zone, or habitat type. Factors, specific to their location, such as microclimate, determine anatomical and physiological characteristics of endemic species. Endemism influences the chemical content (phytochemicals) of the plant species and this makes endemic plants the focus of interest for their use in medicine [14-16].

Endemic plant species of Georgia does not make exclusion with this respect. Various plants have been used as a source of herbal medicines for centuries and investigation of Georgian medicinal manuscripts of X-XVIII centuries confirmed the use of various plant species for medical purposes [17,18]. Earlier, in unpublished study, we collected information on some plant species, endemic to Georgia (*Galanthus lagodechianus* Kem-Nath., *Campanulla kachetica* Kantsch., *Gypsophila steveni* Fisch. & Schranl and *Gymnospermium smirnowii* (Trautv)Takht.) from local dwellers in the territory of the spread of these endemic species. Respondents were questioned concerning the use of the plant species as a phytomedicines. Respondents witnessed the use of extracts of the plant species of interest in combination with other plant extracts as sedatives. At the same time, respondents provided information about anatomical parts (roots, leaves etc.), usable for medicinal purposes, the period (season) when species should be collected and what is a method of preparation of the herbal medicine. Further research is necessary, however, to get scientifically relevant data confirming the medical effects of these endemic plants, and their anxiolytic capacity in particular.

Usually, the treating capacity of plant derived phytochemicals is studied on animal models. For example, Zebra fish animal model is used in research of traditional Chinese medicines [19]. Here we concentrate on two models - rat grooming and rat behavior in elevated maze.

Rat grooming as well as rat behavior in Elevated Plus-Maze (EPM) are good candidates for studying the effect of plant derived medicines on anxiety. Both, grooming as well as behavior in EPM belong to the ethologically based animal models of fear and anxiety. Models of this kind attempt to approximate the natural conditions under which such emotional states are elicited. By employing nonpainful aversive stimuli to induce fear and anxiety, ethological tests minimize possible confounding effects of motivational or perceptual states arising from interference with learning/memory, basic physiological needs or pain-inducing mechanisms. In comparison to conditioned models, ethologically based tests are suggested better analogs of human anxiety [20].

Behavior, expressed in strokes, licks and scratching of entire body is known as "Grooming". Grooming is an innate behavior which may be expressed as either self-grooming or allogrooming - care of conspecific's body [21-26] and is represented across species of mammals and birds, as well as in some representatives of insects [22]. Grooming plays an important role in the care of body surface and regulation of body temperature, as well as in social communication among conspecifics [22,26]. At the same time, grooming lowers the level of anxiety and helps in adaptation to stressogenic environment [27-29]. Rodents are distinguished by rich grooming repertoire and for this reason scientists focus on grooming in laboratory rats [30].

Grooming in rats consists of two principal patterns such as syntactic chain embedded in other forms of grooming behavior. Syntactic chain represents grooming movements into distinct, predictable phases that follow the cephalic-caudal direction, starting from the head and ending in genitals and the tail. The phases of syntactic chain are such as 1. licking the paws, 2. nose and face grooming (strokes along the snout), 3. head washing (strokes over the top of the head and behind the ears by the forepaws), 4. scratching body by hind paws and licking the body fur and 5. licking hind paws, tail and genitals. Some authors distinguish 3 phases such as rostral grooming (grooming of forepaws, nose and face), head washing and body grooming - grooming of body, hind paws and tail/genitals [23].

Natural grooming develops spontaneously and may be observed in everyday situation, while in the experiment grooming may be provoked by various stressors. Stressors used for experimental provocation of grooming behavior are such as handling by the tail, restraints, placement of the animal in novel chamber, fur moistening, delivering noise, exposition to the bright light, predator or larger conspecific.

Grooming behavioral microstructure undergoes predictable changes in stressful situations [21,24]. Increase in the percentage of interrupted grooming bouts, incorrect transitions from one grooming phase to another as well as more rostral pattern of grooming is described as a reaction to the stressor. These changes in grooming microstructure are believed a behavioral marker of stress in rats [29]. At the same time, the change in grooming microstructure depends on the strength of stressor. For example, fur moistening, as a stressor affects grooming much less as compared to the exposition to the bright light [30-32], the data confirming the earlier reports of other authors [33] and in accordance to the more recent reports [34-36]. Therefore, the change in the grooming pattern may identify the stress in the rats and at the same time, may serve as a measure of the strength of stress-induced disorders.

Stress-induced grooming was found to significantly decrease in rats, treated with *Citrus bergamia* essential oil as compared to the control non-treated animals [37]. The same anxiolytic effect was confirmed for aqueous extract of *Alafia multiflora* stem barks in rodents [38]. Significant decrease in grooming activity in Open Field Test was registered in rats, treated with *Arnica montana* [39], *Cinnamomum tamala* and *Withania somnifera* extracts [40]. We reported on anxiolytic effect of the tincture of *Melissa officinalis* in rats, exposed to the bright light as compared to the control group, not treated with the tincture [41].

EPM method relies upon rodents' unconditioned fear of heights/open spaces (avoidance). Behavioral responses in the EPM are easily assessed and quantified by an observer. Briefly, rodents are placed in the intersection of the four arms of the EPM and their behavior is typically recorded for 5 min. The behaviors that are typically recorded when rodents are in the EPM are the time spent and entries made on the open and closed arms. Behavior in this task (i.e., activity in the open arms) reflects a conflict between the rodent's preference for protected areas (e.g., closed arms) and their innate motivation to explore novel environments. Anti-anxiety behavior (increased open arm time and/or open arm entries) is determined simultaneously with a measure of spontaneous motor activity (total and/or closed arm entries). An increase of the time and the proportion of the entrances into the open arms without a changed locomotor activity are regarded as a powerful marker for an anxiolytic substance effect. Other ethological measures that can be observed in rodents in the maze are the number of rears, head dips, fecal boli, freezing or stretched-attend postures [42-44].

The per or administration of *Mercurialis annua* extract showed an anxiolytic effect by increasing the time spent on open arms and the percentage of open arm entries compared to the control group [45,46]. Aqueous extract of leaves of *Coriandrum sativum* is shown to possess dose-dependent anxiolytic activity [47]. Aqueous extract of *Urtica urens* is suggested to increase the time spent on open arms and the percentage of open arm entries in plus maze as compared to control group. Treatments of experimental rats with the aqueous/alcoholic extract of *Plectranthus amboinicus* significantly increased the time-spent and entries into open arms of the EPM, and reduced the time-spent and entries into the closed arms versus saline controls [48]. Significant anxiolytic effects of *Lagenaria cineraria* extracts in rats was demonstrated in EPM [49]. *Allium jesdianum* extract increased time spent and the number of entrance in the open areas of EPM [50]. Treatment with the extract of *Ficus sycomorus* reduced the time and the number of entries into the closed arms of plus maze in rats [51]. The per or administration of the extract of ethanolic extract of Indian *Abies pindrow* leaf showed significant anxiolytic effects on all the paradigms of anxiety, reflected in the significant increase in open field ambulation and slight increase in rearing and activity in the center [52]. Rats treated with the extract of *Melissa officinalis* displayed high percentage of open-arm entries in the EPM as compared to the control non-treated animals [53].

2. Conclusion

The anxiolytic effects of several phytomedicines is demonstrated in medical trials as well as in experimental trials in animal models of anxiety. Taken together, these data are convincing, that plant extracts are efficient in the treatment of anxiety and plant-derived medicines may be considered an alternative to the prescription drug therapies for anxiety. This knowledge is beneficial for society, as long as herbal alternatives to prescription drugs are distinguished with less side effects as well as lower costs and consequent availability for wider community.

Compliance with ethical standards

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Disclosure of conflict of interest

There is no conflict of interests to declare.

Statement of ethical approval

The content of the manuscript is approved by the Ethic Committee of the Ivane Beritashvili Center of Experimental Biomedicine, Tbilisi, Georgia.

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