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Short Communication

Investigation on antidepressant properties of *Arnica montana* in mice model of depression using forced swimming test

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ABSTRACT

Arnica montana have been used as traditional medicine to treat inflammation and minor skin bruises as well as treatment for mental health disorder in homeopathic practice. To date, the role of *Arnica montana* in psychological disorder such as depression is not scientifically evaluated. Hence, this research study evaluates the antioxidant and investigate the antidepressant properties of *Arnica montana* using forced swimming test (FST). The DPPH test is a simple and quick technique to evaluate antioxidants using spectrophotometry. *Arnica montana*. In this research, mice are treated according to their five treatment groups (n = 6) for seven days while the FST were conducted on the 1st and 7th day of the experiment. Overall, the present study clearly demonstrated that *Arnica montana* exerts an antidepressant property in animal behavioral testing.

Key words: *Arnica montana*, escitalopram, immobility time, forced swimming test, antidepressant activity

1. Introduction

Depression is distinct from normal mood swings and short-term emotional responses to ordinary stressors. Depression can be dangerous to one's health, especially if it is persistent and has a moderate-to-severe intensity. It can make the individual who is affected suffer greatly and perform poorly at job, school, and in the family. Depression can lead to suicide at its worst. Every year, around 700,000 people die by suicide. Suicide is the fourth highest cause of death among those aged 15 to 29 WHO (World Health Organization) 2021.

In Malaysia, depression is estimated to affect 8 to 12 percent of the population. Women from low socioeconomic backgrounds and those dealing with comorbid medical conditions registered higher rates (Ng, 2014). The current treatment regimen of clinical depression with anti-depressants is widely prescribed. These anti- depressants have many unpleasant side effects (Claire et al., 2016). These medications' side effects, as well as their low tolerability profile, have limited their clinical use (Claire et al., 2016). Previously, the role of *Arnica montana* homeopathy drug in treatment of clinical depression has never been fully understood.

This research can help to bridge the understanding on the use of alternative medicine in the treatment of clinical depression. The findings of this study can directly benefit clinically depressive patient to have a better treatment option or an add on treatment option because, as aforementioned available anti-depressants have low benefit and unfavorable side effects. *Arnica montana* is a rhizomatous herbaceous perennial herb and a medicinal plant found in Russia, Siberia upland meadows of Central Europe and sparsely found in North Western part of the United States. It can be commonly found in grasslands and shrub lands and alpine mountain environments. *Arnica montana* has been used in homoeopathic medicine for decades. It is used to treat 66 different diseases, but it is most widely used for contusions, burns, rheumatism, and inflammation (Kriplani et al., 2017).

Homeopathy is a form of medicine that is basically founded on the "Principle of Similars": a substance that might cause sickness in a healthy subject can be used as a medicine to treat similar symptom patterns in a sick person; homoeopathic medications are thought to trigger a patient's own self-regulatory healing response (Merrell and Shalts, 2002).

2. Materials and Methods

2.1. Sources of Arnica montana mother tincture

Arnica montana mother tincture was purchased from a local homeopathy center named Jasmin Foundation Homeopathic & Integrative Healthcare Centre. Manufactured

by BM Homeopathic Pharmaceuticals situated in Lahore, Pakistan

2.2. Preparation of Arnica montana 5C, 9C, 30C homeopathy drug

In this current study, the mother tincture was diluted using the Centesimal scale, which is based on the principle that the first potency of mother tincture dilution contains one hundredth part of the original tincture and succeeding potency shall contain one hundredth part of the preceding potency. The Centesimal Scale started off with six minims of tincture and 94 minims of dilute alcohol to give its first potency followed by mixing one minim of first potency with 99 minims of alcohol which gives the second potency. Thereafter subsequent potencies are prepared with one minim of preceding potency with nine minims of dilute alcohol. Minim is a measurement equivalent to one drop of water used in apothecary practice. This method is known by the suffix C indicating its potency.

Potency	Dilution
1C	1. 6 minims of *Arnica montana* mother tincture was added 2. Mixed with 94 minims of normal saline
2C	1. 1 minim of 1C tincture was added 2. Mixed with 99 minims of normal saline
5C	1. 1 minim of 4C tincture was added 2. Mixed with 9 minims of normal saline
9C	1. 1 minim of 8C tincture was added 2. Mixed with 9 minims of normal saline
30C	1. 1 minim of 29C tincture was added 2. Mixed with 99 minims of normal saline

2.3. 2,2-diphenyl-1-picrylhydrazyl test (DPPH test)

The 2,2-diphenyl-1-picryl-hydrazyl (DPPH) assay is commonly employed to determine the free-radical-scavenging activity of crude extracts and single compounds. *Arnica montana* homeopathy drug was too diluted to be used for the DPPH test hence the mother tincture was used which consists of ethanol and *Arnica montana* extract. The study method and ratio of mother tincture volume was adapted from the Ahmad et al., 2017 study. The DPPH stock solution was prepared by adding 3.92 mg DPPH in 100 ml of 82% methanol. After that, 180 µl of DPPH solution (in methanol) was added to 5, 10, 20 µl of *Arnica montana* mother tincture solution in different test tubes. The mixture was thoroughly mixed before being incubated for 15 minutes at 37°C in the dark. The control solution was prepared by mixing methanol (0.1 ml) and DPPH solution (4 ml). A spectrophotometer was used to measure the absorbance at 517 nm. Ascorbic acid was used as positive control and each test was carried out three times. The changes in colour from deep violet to light yellow was observed. Percentage of Scavenging Activity was calculated using EQUATION 1. One-way ANOVA test followed by post hoc Tukey's test was conducted to evaluate the statistically significant difference between all groups of samples.

$$\text{Scavenging activity}(\%) = \left(\frac{A_{517}\text{ of control} - A_{517}\text{ of sample}}{A_{517}\text{ of control}}\right) \times 100$$

Equation 1

2.4. Animals

30 male imprinting control region (ICR) mice weighing 30–40 g were used. Six animal per cage maintained under the standard conditions: room temperature (25 ± 3)°C, humidity 25% ± 5%, 12/12 hr light/dark cycle. Animals were fed with commercially available mouse pellet diet and water was accessible at all time. Animals were placed at the Animal house of University of Cyberjaya, Cyberjaya, Selangor. The study was done in accordance with Malaysian Code of Practice for the Care and Use of Animals for Scientific Purposes by Laboratory Animal Science Association of Malaysia (LASAM) and UOC Animal Care & Use Committee. Ethics approval was obtained from UOC Animal Care & Use Committee. All efforts were made to minimize the number of animals used and minimize suffering induced upon the animals.

2.5. Drug administration

Animals were randomly divided into 5 groups of 6 each and received drugs as follows: vehicle treated: normal saline (NaCl 0.9%); Escitalopram 15 mg/kg; *Arnica montana* 5C *Arnica montana* 9C *Arnica montana* 30C. The treatment drug was administered intraperitoneally.

Animals were randomly divided into five groups (six mice per group) and received treatment as follows: vehicle treated, normal saline (NaCl 0.9%); escitalopram 15 mg/kg; *Arnica montana* 5C 15 mg/kg, *Arnica montana* 9C 15 mg/kg, and *Arnica montana* 30C 15 mg/kg. The treatment drug was administered intraperitoneally once a day, an hour prior to undertaking forced-swimming test.

2.6. Forced swimming test (FST)

This training procedure was conducted based on the method reported in the Roni Yankelevitch-Yahav et al., 2015 study. On day 0, a pilot test was conducted, where all the groups of animals were subjected to the FST after administering the respective drug solutions 1 hour before the test. In training session, mice were forced to swim individually in a 2L Beaker, containing fresh water up to 15 cm, maintained at 25°C for 6 minutes. In this test, after a brief spell of vigorous activity, animals showed a posture of immobility which was characterized by floating motionless in the water making only those movements necessary to keep the head above the water. This immobility reflects the state of depression, and the duration of immobility was recorded

for 6 minutes. Actual test recordings were done on 1st, 7th day of treatment. After recording of mobility-immobility time, each mouse was removed, wiped with dry cloth and allowed to dry before being returned to their home cages. After every session was ended, fresh new water was replaced to avoid any influence on the next mice.

2.7. Statistical analysis

The analysis was done by using IBM SPSS version 27 software. Data were expressed as the mean ± standard error of mean (S.E.M). Comparisons between experimental and control groups were performed by the one-way analysis of variance (ANOVA) followed by post-hoc Tukey's test, $P < 0.005$ was considered significant.

3. Results

3.1. Antioxidant Property of Arnica montana

Arnica montana mother tincture with the highest volume 20 µl had a better antioxidant potential by reporting the highest percentage of DPPH inhibition with a mean value of (81.45% ± 0.22) compared to the other volumes and ascorbic acid. *Arnica montana* mother tincture 5 µl (40.36 ± 0.14), *Arnica montana* mother tincture 10 µl (80.66 ± 0.15) and ascorbic acid 20 µl (74.33 ± 0.59) respectively. ANOVA test revealed that there was a significant difference ($p < 0.001$) between all three volumes of *Arnica montana* mother tincture and positive control. In comparison post hoc test Tukey's test reported that the ascorbic acid 20 µl (positive control) is significantly different with *Arnica montana* 5 µl ($p < 0.001$), *Arnica montana* 10 µl ($p < 0.001$) and *Arnica montana* 20 µl ($p < 0.001$) respectively.

3.2. Effect of Arnica montana 5C, 9C & 30C when mice exposed to FST

Based on the Figure 1, in increasing order of mean immobility time, on day 1 of the treatment, the mean immobility time for *Arnica Montana* 30C was the lowest with a mean immobility time of 184 seconds. The second lowest immobility time was by the positive control group (escitalopram) with a mean immobility time of 204 seconds.

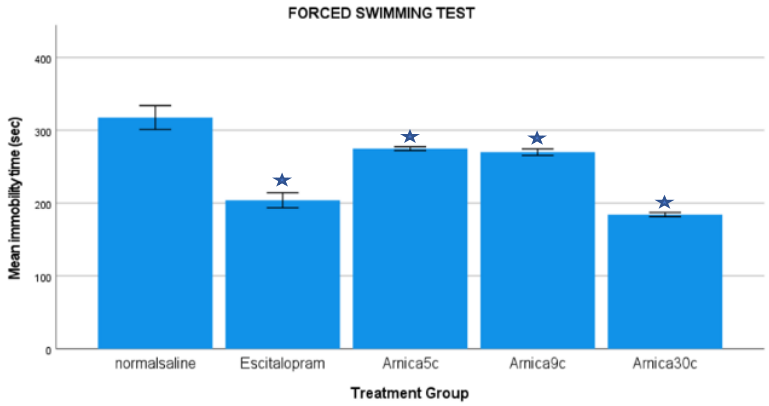

Figure 1. Day1, Effects of Normal saline, Escitalopram, *Arnica montana* 5C, 9C, 30C that was intraperitoneally (i.p) administered 1 hour before the test. Each column represents the mean ± S.E.M of the immobility time (sec) of 6 ICR mouse in each group (n = 6) *P < 0.05 compared to (Normal saline). The ANOVA test followed by post hoc Tukey's test was used to analyse the statistical differences between the groups.

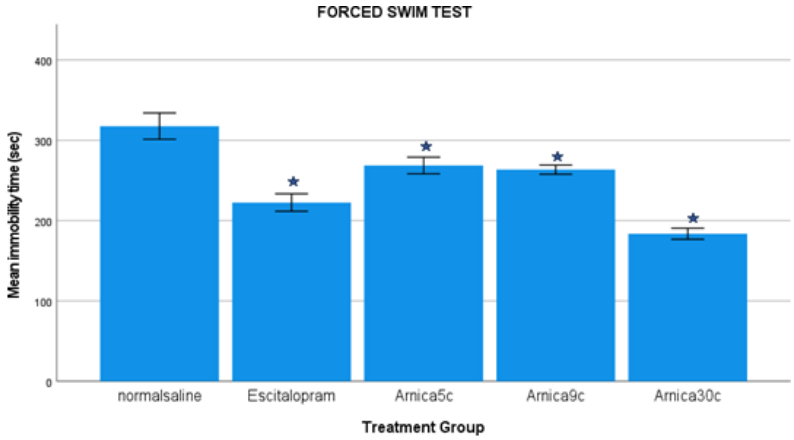

Figure 2. Day 7, Effects of Normal saline, Escitalopram, *Arnica montana* 5C, 9C, 30C that was intraperitoneally (i.p) administered 1 hour before the test. Each column represents the mean ± S.E.M of the immobility time (sec) of 6 ICR mouse in each group (n = 6), *P < 0.05 compared to (Normal saline). The ANOVA test followed by post hoc Tukey's test was used to analyse the statistical differences between the groups.

Thirdly, the *Arnica montana* 9C group recorded a mean immobility time of 270 seconds. Followed by *Arnica montana* 5C recorded a mean immobility time of 274 seconds. Lastly the negative control group (normal saline) recorded a mean immobility 317 seconds. Therefore, *Arnica montana* 30C and positive control group (escitalopram) showed the most reduction in duration of immobility time when compared to the negative control group (normal saline).

Referring to Figure 2, in increasing order of mean immobility time, on day 7 of the treatment, the mean immobility time for *Arnica montana* 30C was the lowest with a mean immobility time of 183 seconds. The second lowest immobility time was by the positive control group (escitalopram) with a mean immobility time 222 seconds. Thirdly, the *Arnica montana* 9C group recorded a mean immobility time of 263 seconds. Followed by *Arnica montana* 5C recorded a mean immobility time of 268 seconds. Lastly the negative control group (normal saline) that recorded 317 seconds. Therefore, *Arnica montana* 30C and positive control group (escitalopram) showed the most reduction in duration of immobility time when compared to the negative control group (normal saline).

4. Discussion

ROS/RNS refers to pro-oxidants/oxidants in general. ROS (radicals formed from oxygen) which is the most important free radicals created during metabolic reactions. Both ROS and RNS can be divided into two types of compounds, radicals and non-radicals. Radicals are species that have at least one unpaired electron in the shells around the atomic nucleus and can exist on their own. The oxygen molecule is a radical, and it is labelled biradical because it consists of two unpaired electrons (Phaniendra et al., 2015). Halliwell B study (as cited in Phaniendra et al., 2015) mentioned that the examples for the radicals include Superoxide (O^2-), Oxygen radical, Hydroxyl (OH·), Alkoxy radical (RO), Peroxyl radical (ROO·), Nitric oxide (nitrogen monoxide) (NO·) and nitrogen dioxide (NO_2) (Halliwell, 1999). It was observed that the highest volume of *Arnica montana* mother tincture 20 µl had a greater percentage of free radical scavenging activity with a mean value of (81.45% ± 0.22 compared to the positive control ascorbic acid with a mean value of (74.33 ± 0.59). This suggests that *Arnica montana* has a better antioxidant potential than ascorbic acid. From a previous study (Dimitrova and Balabanova, 2012) it has been observed that *Arnica montana* flower heads extracts, cultivated in Bulgaria were compared with those of BHT (Butylated hydroxytoluene) as positive controls, it was shown that the scavenging activity of *Arnica montana* was stronger with a IC50 values of 44.65 µg mL^{-1} (DPPH) and 9.87 µg mL^{-1}. Another study also mentioned that against superoxide, hydrogen peroxide, and nitric oxide radicals, it was found that *Arnica montana* extracts had antioxidant activity, which was equivalent or more than standard antioxidants, BHT and ascorbic acid (Shika et al., 2017 as cited in Flórez-Fernández et al., 2021). Hence, we can somewhat say that *Arnica montana* has a great antioxidant potential. The antioxidant potential of *Arnica montana* was suggested due to the presence of tanins and polyphenols which was seen in the (Dimitrova and Balabanova, 2012) study. Valan et al., 2010 study as cited in Priyanka et al., 2017 stated that the presence of flavonoids and phenolic compounds in *Arnica montana* results in 71.52 percent DPPH scavenging potential. Hence it can be said that the antioxidant potential of *Arnica montana* that was seen in this study could be due to the presence of tannins, polyphenols & flavonoids.

In comparison to a study done by Ahmad et al. (2013), the mean immobility of the *Arnica montana* extract 500 mg/kg treated mice with the FST test showed 39 seconds whereas, the mean immobility time of the extract 300 mg/kg of *Arnica montana* treated group was 218 seconds which suggested an increase in the immobility time when concentration of *Arnica montana* decreases. When a homeopathy drug with a higher concentration of active ingredient, has the lower numeral preceding. Therefore, the *Arnica montana* 5C had the highest concentration of active ingredients and reported the highest mean of immobility time. From the study by Ahmad et al., 2013, *Arnica montana* in a higher concentration showed an in mean immobility time which actually showed an anxiolytic like effect but when the concentration decreased it exhibited an antidepressant like effect. Currently there are no studies that could explain as to why the concentration difference in *Arnica montana* exhibits different effects.

A couple of compounds found in *Arnica montana* could be contributing to its antidepressant like effect. When explaining ancient homoeopathic treatments for depression, it has been mentioned that *Arnica montana* can be used to treat depression and anxiety (Gromova, 2013). This could be attributed to the availability of flavonoids (quercetin 3-O-glucoside) in *Arnica montana* mother tincture (Ganzera et al., 2008). Quercetin increases the availability of 5-HT and norepinephrine in the synaptic cleft, which appears to be dysregulated in depression & antidepressant-like properties of quercetin seem to be independent of the HPA axis (Demir et al., 2016). In olfactory bulbectomized rats, it was discovered that quercetin inhibited the microglial neuroin-flammatory response and produces an antidepressant-like effect (Rinwa et al., 2013). Also, Quercetin had no influence on MAO-A activity in mice intestinal mitochondria, implying that it has no side effect on dietary monoamine metabolism in the gut. These findings imply that quercetin acts as a weak (but safe) MAO-A inhibitor in the brain, modulating 5-HT levels (Bandaruk et al., 2012). *Arnica montana* extracts has luteolin another flavonoid which was shown to suppressed the expression of endoplasmic reticulum stress-related proteins in hippocampus of corticosterone-treated depression model mice (Ishisaka et al., 2011). The antidepressant effects seen in Cirsium japonicum Fisch. ex DC are also produced

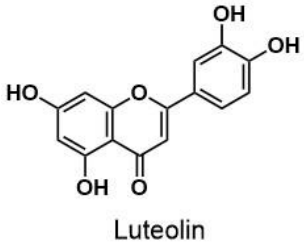

Figure 3. Chemical structure of Luteolin.

by luteolin, probably by potentiation of the GABA A receptor-Cl-ion channel complex (de la Peña et al., 2014) (Figure 3).

5. Conclusion

Arnica montana has been used in many studies and the plant has shown to have medicinal properties which include, antioxidant, anti-inflammatory, anti-anxiolytic, antimicrobial, antifungal property , anti-osteoarthritic. The potential of this plant as an antioxidant and antidepressants needs to be further explored by studying its bioactive compounds and it possible mechanism of action as an antidepressant.

From this current study there may be a potential for homeopathy remedy of *Arnica montana* to be used in depression treatment, but more investigation and research needed to study and justify its appropriateness as an adjuvant or alternative therapy to treat depressed patients.

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