Journal of Innovations in Pharmaceutical and Biological Sciences (JIPBS)
ISSN: 2349-2759
Available online at www.jipbs.com

Research article

Antidepressant activity of *A. hierochuntica L.* effervescent granules using forced swimming test

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Key words: Effervescent granules, Force Swimming Test, Herbal medicines, Anti-depressant.

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Vol. 6 (2), 38-44, Apr-Jun, 2019.

Abstract

People in modern society suffer from different psychiatric disorders, in particular depression. Recently, there is a global increase in the use of herbal medicines for psychiatric reasons especial depression. *Anastatica hierochuntica L.* (Kaff-E-Maryam) is a well-known desert zone medicinal plant widely used as traditional drug in Central Asia, Africa, and elsewhere. The study objective is to assess the antidepressant activity of effervescent granules prepared from aqueous extract of *A. hierochuntica* effervescent granules were evaluated for flow property (i.e angle of repose, bulk density, tapped density and Hausner's ratio), particle size distribution and effervescence time. The antidepressant like activity of *A. hierochuntica* aqueous extract and granules was studied in albino mice using forced swimming test (FST). The formulated effervescent granules exhibited excellent flow properties and bulk density. Effervescence time was less than 15 sec. *A. hierochuntica* aqueous extract and effervescent granular formula at dose level 20 mg/kg exhibited significant decrease in duration of immobility in FST as compared to – ve control group ($p < 0.05$). The effect of *A. hierochuntica* was similar to that of imipramine dose 10mg/kg ($p < 0.05$). No significant difference was observed in antidepressant activity of *A. hierochuntica* aqueous extract and the granule form ($p > 0.05$). Effervescent granules showed that it can be used as a novel approach for using *A. hierochuntica* for treatment of depression.

Introduction

Depression is a common mental illness with severe consequences to human fitness. For most synthetic antidepressants, there are serious defects such as a narrow spectrum of antidepressants, adverse reactions, high drug prices and easy recurrence [1].

Many people gradually turn to herbal medicine to find out other alternative antidepressants with a low toxicity [2].

Many studies have been conducted to investigate the antidepressant activity of many herbs. In many countries, herbal medicines have been widely used, as many people around theworld prefer herbal treatments over chemical medicines [3-4].

In developing countries, herbal medicines are relatively more popular because they are culturally acceptable and people believe they are natural, safe and useful compared to other chemical medicines [2, 4, 6]. *Anastatica hierochuntica L.* (Family: Brassicaceae) locally called 'Kaff-e-Maryam', is a well-known medicinal plant growing in the desert area. [4-5]. *A. hierochuntica* are used traditionally for its various medical benefits [5, 7]. People thought that *A. hierochuntica* plays a role in the treatment of reproductive system, menstrual cramps, uterine bleeding, diabetes mellitus, epilepsy, gastric disorder and depression. Based on its frequent medical use and popularity among the people of Central Asia, Africa, the Arab Peninsula and elsewhere, Kaff-e-Maryam was selected for this study [2, 5]. Effervescent granules are fast dissolving, highly soluble, convenient dosage forms for patients with difficulty swallowing [8]. Therefore, effervescent preparations are interesting alternatives for the pharmaceutical administration of herbal extracts because they can incorporate large quantities of material in a single dose, musk unpleasant taste and provide an easy and accepted method of herbal drug use. Additionally, they are easy to take, exhibit more stability than liquid dosage forms and offer the possibility to improve the absorption of the active ingredients due to prior dissolution [9, 11]. This study is to prepare an effervescent granule of aqueous extract of *A. hierochuntica* in addition to assess the antidepressant activity using FST in mice.

Experiments

Material and method

The whole dried plant was purchased from medicinal plant shop in Qassim region. The authenticity of the plant

was identified at medicinal chemistry and natural product department, College of pharmacy, Qassim university. Imipramine hydrochloride, a tricyclic antidepressant, was purchased from Sigma–Aldrich (St. Louis, MO USA), Tartaric acid, Citric acid and Sodium bicarbonate (Colorcon Asia Pvt. Ltd - Mumbai – India), PEG 4000, Mannitol and aspartame Sigma-Aldrich Co. (St. Louis, MO, USA).

Plant extracting

A. hierochuntica aqueous extract was prepared as reported by Asuzu in 1986 [10], with a minor modifications. Ten grams of dried plant material were added to one hundred milliliters of sterile distilled water in a round bottom flask with a glass stopper. The mixture was then shaken well and allowed to stand at 25^{0}C for 2 h. Then a reflux condenser was attached to the flask and boiled gently for 1h, cooled, shaken well and filtered through a dry Whatman filter paper No 1. The filtrate was then poured into a sterile glass beaker and kept in hot air oven at 50°C to evaporate till dryness and reach a constant weight. Dried extract stored in an air tied container at – 80°C until further analysis.

Preparation of effervescent granules using the wet method

A. hierochuntica effervescent granules were prepared by wet granulation method. Ten grams of dried extract was weighed accurately, passed through sieve no 20 and blended with tartaric acid, citric acid and sodium bicarbonate mixture. PEG 4000 dissolved in absolute ethanol, dried with heating in water bath, sieved through sieve 20 and added to the mixture. Finally mannitol and aspartame added to the mixture powder. The mixture powder was granulated by 95% ethanol and sieved through 80-mesh sieve and dried with heating in water bath under 50°C. The dried granules are again screened through a 40-mesh sieve and packaged in air tight containers.

Identification of chemical composition using FTIR analysis

Both dried aqueous extract powder and effervescent granules *of A. hierochuntica* was subjected to FTIR analysis. One milligram of dried extract powder/ granules was encapsulated in 10 mg of KBr pellet, in order to prepare translucent sample discs. The powdered sample of the pellet was loaded in FTIR spectroscope (Shimadzu, Japan), with a Scan range from 500 to 4000 cm^{-1}.

Evaluation of effervescent granules

Particle size analysis

Particle size distribution and mean particle size of granules were determined using sieve analysis method as mentioned in Martin, 1993 [12]. The experiment was performed in triplicate. The mean, standard deviation and coefficient of variation were calculated from the data obtained. The mean particle size was calculated after sieving as follows Parrott, *et al.*, 1986 [13]:

$$d_{ave} = \sum nd / \sum n$$

Where, d_{ave} : is the average mean diameter of granules.
n: is percent weight fraction retained on smaller sieve.
d: is the arithmetic mean size of sieve opening.

Flow property of granules

Angle of repose (Θ)

The fixed height cone method was adopted. A cut-stem glass funnel was tightened at a height 2.5 cm from the horizontal plane. The Granules sample was allowed to flow gently through the funnel till a cone was formed and reached the funnel surface orifice Powder flow was then stopped and the average diameter of the formed cone (d) was determined [12]:

$$\text{Tan } \Theta = 2h/d$$

Where " h" and "d" are the height and the diameter of the cone, respectively, the procedure was done in triplicates, and the average angle of repose was calculated for each powder.

The percentage Compressibility (Carr's Index) & Hausner ratio

Five grams of each powder sample of each formula were placed in a graduated cylinder and the volume occupied was measured as V_O (initial bulk volume), and the final tapped volume, V_f, of the powder after tapping the material until no further volume changes occur was measured. An average of three determinations was calculated. The percentage compressibility index was then calculated according to the following equation [13]:

$$\textit{Carr's Index} = \left[1 - \left(\frac{Vf}{Vo}\right)\right] \text{x} 100$$

$$\textit{Hausner ratio} = \frac{Vi}{Vf}$$

Effervescence duration and volume

To one hundred ml of distilled water, 10 gm of effervescent granules were poured, duration of effervescence in seconds was determined and clarity of solution was visually observed.

Antidepressant activity in mice using forced swimming test

Twenty four swiss albino mice weighing 25-35 grams of either sex were randomly selected and grouped into four groups (n=6) as mentioned into table 1. Group I was used as a – ve control, which received 0.2 ml saline, group II (+ ve control) received Imipramine drug solution

(10mg/kg) orally, while group III and IV received an oral solution equivalent to (20 mg/kg) of *A. hierochuntica* aqueous extract and effervescent granules respectively. All groups were received the medication dose 60 minutes before the beginning of the test. All mice were housed in animal house at 27±2°C temperature and 45-55% relative humidity. Food and water were available ad libitum. All the experiments were conducted according to the ethical guidelines approved by laboratory animals use and care committee in our university.

Table 1. Study groups.

Experimental model	Drug and Dose
Group 1 (- ve control)	Saline (0.2 ml)
Group 2 (+ve control)	Imipramine drug solution (10mg/kg)
Group 3 (Test group)	Aqueous extract of *A. hierochuntica* equivalent to (20 mg/kg)
Group 4 (Test group)	Effervescent granules of *A. hierochuntica* equivalent to (20 mg/kg)

Forced swimming test

Forced Swim test (FST) was performed according to the method described by Porsolt *et al.* [15-16]. Mice were forced to swim individually for 10 min, in a glass beaker of 11cm diameter, 15cm height containing fresh water up to a height of 6cm, at a temperature of 27±2°C. This constituted the "pre-test" session. The test-session was conducted 60 minutes after treatment administration. The mouse was considered immobile when it floats motionlessly or made only those movements necessary to keep its head above the water surface [17]. The total duration of the immobility during the last 4 min of the 6 min test was recorded.

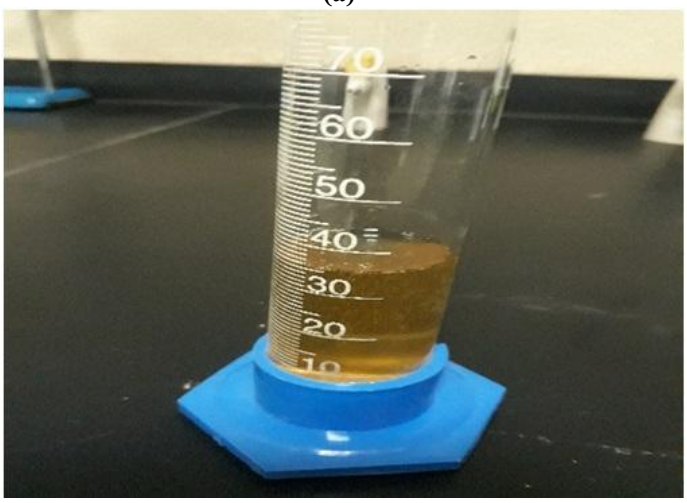
Statistical Analysis

All data were analyzed using ANOVA test, followed by Kruskal Wallis test t. The level of significance was p-value ≤ 0.05.

Results and discussion

Aqueous extract of *A. hierochuntica* was prepared as reported by Asuzu. It gave about 20.25% yield. Wet granulation method was used to prepare effervescent granules of *A. hierochuntica*. Effervescent granules of *A. hierochuntica* were yellowish brown freely flowing granules with accepted appearance and odour (Figure 1). After effervescent it gives yellowish clear solution without any residues of turbidity. Accepted appearance and ease of use may increase patient acceptance to use *A. hierochuntica* for treatment purpose regularly.

(a)



(b)

Figure 1. (a) Effervescent granules of aqueous extract of *A. hierochuntica*. (b) Solution of effervescent granules of aqueous extract of *A. hierochuntica* after dissolution.

Pharmaceutical characterization of prepared effervescent granules

Particle size distribution and mean particle size of granules were determined using sieve analysis method as mentioned in Martin, 1993 [12]. Particle size analysis showed maximum retention in size range 149-210 µm as shown in Figure 2. The mean diameter of the granules was about 177 µm. The values of the angles of repose, Carr's Index (%) and Hausner Ratio was 31 ± 0.5, 10.75 ± 0.51 and 1.09 ± 0.04 respectively indicates good flow properties of the formula. Angle of repose of granules indicating good flow properties and non-cohesive characteristics of the granular formula which may be due to the mannitol and PEG content in the formulae [20]. Percentage compressibility of granules predicting good flow characteristics with minimum cohesion indicating low antiparticle friction and adhesion during single dosage packaging, higher porosity revealed that the disintegration time would be lower. Effervescent granules with effervescence time less than 15 sec indicating good

effervescent ability as mentioned by Loyd VA, 2011 [21]. The study showed that prepared effervescent granules has effervescent time less than eight seconds indicating low moisture content and good flowing ability.

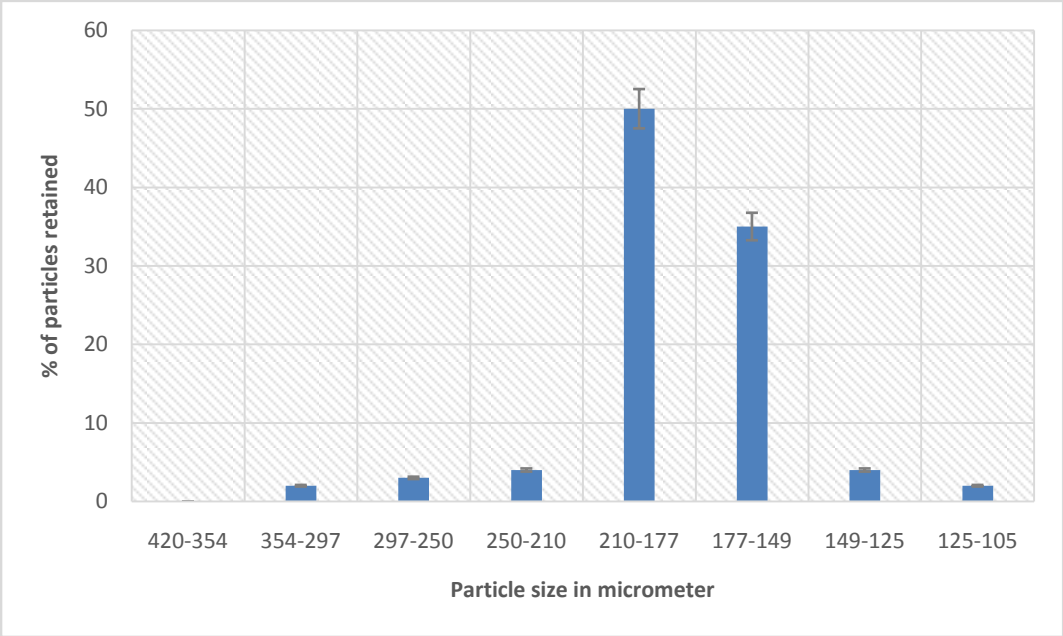


Figure 2. Particle size analysis results of effervescent granules of aqueous extract of *A. hierochuntica.*

Results of FTIR analysis

FTIR spectrum was used to identify the functional groups of the active components present in *A. hierochuntica* extract based on the peaks values in the IR radiation. Figure 3 and 4 illustrates the FTIR spectrum profile of *A. hierochuntica* aqueous extract and granules respectively. Figures show maximum peaks at 3334.1, 1637.5, 1485.23cm^{-1} and 3271, 1599.1, 1395.58cm^{-1} for the aqueous extract and granules respectively. The FTIR spectrum gave broad peak at 3334.1cm^{-1} and 3271cm^{-1} which indicated the presence of N-H stretching. It gave another peak at 1637.5cm^{-1} and 1599.1cm^{-1} which indicated the presence of alkenes C=C stretching, 1485.23cm^{-1} and 1591cm^{-1} attributed to alkyl group. No absorbance was observed in the region 2220cm^{-1} - 2260cm^{-1} indicates the absence of cyanide group in the extract. This results shows that *A. hierochuntica* does not contain any toxic substances and the extract is safe to be used orally. The FTIR spectrum confirmed the presence of phenols, alkenes, alkynes, aromatics, nitro compounds and amines. High phenolic content in *A. hierochuntica* was reported in Youshaki *et al.*, study [23] and Farid C. *et al.* studies [5]; which is comparable with other herbs from the *Asteraceae* family. In accordance with absorbance spectrum, similar peak at the same wave length was observed and identified as Luteolin-O-glucoside, Luteolin-O-glucuronide, Kaempferol-3-O-glucoside and Luteolin-O-glucuronide content of the herbs as reported by AlGamdi N., *et al.,* 2011 [22]. Flavonoid content of have been reported at similar wave length by Yoshikawa *et al.* 2003 [23] who isolated two novel skeletal flavanones, anastatins A and B, from *A. hierochuntica*. The FST model is the more widely used animal model for screening antidepressant activity. Antidepressant activity of *A. hierochuntica*, in the doses studied is similar to that of imipramine (10 mg/kg). As shown in Figure 5, Imipramine (10 mg/kg), *A. hierochuntica* aqueous extract and effervescent granule loaded with the aqueous extract significantly reduced immobility time as compared with control mice. Results also gave an evident on, the absence of significant difference in the reduction of immobility by both the *A. hierochuntica* aqueous extract and effervescent granule loaded with the aqueous extract. Anti-depressant activity of *A. hierochuntica* could be attributed on the base of high polyphenolic and flavonoid compound (flavone C-glycosides, O-glycosides, C-diglycosides and O-glycoside-C-glycosides) [22].

Forced swimming test (FST)

The study aimed to investigate the antidepressant like activity of aqueous extract *of A. hierochuntica* and effervescent granule loaded with the aqueous extract in mice using the forced swimming test. The study showed that There was a statistically significant decrease in the immobility (155.33 ± 32.24 seconds), (154.53 ± 23.24 seconds) and (156.13 ± 20.84 seconds) for group 2, 3 and group 4 respectively when compared to the control group (205 ± 23.10 seconds) ($p < 0.05$). The reduction in immobility was no statistically different for group 4 upon comparing with group 3 ($p < 0.5$). The FST model is the more widely used animal model for screening antidepressant activity. Antidepressant activity of *A. hierochuntica*, in the doses studied is similar to that of

imipramine (10 mg/kg). As shown in Figure 5, Imipramine (10 mg/kg), *A. hierochuntica* aqueous extract and effervescent granule loaded with the aqueous extract significantly reduced immobility time as compared with control mice. Results also gave an evident on, the absence of significant difference in the reduction of immobility by both the *A. hierochuntica* aqueous extract and effervescent granule loaded with the aqueous extract. Anti-depressant activity of *A. hierochuntica* could be attributed on the base of high polyphenolic and flavonoid compound (flavone C-glycosides, O-glycosides, C-diglycosides and O-glycoside-C-glycosides) [22]. Many studies used FST to prove the anti-depressive like activity of herbal medicines. Zhang, M., mentioned in his study, the anti-depressive activity of Paeonia lactiflora P. (Ranunculaceae) [25]. Other studies showed the antidepressant activity of peony extract in rodents [26]. Albizia julibrissin, widely widespread throughout Asia, found that it remarkably decreased the immobility time in the forced swimming tests showing an antidepressant activity [27]. Safranal and crocin are two main constituents of Saffron (stigmates of Crocus sativus L.) safranal and crocin exhibited antidepressant-like activity based on FST as reported by Hossesein.*et al.*, study [28].

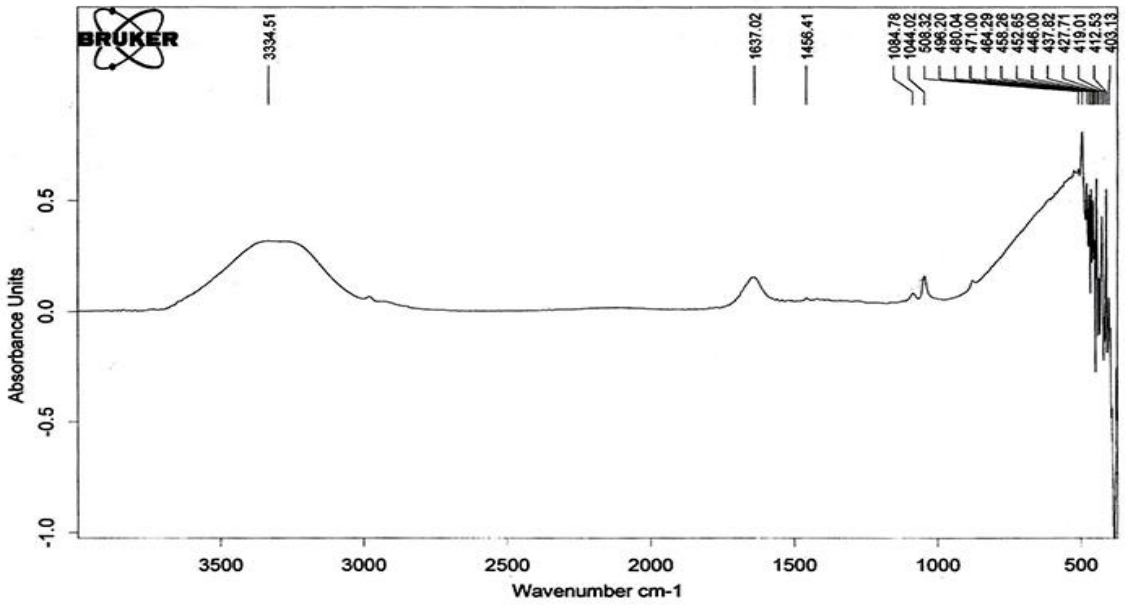


Figure 3. FTIR Spectrum of *A. hierochuntica* aqueous extract.

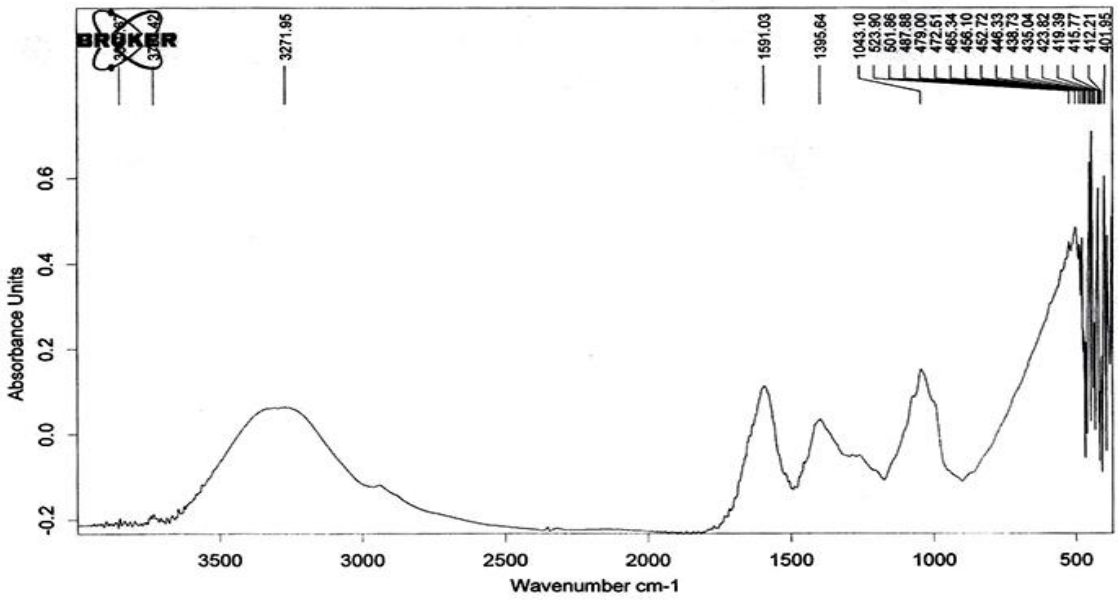


Figure 4. FTIR Spectrum of *A. hierochuntica* aqueous extract embedded in effervescence granules dosage form.

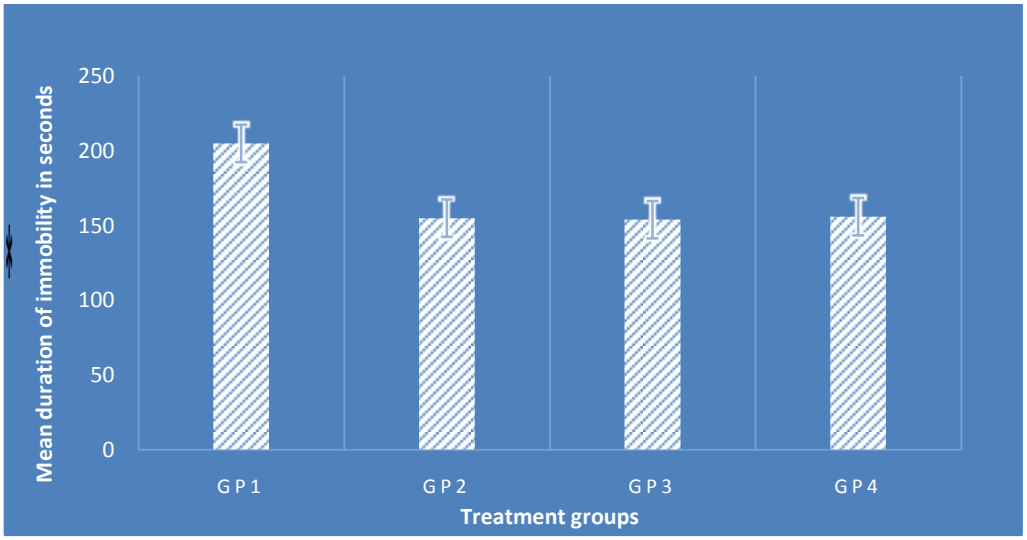


Figure 5. Effect of drugs on FST in albino mice, GP1 (saline), GP2 (Imipramine drug solution (10mg/kg), GP3 (Aqueous extract of *A. hierochuntica* equivalent to (20 mg/kg), GP4 (Effervescent granules of *A. hierochuntica* equivalent to (20 mg/kg), N =6, values are expressed as Mean ±SEM.

Conclusion

The present study was undertaken to investigate the antidepressant-like effects of effervescent granules of aqueous extract of *A. hierochuntica* using Forced swimming test (FST) in mice. Acute treatment with *A. hierochuntica* aqueous extract and effervescent granules at 20 mg/kg significantly reduced the immobility time compared with the control group, and thus showed an antidepressant-like effect. This effect was comparable to that of imipramine at 10 mg/kg. Effervescent granules showed that it can be used as a novel approach for using *A. hierochuntica* for treatment of depression as they offer advantages such as good flow properties, faster disintegration and dissolution in addition to be palatable dosage form as compared to other dosage forms. Hence, it can be widely accepted by patients specially geriatric patients due to lesser risk of choking, in addition to lower time and cost required for their manufacture in comparing with other solid dosage forms.

Conflict of interest

The authors confirm that this article content has no conflict of interest.

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