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ANTIDEPRESSANT ACTIVITY OF EMBELIN ISOLATED FROM THE METHANOL EXTRACT OF FRUITS OF *EMBELIA RIBES* BURM.

Dr. Dhanapal Venkatachalam

Sree Sastha Pharmacy College, Chembarambakkam, Chennai-600123.

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ABSTRACT

Objective: The objective of the study was to evaluate the major known active principleembelin of *Embeliaribes* for possible antidepressant activity..*EmbeliaribesBurm* (Family: Myrsinaceae) is extensively used in Indian traditional medicine for treating various disease conditions including chronic inflammatory disorders, heart and urinary conditions, snake and insect bites and tumour. Methodology: Fractionation of the methanlic extract of dried powdered fruits using column chromatography over silica gel afforded embelin. Experimental depression was induced by subjecting mice to tail suspension test (TST) and forced swimming test (FST) experimental models. Intraperitonial administration of embelin (2.5 and 5 mg/kg) 30 min prior to induction of experimental depression resulted in dose-dependent reduction of immobility under both test conditions. Results: The effects of embelin on immobility time of mice in the TST at the doses of 2.5 and 5 mg/kg, embelin induced antidepressant-like effect with significance level of $p < 0.05$; $p < 0.001$ respectively when compared with the control group. The positive control, imipramine, administered at the dose of 15 mg/kg did also show antidepressant-like effect comparable with that of 5 mg/kg embelin ($p > 0.001$).As with the FST, treatment of mice with 2.5 and 5 mg/kg of embelin given by intra peritoneal route significantly decreased immobility in the FST. The data obtained at these two doses were significantly different from the control group ($p < 0.01$ and $p < 0.001$ respectively). The positive control, imipramine, did also shorten immobility time in the FST ($p < 0.001$). Conclusions: The result concludes that,the major bioactive constituent of *Embeliaribes*, embelin, exhibited significant activity in mice TST and FST experimental models. The observed potent activity at doses lower than the standard antidepressant drug, imipramine, suggests the potential of embelin and *Embeliaribes* for treating mental depression.

Corresponding author
Dr Dhanapal Venkatachalam
Principal,
Sree Sastha College of Pharmacy,
Chennai –Bangalore High Way,
Chembarambakkam, Chennai – 600123.
vddpaul@gmail.com
+ 919443952113
+91-7904937309

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INTRODUCTION

Depression is a common disorder that is projected to become the second biggest contributor to the global health problem and disability by the year 2020 (WHO, 1999). There is now an ever increasing rate of depression-related death both by suicide and due to the associated physical/physiological disorders (Paykel, 2006). The primary symptoms of depression are unhappiness, low mood or reduced interest. Most of the today's antidepressant therapies have limitations due to either their inadequate efficacy over prolonged usage or unwanted side effects. Consequently, recent studies have given emphasis to the identification new drugs, preferably from natural sources, that mitigate depressive-like symptoms.

Embeliaribes Burm (Family: Myrsinaceae) is extensively used in Indian traditional medicine for treating various disease conditions including chronic inflammatory disorders, heart and urinary conditions, snake and insect bites and tumour (Kapoor et al., 1983; Varier, 2006). Various preparations of the plant are also known to be used as brain tonic and for treating mental disorders (Afzal et al, 2012). Recent studies on this plant mainly focus on its principal active constituent, embelin (Figure 1), due to its unique chemical and pharmacological properties. Among the various pharmacological activities reported for embelin are antimicrobial (Chitra et al., 2003), antifertility (Krishnaswamy and Purushothaman, 1980; Radhakrishnan and Alam, 1975), anti-tumour (Chitra et al., 1994), anti-inflammatory (Kapoor et al., 1983), antioxidant (Joshi et al., 2007; Dharmendra, et al., 2009), anti-diabetic (Mahendran et al., 2011) and wound healing (Kumara Swamy et al., 2007) effects. Some studies related to the effect of embelin on the central nervous system such as anticonvulsant (Mahendran et al., 2011) and anxiolytic (Afzal et al., 2012) activities have also been reported. To the best of the authors' knowledge, however, the antidepressant activity of this important lead natural product has not yet been studied. Herewith, the antidepressant potential of embelin was evaluated by using two universally accepted experimental models: mice tail suspension (TST) and forced swimming (FST) tests.

MATERIALS AND METHODS

Animal's Swiss albino mice (20–25 g) of either sex were used for the study. Animals were obtained from Siddhartha Institute of Pharmacy, Dehra-dun, India. Animals were maintained at 25±1◦ C, 55±5% humidity and under a 12:12 h light/dark cycle (lights on at 07:00 h). The animals had free access to standard pellet diet (Lip-ton rat feed, Ltd., Pune) and water ad libitum. All experiments were conducted in accordance with the Institutional Animal Ethics Committee regulations approved by the Committee for the purpose of Control and Supervision of Experiments on Animals (Reg. No. 1423/PO/a /23/CPCSEA). The animals were divided into four groups of six mice each. Group I served as solvent control and received 1% Tween 80 (v/v) in a 0.9% (w/v) saline (1 ml/100 g). Group II mice served as a positive control and received 15 mg/kg imipramine. Group III and IV received embelin suspension (1% Tween 80 (v/v)) at a dose of 2.5 and 5 mg/kg respectively. All treatments were administered intra-peritoneal 30 min prior to induction of experimental depression.

Plant material

Fresh fruits of *EmbeliaribesBurm* (Myrsinaceae) were purchased from the local market of Chennai, India, in Feb 2023, were authenticated by Taxonomist. The material was air dried in shade, powdered mechanically and stored in airtight containers.

Extraction and isolation of embelin

Our published method for plant processing, extraction and isolation procedures has been adopted (Afzal et al., 2012). Briefly, dried powder fruits of *Embeliaribes* (4 kg) were extractedwith methanol (12 L) at 50 ◦ C for 2 days. Removal of the solvent under reduced pressure resulted in 614 g of the extract residue. Column chromatography of the crude extract using silica gel (60–120 mesh) and elution with petroleum ether and benzene (2:3) afford 9.5 g (0.425%) of embelin (Afzal et al., 2012). It was crystallized from (petroleum ether) as shiny orange compound, which showed over 99% purity when analysed by HPTLC (solvent, petroleum ether:benzene; 99:1). The structure of the isolated compound was established by comparison of spectral data with those previously reported for embelin (Hao, 2005). In the experimentation, embelin crystals were dissolved in olive oil for oral dosage given to the mice.

Assessment of antidepressant-like activity

Tail suspension test (TST)

The TST was performed by following the method of Steru et al (Steru et al, 1985). Mice were suspended on the edge of a table, 58 cm above the floor, by placing adhesive tape approximately 1-2 cm from the tip of the tail. Immobility time as an indicative of a state of depression was recorded during 6 min experimental period. Mice were considered immobile when they did not show any body movement and hanged passively.

Forced swimming test (FST)

The FST method described by Porsolt., et al. (1977) was adopted. Mice were individually forced to swim in an open cylindrical container (diameter 20 cm, height 50 cm) containing 25 cm of water at 25 °C. After an initial 2 min period of vigorous activity, mice normally take a typical immobile posture. Animals were considered immobile when they float in an upright position, making only small movements to keep their head above water. The total duration of immobility was recorded during the next 4 min of the total 6 min test period. The changes in immobility duration were studied for the various treatment groups. In this experimental model, animal were used only once (Porsolt et al, 1977).

Statistical Analysis

The data were expressed as mean ± S.E.M. Statistical comparisons were performed by one-way ANOVA followed by Dunnett's-test using Graph Pad Prism version 5.0, USA. $P < 0.05$ was considered significant.

Results

The methanol extract of fruits of *Embeliaribes* was used for the isolation of the bioactive natural product, embelin. Column chromatography of the crude extract resulted in the isolation of embelin (9.5 g, 0.425%). Based on spectroscopic analysis and comparison with our previously published data (Afzal et al., 2012) and others (Hao, 2005), the com-pound was identified as embelin (Figure 1)

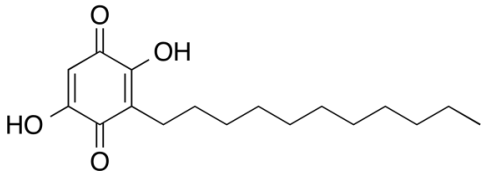

Figure 1.Chemical structure of embelin.

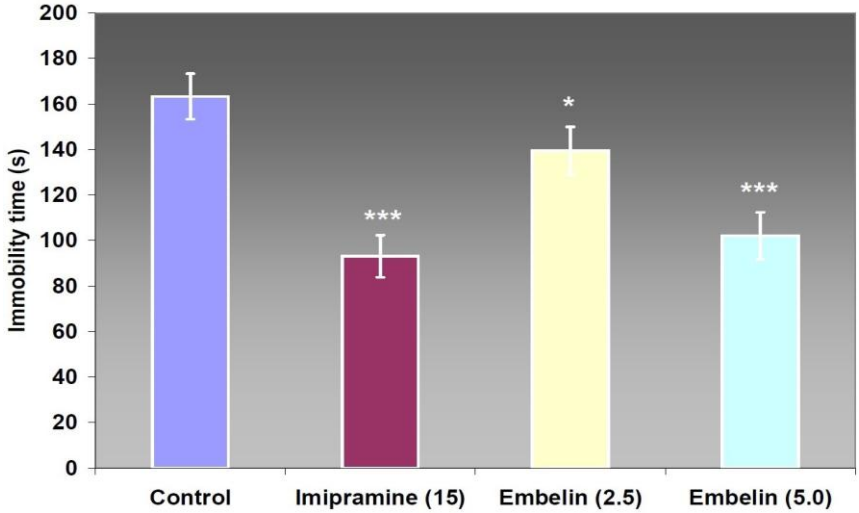

Figure 2. Effect of embelin in tail suspension test (TST) for depressive behaviour. Data represents the mean±SEM for each group (n=6).

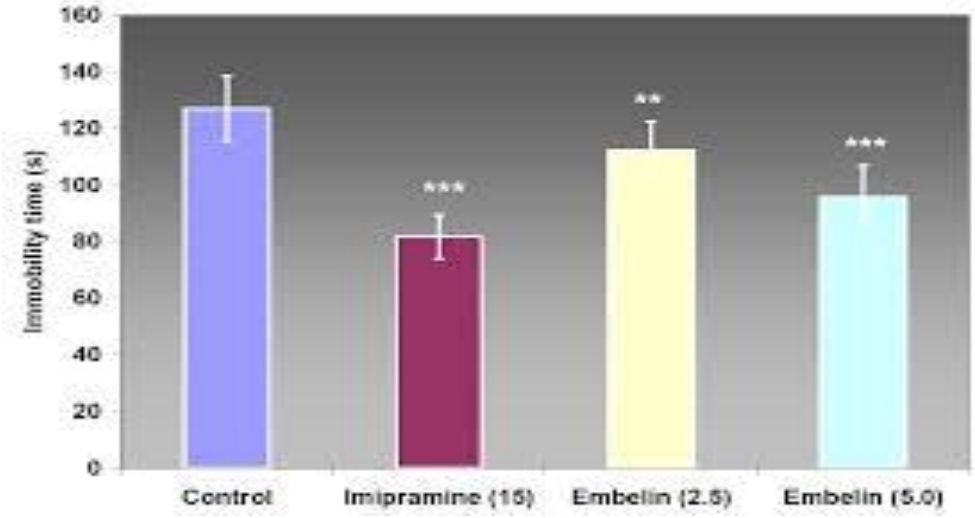

Figure 3. Effect of embelin in forced swim test (FST) for depressive behaviour. Data represents the mean±SEM for each group (n=6).

Figure 2 shows the effects of embelin on immobility time of mice in the TST. At the doses of 2.5 and 5 mg/kg, embelin induced antidepressant-like effect with significance level of $p < 0.05$; $p < 0.001$ respectively when compared with the control group. The positive control, imipramine, administered at the dose of 15 mg/kg did also show antidepressant-like effect comparable with that of 5 mg/kg embelin ($p > 0.001$).

As with the FST, treatment of mice with 2.5 and 5 mg/kg of embelin given by intraperitoneal route significantly decreased immobility in the FST. The data obtained at these two doses were significantly different from the control group ($p < 0.01$ and $p < 0.001$ respectively). The positive control, imipramine, did also shorten immobility time in the FST ($p < 0.001$).

DISCUSSION

Embelin (2,5-dihydroxy-3-undecyl-1,4-benzoquinone; Figure 1.) is a major constituent and active principle of the well-known medicinal plant, *Embeliaribes.* Numerous recent studies on embelin and the crude extracts of *Embeliaribes* have laid the scientific foundation for most of the traditional uses of the plant in Asia. Apart from limited studies on the anticonvulsant (Mahendran et al., 2011) and anxiolytic (Afzal et al., 2012) activities of the plant, however, scientific data in support of the claimed traditional use for depression-related CNS disorders are not available. The present study was therefore designed to evaluate the antidepressant potential of embelin through TST and FST experimental models. All major antidepressant drugs including the tricyclic 5-hydroxytryptamine reuptake and monoamine oxidase inhibitors have been shown to display activity in the validated TST and FST experimental model of depression (Ishola et al., 2012). Mice suspended by their tail in the TST or placed in cylinder of water in the FST show immobility that is attributed to cessation of their persistent escape-directed behaviour (Cryan and Holmes, 2005). The druginduced decrease in this experimentally induced immobility is therefore taken as a measure of antidepressant property. In the present study, administration of embelin just 30 min perior to the experiment appears to reduce immobility in both experimental models suggesting its antidepressant-like potential. Interestingly, the observed effect of embelin at the dose of 5 mg/kg was comparable with the tricyclic antidepressant positive control drug, imipramine, administered at the dose of 15 mg/kg. The other relevant finding of the present study was the demonstration of the anti-immobility effect by embelin as dose dependent. Recent report from our laboratory has also revealed the anxiolytic effect of embelin (Afzal et al., 20012); further suggesting the therapeutic potential of the compound for various CNS disorders. It is well known that compounds that selectively bind with high affinity benzodiazepine receptors possess both anxiolytic and antidepressant effects (Ishola et al., 2012; Kavvadis et al., 2003; Svenningsen, et al., 2006). There is also an emerging body of evidence to suggest the role of gammaaminobutyric acid (GABA) in the pathophysiology of various mood disorders including depression (Petty, 1995; Pilc, 2005). Since, the anxiolytic effect of embelin was shown to be mediated through effect on the GABA system (Afzal et al, 2012), similar mechanism of antidepressant action cannot be ruled out. It is widely accepted that monoamine neurotransmitters and their receptors dysregulation in the brain are the major pathological hallmark of mental depression. Recent evidence however suggests that oxidative stress in the brain cortex is also associated with patients suffering from recurrent depressive disorder (Michel et al., 2007). Smaga et al (2012) have recently tested the involvement of oxidative stress in depression by studying the effect of the antioxidant N-acetylcysteine on animal behaviors, including FST. The study unequivocally revealed that chronic administration of N-acetylcysteine and imipramine's induce antidepressant effect by increasing cellular antioxidant mechanisms (e.g. superoxide dismutase activity) in the brain (Smaga et al (2012). Several other studies have further substantiated the beneficial effect of antioxidants in treating depression (Berk et al., 2011; Brocardo ET AL., 2012; Selek et al., 2012). It is therefore reasonable to assume that the observed antidepressant-like activity of embelin could be attributed to its known antioxidant effect ((Joshi et al., 2007; Dharmendra, et al., 2009). A 3D-structural model similarity search further shows that embelin is closely related with the well-known antioxidant alpha-tocopherol (AT, vitamin E), especially in the polar phenolic heads (Figure 4) and long-chain non-polar tails (Figure 5). Interestingly, AT has been shown to display antidepressant-like activity in experimental animals including in the TST and FST experimental models (Lobato et al., 2010). It was further reported that AT administered orally was not only effective in long-term treatment (28 days) but also able to potentiate the effect of low doses of Fluoxetine (Lobato et al., 2010). The antioxidant, antidepressant and various other biological activities shared by embelin and AT could therefore be due to their electrostatic and steric resemblance (Figure 4). The observation of anti-depressant like activities of embelin at relatively low doses could also be explained by its amphiphilic (dual hydrophobic tail-hydrophilic polar head) behaviour. Such physicochemical profile is likely to enable embelin to readily cross the blood-brain barrier and induce antidepressant effects.

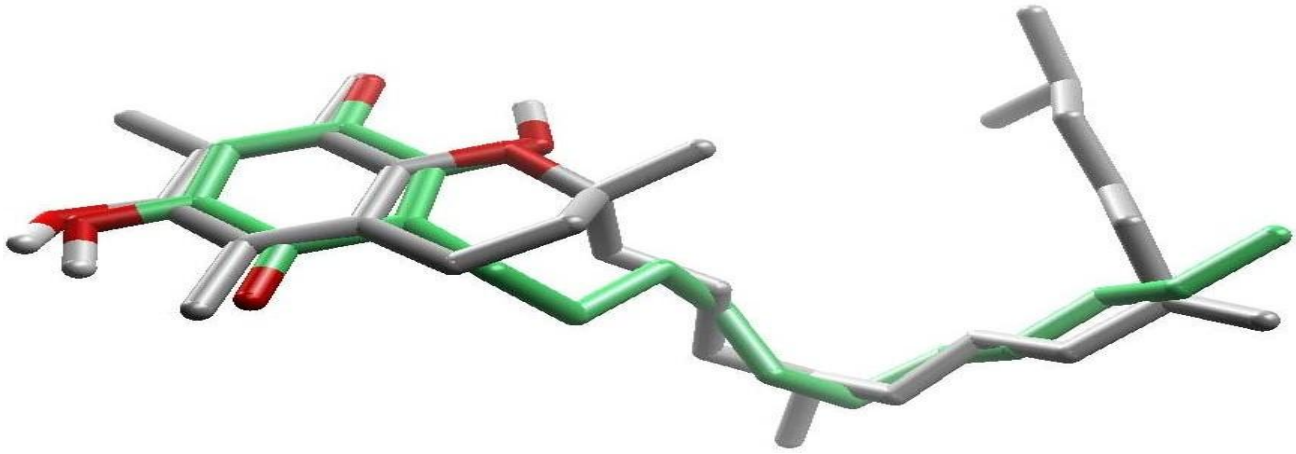

Figure 4. 3D-molecular alignment of AT (grey coloured carbon atoms) and embelin (green coloured carbonatoms) reveals similar electrostatic and steric profile. Red colour indicates oxygen atoms, while white colourindicates polar hydrogen atoms attached to oxygen atoms. Hydrogen atoms (except polar ones) are omitted forclarity

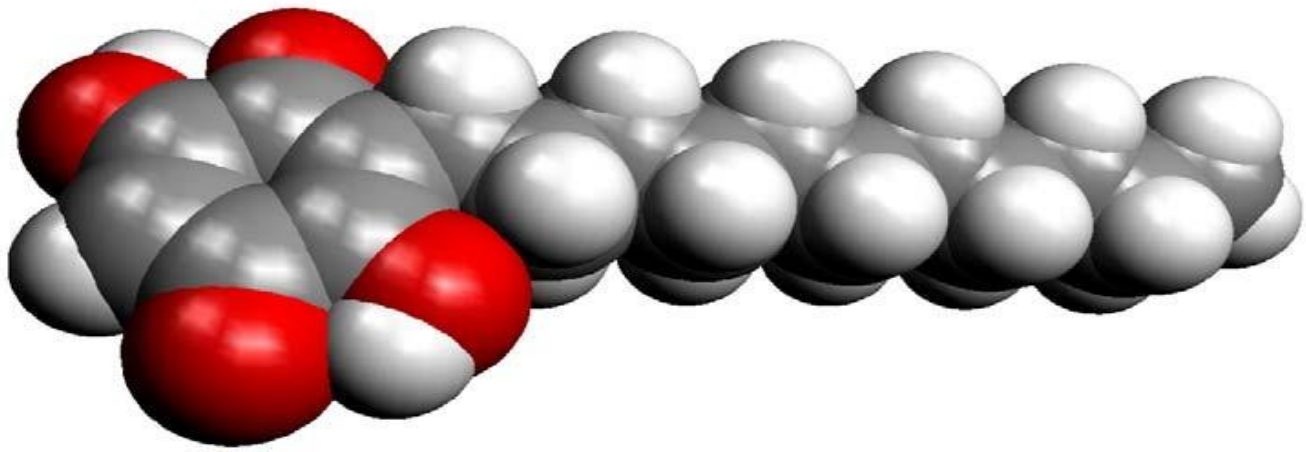

Figure 5. Polar head and non-polar tail of embelin carries amphiphilic (dual solubility or hydrophobic-hydroph-ilic) behaviourto rapidlypenetratethecell membranesincludingbloodbrainbarrier

CONCLUSION

In conclusion, the major bioactive constituent of *Embeliaribes*, embelin, exhibited significant activity in mice TST and FST experimental models. The observed potent activity at doses lower than the standard antidepressant drug, imipramine, suggests the potential of embelin and *Embeliaribes* for treating mental depression. Future studies are required to ascertain the exact mechanism(s) of action of embelin's antidepressant-like effect.

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Competing interests:
Author has declared that no competing interests exist.

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