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Research Article
Evaluation of the Antidepressant Activity of *Beta vulgaris* Alone and in Combination with Fluoxetine in Mice

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Abstract

Background and Objective: *Beta vulgaris* (BV) possesses strong antioxidant and anti-inflammatory properties that may play a vital role in mitigating mental disorders like depression. The present study was designed to evaluate the antidepressant effects of aqueous and methanolic extracts of BV using standardised mouse models of depression. **Methodology:** The extracts were analysed for phytochemical ingredients and *in vitro* experiments were done to determine antioxidant properties of BV extracts. After preliminary dose range finding studies for any adverse effects, the antidepressant activities of aqueous and methanolic extracts of BV were evaluated in mouse models of depression. Animals were randomly divided into 8 groups (6 animals per group): Group 1 and 2 served as vehicle control and fluoxetine (20 mg kg^{-1}) standard control, respectively. Groups 3 and 4 were given aqueous extract of BV orally at doses of 100 and 200 mg kg^{-1} day^{-1}, respectively. Groups 5 and 6, received methanolic extract of BV at doses of 200 and 400 mg kg^{-1} day^{-1}, respectively. Groups 7 and 8 received 200 and 400 mg kg^{-1} day^{-1} methanolic extract of BV, respectively, +10 mg kg^{-1} day^{-1} dose of fluoxetine. Following 14 days daily dosing, all animals were tested using behavioural tests of depression on day 15th using Forced Swim Test (FST), Tail Suspension Test (TST) and Locomotor Activity Test (LAT). **Results:** In comparison with the control groups 1 and 2, marked changes were observed in all parameters in extract-dosed mice. Especially, significant antidepressant effects were found in mice given simultaneously combined doses of 200 or 400 mg kg^{-1} day^{-1} of BV methanolic extract+10 mg kg^{-1} day^{-1} fluoxetine, suggesting an additive serotonergic effect. **Conclusion:** Overall, the findings suggest that *Beta vulgaris* has a potential for developing an alternative plant-derived antidepressant therapy.

Key words: Beetroot extract, *Beta vulgaris*, antidepressant activity of beetroot, fluoxetine, behavioural model of depression

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

According to the World Health Organization (WHO)[1] mild to moderate depression is gaining the status of one of the most widely growing mental illness in humans affecting more than 100 million individuals each year. The latest global epidemiological study by WHO estimated that mental health disorders contribute around 7.4% of global disability-adjusted life years (DALYs) and about 22.7% of global years lived with disability (YLDs). Overall, of all the YLDs, 9.6% are attributed to depression alone, followed by anxiety (3.5% of all YLDs), schizophrenia, substances of abuse and bipolar disorder (just over 2% of all YLDs). In South Asia, including India, more than 50% of all the mental health related DALYs are related to depression[2]. In adult humans, the incidence of depression is nearly 3-5% with a life-time prevalence[3,4] of 5-20%. According to the global WHO report, India ranks 5th as far as the prevalence of mental illness and depression is concerned, with an estimated DALY of 1,400 per 1,00,00 persons[5]. Depression has been linked with both inherent physiological factors as well as situations causing mental stress, modern stressful lifestyle and dietary factors[6]. Clinical depression is generally associated with lower levels of monoamines in the CNS, e.g., serotonin, epinephrine and norepinephrine[7]. The CNS oxidative stress is also involved in the etiology of depression and other mental disorders due to the generation of free radicals and neurotoxins, which may alter neuronal plasticity in the cortico-limbic regions of the brain, leading to neuronal damage[6].

A wide variety of psychotherapeutic agents are used for treating depression, including SSRI-type drugs like fluoxetine. However, the SSRI category of drugs sometimes produce serious adverse effects such as excessive fatigue and altered behaviour associated with increased suicidal tendency in patients. Most of the tricyclic antidepressants cause unwanted side effects like dry mouth, urinary retention, constipation, blurred vision and glaucoma, weight gain and increased heart rate, which reduces patient compliance. Hence, alternative remedies for treating depression are often sought by both patients and health care professionals, which may serve as an add-on to the existing antidepressant psychotherapies[6]. Recently, herbal medicines and/or nutraceuticals have been promoted for treating depression and have found better patient acceptability due to lesser side effects, easy availability and lower cost. Many herbal alcoholic extracts of plants such as *Centella aciatica*, *Hibiscus tilaceous*, *Moringa oliefera* have shown antidepressant activities in experimental mouse models[7,8]. The use of herbal extracts as antidepressants may aid in reducing the dose of the corresponding synthetic drug[6]. Several plant products and botanicals like St. John's wort (*Hypericum perforatum*), brahmi (*Centella asiatica*), turmeric (*Curcuma longa*), tulsi (*Occimum sanctum*) have shown significant antidepressant activities, which are attributed to their inherently rich antioxidant ingredients and flavonoids. The flavonoids have been reported to possess neuroprotective effects through reduction of oxidative stress in the CNS and amelioration of depression[8-10].

Beetroot, *Beta vulgaris* (BV), belongs to the family Chenopodiacae and beetroot juice is purported as promising candidate for developing natural health products due to its antioxidant, chemoprotective properties and free-radical scavenging activity[11]. The BV is a rich source of many valuable phytoconstitutents and contains large amount of nitrates, antioxidants like betaxanthin, anthocyanins, betacyanin, vulgaxanthin, vitamin C, flavonoids and tryptophan[11,12]. The flavonoids are known to have a wide array of beneficial activities against a variety of diseases, including cardiovascular disorders, chronic inflammatory diseases and cancer. The BV also contains folate (present as folic acid) which can help reduce the risk of anxiety and depression[13]. Tryptophan has also been found in BV[11] and an enzyme present in BV aids in the conversion of tryptophan to serotonin[12]. Beetroot nitrates are reported to have a range of beneficial cardiovascular effects, including reduction of blood pressure, inhibiting platelet aggregation, preserving or improving endothelial dysfunction, enhancing exercise performance in healthy individuals and patients with peripheral arterial disease[14]. The BV has been used in traditional Indian medicine for a number of ailments such as expectorant, diuretic, sedative and in the treatment of mental disorders. In addition, BV has the ability to produce hepatoprotective[15], antihypertensive[16] and antihyperlipidemic effects[17], however, its potential to act against depression remains unexplored.

The present study was designed to investigate the antidepressant potential of *Beta vulgaris* in experimental model of depression. Forced Swimming Test (FST), Tail Suspension Test (TST) and locomotor activity (LAT) were used as endpoints to determine the antidepressant activity of aqueous and methanolic extracts of BV in adult Swiss albino mice.

MATERIALS AND METHODS

Identification and authentication of the beetroot plant: Fresh beetroots, *Beta vulgaris* were purchased from the local market of Santacruz (West), Mumbai. Samples were authenticated by Department of Life sciences, Ramnarain Ruia College, Mumbai. All the solvents used were of analytical

grade. Several physicochemical parameters such as extractive value, ash value, foreign organic matter and test for heavy metals were conducted employing procedures mentioned in the Indian Pharmacopoeia (Indian Pharmacopoeia, 2014)[18].

Extraction procedures

Aqueous extraction: Fresh beetroots (50 g) were grated and exhaustively macerated with 250 mL double distilled water for 48 h. The extract was filtered through the muslin cloth and marc was pressed. The filtrate was subjected to spray drying at 140°C inlet temperature to get the solid residue.

Methanolic extraction: Fifty grams of fresh beetroots were grated and exhaustively macerated with 250 mL of methanol for 72 h. The extract was filtered through the muslin cloth and marc was pressed. The filtrate was subjected to rotary evaporation at 60°C to get a concentrated extract. The remaining solvent was evaporated on a water bath at 60°C in order to get a solid residue.

Phytochemical screening of the extracts: Both extracts were screened for major phytochemical ingredients, namely betalains, flavonoids, phenolic compounds and saponins. The total amounts of phenolics and flavonoids from extracts were quantified using the methods of Khandelwal[19] and John *et al.*[20].

Betanins as betacyanin and betaxanthins were detected by determining maximum absorbance of the 10% solutions of each extract at 537 and 480 nm, respectively[21]. Saponins were detected by foam test. The presence of tryptophan and tyrosine was assessed by xanthoprotein test.

***In vitro* antioxidant studies:** The *in vitro* antioxidant studies of both aqueous and methanolic extracts were done by diphenyl-picryl-hrzyl (DPPH) free radical scavenging and hydrogen peroxide (H_2O_2) non free-radical scavenging activities. The abilities of the extracts to scavenge these radicals are the measure of their antioxidant potential. The DPPH and hydrogen peroxide scavenging activities were carried out by the methods described by Badami *et al.*[22] and Glucin *et al.*[23], respectively. The antioxidant activities were calculated based on the IC_{50} values. The IC_{50} values are the concentrations of extracts needed to inhibit or scavenge 50% of these free-radicals. They are indicative of the antioxidant activity of the free-radical scavenging moiety with higher values indicating lesser potential for antioxidant activity.

Animal husbandry of experimental animals: Adult male Swiss Albino mice weighing 25-30 g were obtained from the Animal Facility of Sppsptm Nmims. The animals were housed in polycarbonate cages at room temperature (25±20°C) and humidity (75±5%) with 12:12 h light-dark cycle. On arrival, mice were acclimatized for 1 week prior to starting the experimental investigation. All studies were initiated after obtaining the approval from the Institutional Animal Ethical Committee (IAEC). Approval No: CPCSEA/IAEC/SPTM/P-06/2015.

Dose finding acute toxicity studies: Acute toxicity studies[24] were carried out according to the OECD guidelines number 423. Mice were randomly divided into three groups consisting of six animals each: Group 1 and 2 received single oral doses of the aqueous and methanolic extracts, respectively, at a dose of 2000 mg kg^{-1}. Sodium carboxymethyl cellulose (0.1%) in distilled water was administered orally to the control group at a volume of 1 mL. The overt general behaviour of the mice was recorded at 1, 4 and 24 h post-dosing and daily thereafter for a total duration of 14 days. Parameters such as changes in behaviour, any signs of convulsions, overt toxicity and death (if any) were recorded and times at which these incidents occurred were also noted.

Preparation of solutions: Fluoxetine was used as a standard antidepressant drug, while the aqueous *Beta vulgaris* extract (BVAE) and methanolic *Beta vulgaris* (BVME) were used as test agents. The standard drug as well as the methanolic extract was suspended in 1.0% sodium carboxymethyl cellulose and the aqueous extract was dissolved in distilled water just before administration. The solutions were administered by gavage in a dosing volume not exceeding 1 mL in all the cases.

Experimental design and treatment: Animals were randomly divided into eight groups containing six animals each. The treatment plan for these groups was as follows:

Group I : Control group (administered 0.2 mL of 1% suspension of sodium carboxymethyl cellulose (CMC) in distilled water)
Group II : Standard drug-fluoxetine at a dose of 20 mg kg^{-1} day^{-1}
Group III : BVAE at a dose of 200 mg kg^{-1} day^{-1}
Group IV : BVAE at a dose of 400 mg kg^{-1} day^{-1}
Group V : BVME at a dose of 200 mg kg^{-1} day^{-1}
Group VI : BVME at a dose of 400 mg kg^{-1} day^{-1}
Group VII : BVME at a dose of 200+fluoxetine 10 mg kg^{-1} day^{-1}
Group VIII : BVME at a dose of 400+fluoxetine 10 mg kg^{-1} day^{-1}

The oral dosing was carried out for a period of 14 consecutive days and the animals were evaluated for antidepressant activity on the 15th day. It was noted that the methanolic extracts produced greater antidepressant activity than that of the aqueous extract. Hence, groups VII and group VIII were given a combination of the BVME and low doses of fluoxetine (10 mg kg^{-1} day^{-1}) for further 14 days.

Evaluation of antidepressant activity

Forced Swim Test (FST): The FST was carried out according to the method described by Porsolt *et al.*[25].

Tail suspension test (TST): Tail suspension test was conducted according to the method described by Porsolt *et al.*[24] with slight modifications[25,26].

Locomotor activity test (LAT): Locomotor activity test was performed as described by Morley-Fletcher *et al.*[27] to assess the Central Nervous System (CNS) inhibitory or stimulatory activity of the BV extracts.

Statistical analysis of data: The differences among experimental and control groups were determined using the Graph Pad INSTAT 3.0 software for windows. Comparisons among different groups were performed by analysis of variance (ANOVA). Statistically significant differences between control and experimental groups were assessed by student's t-test and differences were considered significant when $p<0.05$. All results are expressed as mean±standard error of mean (SEM).

RESULTS

Phytochemical screening of aqueous and methanolic extracts: Qualitative tests used for the detection of betalains are shown in Table 1. Phytochemical screening revealed the presence of flavonoids, phenols and betalains in both aqueous and methanolic extracts. However, the presence of saponins and amino acid tryptophan were detected only in methanolic extracts (Table 2). Quantification of phenolics and flavonoids revealed significantly higher amounts in the methanolic extract than aqueous extract (Table 3).

***In vitro* antioxidant studies of beetroot extracts:** The IC_{50} values obtained for the scavenging of free-radicals are summarised in Table 4. In both radical scavenging assays, the respective mean IC_{50} values (ppm) were 2.3-2.6 times smaller for the methanolic extract than the aqueous extract, viz., (BVME 103.56 vs BVAE 241.40 ppm and BVME 121.32 vs BVAE 314.21 ppm). These observations suggest that the methanolic beetroot extract has far greater antioxidant ability for removing the free-radicals than that of the aqueous extract.

Dose finding acute toxicity studies: The acute toxicity studies, done with both BV extracts (at single oral doses of 2000 mg kg^{-1}), demonstrated no mortality, change in body weight, overt behaviour or clinical symptoms of toxicity throughout the 14 day observation period. Thus both extracts were considered to be safe in mice at high dose level. For the evaluation of antidepressant activity, both extracts were administered orally to mice with the doses reduced to 1/10th and 1/5th of the 2000 mg kg^{-1} dose level.

Forced swim test (FST): Once daily oral administration of both BVAE and BVME for 14 consecutive days at different doses either alone or in combination with fluoxetine resulted in decreased immobility time in mice (Fig. 1). However, BVME was more effective in causing immobility in the forced swim test. Standard fluoxetine (20 mg kg^{-1} day^{-1}) also caused significant reduction in the immobility time. As opposed to the controls, statistically significant reductions in the immobility time were observed in animals given combinations of BVME at 200 and 400 mg kg^{-1} day^{-1} along with 10 mg kg^{-1} day^{-1} dose of fluoxetine ($p<0.001$). On the other hand, the immobility time was not significantly reduced in these groups when compared with the standard fluoxetine dose of 20 mg kg^{-1} day^{-1} ($p>0.05$). However, numerically speaking, an increased reduction in immobility time by about 85% (compared to the normal control) was observed in the group administer a combination of 400 mg kg^{-1} day^{-1} BVME+10 mg kg^{-1} day^{-1} fluoxetine (Fig. 1).

Table 1: Qualitative tests used for the detection of betalains in aqueous and methanolic

Test	Procedure	Methanolic extract	Aqueous extract	Inference
Test for betalain pigments (betacyanins and betaxanthins)	Two milliliters of the extract solution+addition of 10% dilute hydrochloric acid	Dark purple colouration	Dark colouration	Betacyanins are present
	Two milliliters of extract+addition of sodium hydroxide solution	Yellow colouration	Yellow colouration	Betacyanins confirmed
	Two milliliters of extract+concentration HCl and observe under long UV	Blue fluoroscence	Blue fluorescence	Betacyanins confirmed

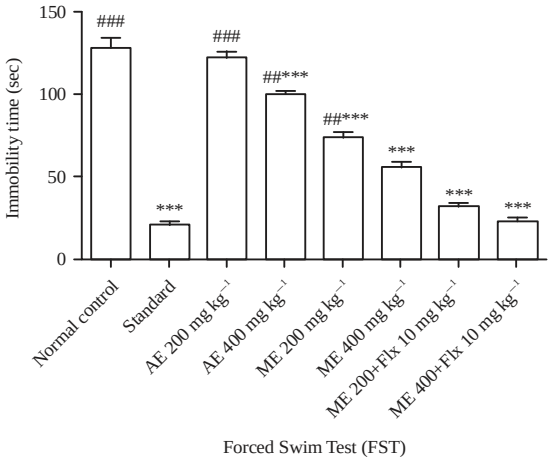

Fig. 1: Graphical representation of results of FST. Statistical analysis was performed with one-way ANOVA followed by Bonferroni's comparison test according to which ***p<0.001 when compared to normal control, ##p<0.01 and ###p<0.001 when compared to standard. Standard: Fluoxetine 20 mg kg^{-1} and ME+Flx: Methanolic extract+fluoxetine

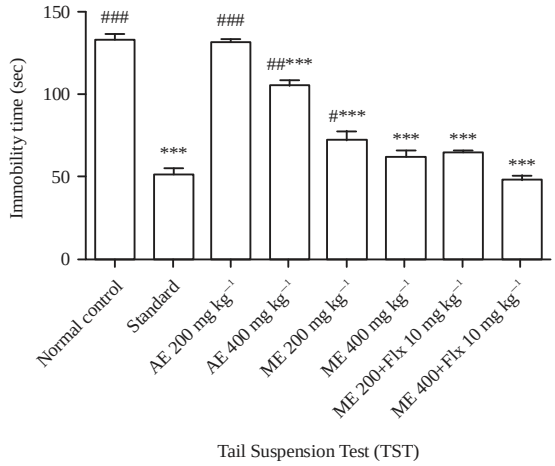

Fig. 2: Graphical representation of results of tail suspension test. Statistical analysis was performed with one-way ANOVA followed by Bonferroni's comparison test according to which ***p<0.001 when compared to normal control, #p<0.05, ##p<0.01 and ###p<0.001 when compared to standard. ME+Flx: Methanolic extract+fluoxetine

Table 2: Phytochemicals found in the aqueous and methanolic extracts of beetroot

Phytochemicals	Aqueous extract	Methanolic
Phenolics	Present	Present (Colour more intense)
Flavonoids	Present	Present (Colour more intense)
Tryptophan	Absent	Present (Intense orange colouration)
Betalains	Present	Present
Saponins	Absent	Present

Table 3: Quantification of phenolics and flavonoids in the aqueous and methanolic extracts of beetroot (Expressed as ±SEM)

Phytochemicals	Aqueous extract (w/w)	Methanolic extract (w/w)
Total phenolic content	2.4±0.89% (GAE)	9.5±0.32% (GAE)
Total flavonoid content	6.2±0.69% (qE)	12.3±0.56% w/w (qE)

Table 4: Antioxidant activities of aqueous and methanolic extracts of beetroot (Expressed as Mean±SEM)

	IC_{50} value (ppm)	
Test samples	DPPH scavenging assay	H_2O_2 scavenging activity
Standards used	(Ascorbic acid) 17.78 ± 0.91	(Quercetine) 31.33±0.97
Methanolic extract	103.56±1.23	121.32±0.99
Aqueous extract	241.40±1.01	314.21±1.12

Tail suspension test (TST): It was observed that with increasing doses of BVAE and BVME, there was a decrease in TST time as compared to control group (Fig. 2). However, both doses of BVME (200 or 400 mg kg^{-1} day^{-1}) produced a more significant reduction in the TST than equivalent doses of the BVAE. In addition, standard fluoxetine showed significant reduction in the immobility period. However, the combination doses of 200 or 400 mg kg^{-1} day^{-1} BVME with 10 mg kg^{-1} day^{-1} fluoxetine (p<0.001) showed a more pronounced effect, that was virtually equivalent to standard 20 mg kg^{-1} day^{-1} fluoxetine alone. As opposed to the normal control, more than 50% reduction in the immobility times were observed in animals give standard doses of fluoxetine (20 mg kg^{-1} day^{-1}) and combined doses of BVME (400 mg kg^{-1} day^{-1}) plus low dose of fluoxetine (10 mg kg^{-1} day^{-1}) (Fig. 2). These results indicated an additive effect of BVME with fluoxetine in the tail suspension test.

Locomotors activity test (LAT): No significant changes in locomotor activities were noticed among all treatment groups (p>0.05), since all groups nearly exhibited similar locomotors count (Fig. 3). These results suggest that antidepressant activity of BV extracts manifested by the reduced immobility times in FST and TST may not be associated with CNS stimulation.

DISCUSSION

Our hypothesis is that beetroot (*Beta vulgaris*, BV) by virtue of its rich antioxidant, flavonoid and anti-inflammatory ingredients (betacyanins, betaxanthins, phenolics, flavonoids and tryptophan) may have a therapeutic potential for treating

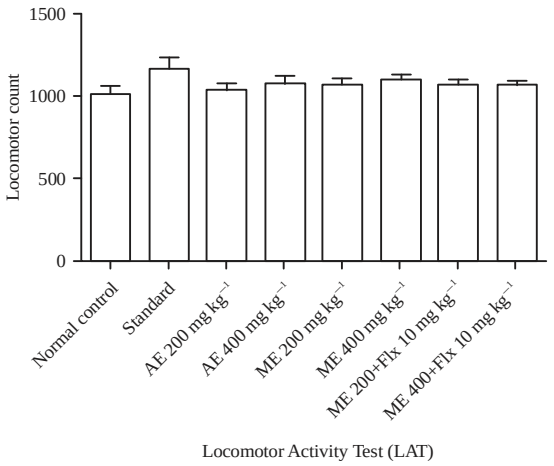

Fig. 3: Graphical representation of results of locomotor activity test

mild to moderate depression and some other minor mental ailments. Hence, the present study was designed to evaluate the antidepressant actions of aqueous and methanolic extracts of BV in a validated behavioural mouse model commonly used for determining the antidepressant effects of synthetic drugs and herbal remedies. Several investigations have suggested that flavonoids have a potential to alleviate some minor and major depressive disorders[28]. It has been reported that flavonoids act through their antioxidant action as well as through protective neurogenesis[29,30]. Many mental disorders such as Alzheimers disease[31], epilepsy[32], schizophrenia[33] and depressions[34] are thought to be associated with the impairment of neurogenesis and neurodegenerative changes in the CNS and can be ameliorated with flavonoids. Additionally, plant-derived flavonoids and antioxidants have been reported to improve the synthesis and storage of brain mono-amines and consequently increase the levels of norepinephrine, dopamine and serotonin and these neurotransmitters are strongly associating with the antidepressant effects of phytochemicals[30,34].

The phytochemical analysis revealed the presence of phenolics and flavonoids in both BVAE and BVME (Table 2). However, nearly 4-fold greater amount of phenolics (2.4 vs 9.5%) and 2-fold greater amount of flavonoids (6.2 vs 12.3%) were found in the BVME (Table 3). The *in vitro* antioxidant assays revealed 2.3-2.6 times greater free-radical scavenging ability of the BVME (Table 4). The results of both antioxidant assays positively correlated with the higher concentrations of phenolic and flavonoid ingredients present in the BVME in comparison with BVAE.

The mice treated with 200 and 400 mg kg^{-1} day^{-1} BVME depicted significant reductions in immobility time in both the FST and TST parameters. However, the groups given a combination of BVME+10 mg kg^{-1} day^{-1} fluoxetine showed more profound effects on immobility period which was comparable with the standard dosing group of 20 mg kg^{-1} day^{-1} fluoxetine alone. The combination of BVME with lower dose of flouxetine produced enhanced effect in both the FST and TST parameters than BVME given alone (Fig. 1, 2). It therefore appears that the simultaneous administration of BVME plus fluoxetine caused an additive antidepressant effect in the mouse model.

The FST is far more stressful than TST and drugs which act by the serotonergic pathway show greater reduction in immobility in the FST than the TST[27]. The methanolic extracts either alone or in combination with fluoxetine showed significant reductions in immobility time compared to the normal control ($p<0.001$), suggesting the involvement of serotonergic pathway in this manoeuvre. It has been reported that TST represents a condition in which antidepressants such as monoamine oxidase inhibitors (MOIs), selective serotonin reuptake inhibitors (SSRIs) and nor-epinephrine reuptake inhibitors are involved[28]. More pronounced effects of beetroot extracts were seen in the TST as compared to the FST. These differences may be attributed to the presence of beetroot betalains, flavonoids and phenolics acting through the reduction of oxidative stress and protecting CNS monoamines[29,30].

No significant changes in the LAT were observed between the control and treated groups. The result suggest that the increased mobility observed in FST and TST manoeuvres may be attributed to the antidepressant effects of treatments administered as well as the possibility of occurrence of false positives due to CNS stimulation and consequently the motor activity may not be altered by the treatments used.

Herbals drugs like St. John's wort *Hypericum perforatum*, *Centella asiatica*, *Curcuma longa* and *Moringa oleifera* are reported to have antidepressant activities which are attributed largely to their flavonoid and serotonergic actions in the CNS[8,35-37]. Previous studies reported in the literature have shown that the flavonoid-induced antioxidant and oxidative stress reducing effects may be translated into antidepressant activities and can be manifested through the reduction in immobility in animal models[30,34]. The presence of antioxidants (phenolics and flavonoids) in BV extracts, high *in vitro* free-radical scavenging properties of both BVME and BVAE coupled with their *in vivo* antidepressant activities lend support to this hypothesis proposed in this study. Markedly high antidepressant activity recorded following the combined administration of low dose of fluoxetine plus BVME suggests an additive effect of the combined treatment.

Flavonoids are reported to cause antidepressant activities through multiple pathways, viz., reduction in the uptake of serotonin, dopamine and inhibiting monoamine oxidase enzymes which oxidize dopamine and nor-epinephrine[27,38]. In addition, the betalains when metabolized in the body produce 2 molecules of L-dopa and norepinephrine[21]. Such may also be the mechanism of phytochemicals detected in BV extracts and manifested in the TST and FST parameters.

The chemical analysis of BVME revealed the presence of tryptophan (Table 2), an essential amino acid not synthesised in the human body and is only available in the diet. Tryptophan is required for protein synthesis and as a precursor of key biomolecules such as serotonin, melatonin, tryptamine, niacin, quinolinic acid and kynurenic acid, nicotinamide adenine dinucleotide, etc. Excessive dietary restriction and malnutrition decreases brain serotonin stores and leads to behavioral changes such as hyperactivity, depression, anxiety, suppression of appetite, anorexia nervosa and behavioral impulsivity. Dietary supplementation of tryptophan may improve these disorders[39]. In the present study, saponins were also detected in the methanolic extract of BV (Table 2). Previously, triterpenoid saponins such as oleanoic acid and hedragenin have been found in the beetroot, which have antidepressant activities[13]. The results of present study confirmed antidepressant activity of BV extracts. However, the quantitative proportion of beetroot ingredients and their individual biological activities remain to be ascertained. Further studies with major components would enhance our understanding about the contribution of antioxidant and antidepressant activities of the beetroot extract. Based on the current findings, it is postulated that the BV extract-induced antidepressant activity may primarily occur through the involvement of serotonergic and adrenergic pathways as well as reduction of oxidative stress in the CNS.

In summary, the presence of antioxidants, flavonoids, along with other aforementioned components may collectively contribute to the antidepressant activity of BV extracts. In other words, the antidepressant and antioxidant activities observed in BVAE and BVME may be due to multiple pharmacological actions resulting from the various components present in the beetroot extracts. While this preliminary behavioural mouse model study provides experimental evidence about the antidepressant activity of beetroot extract, further well designed psychopharmacological investigations in experimental animal models and humans are needed to get deeper insights into the antidepressant mechanisms involved in the beetroot extract.

CONCLUSION

The pivotal finding of this study is that *Beta vulgaris* either alone, or in combination with low dose of fluoxetine causes marked antidepressant activity, as noticed in the behavioural mouse models. To the best of our knowledge, this study is the first of its kind to demonstrate antidepressant activity in the standardised behavioural mouse models of depression. While this behavioural mouse model study showed positive signals for the antidepressant activity of beetroot, further investigations are needed to understand the underlying mechanism through which beetroot extract produces antidepressant effects. The results of this novel study suggest that there is a tremendous potential to develop antidepressant therapy with *Beta vulgaris* extracts either alone or its combination with fluoxetine-type antidepressant drugs. The BV extract alone or combined with low doses of fluoxetine seems to have a promising potential for the development of cost-effective alternative therapy for treating mild to moderate depression in humans. The developing countries are desperately looking for alternative therapies for treating depression in view of the high cost of synthetic drugs.

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