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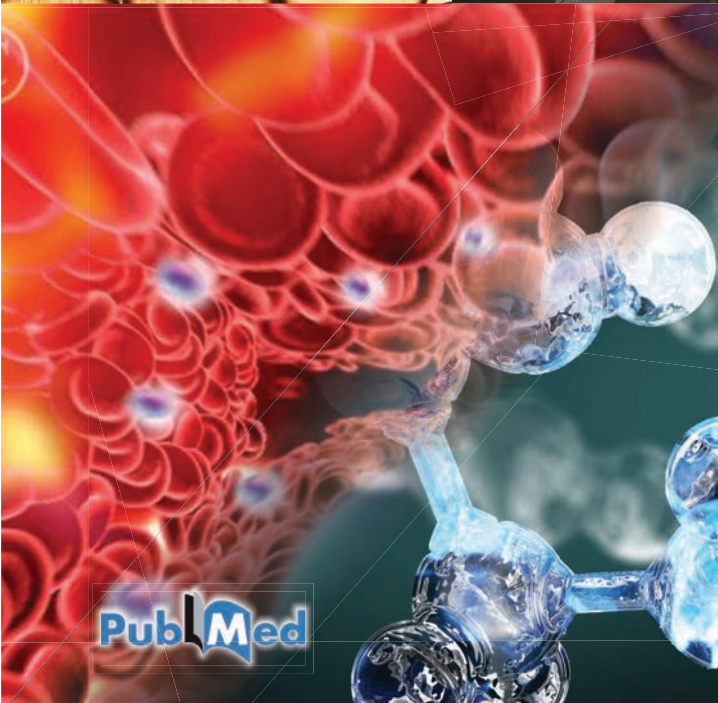
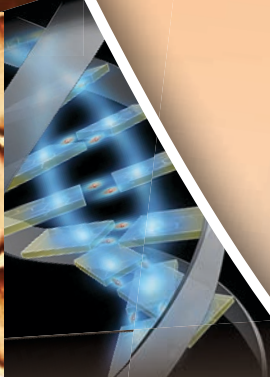
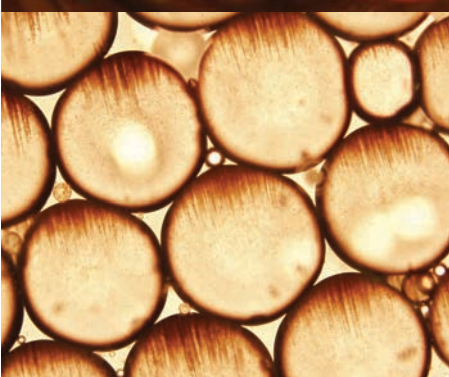


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# Phytotherapy of experimental depression: *Kalanchoe integra* Var. *Crenata* (Andr.) Cuf Leaf Extract

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## ABSTRACT

**Context:** *Kalanchoe* sp. have been used since 1921 for central nervous system (CNS) disorders such as psychosis and depression. It is known to possess CNS depressant effects. **Aims:** To investigate the antidepressant properties of the aqueous leaf extract of *Kalanchoe integra*. **Settings and Design:** The study was carried out at the Kwame Nkrumah University of Science and Technology between 6 a.m. and 3 p.m. **Materials and Methods:** ICR mice were subjected to the forced swimming test (FST) and tail suspension test (TST) after they had received extract (30-300 mg/kg), fluoxetine (3-30 mg/kg), desipramine (3-30 mg/kg) orally, or water (as vehicle). In a separate experiment, mice were pre-treated with reserpine (1 mg/kg),  $\alpha$ -methyl paratyrosine (AMPT; 400 mg/kg), both reserpine (1 mg/kg) and AMPT (200 mg/kg) concomitantly, or *p*-chlorophenylalanine (pCPA; 200 mg/kg) to ascertain the role of the noradrenergic and serotonergic systems in the mode of action of the extract. **Statistical analysis used:** Means were analyzed by analysis of variance (ANOVA) followed by Newman-Keuls' *post hoc* test.  $P < 0.05$  was considered significant. **Results:** In both FST and TST, the extract induced a decline in immobility, indicative of antidepressant-like effect. This diminution in immobility was reversed by pCPA, but not by reserpine and/or AMPT. The extract increased the swimming and climbing scores in the FST, suggestive of possible interaction with serotonergic and noradrenergic systems. In the TST, the extract produced increases in both curling and swinging scores, suggestive of opioidergic monoaminergic activity, respectively. **Conclusions:** The present study has demonstrated the antidepressant potential of the aqueous leaf extract of *K. integra* is mediated possibly by a complex interplay between serotonergic, opioidergic, and noradrenergic systems.

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**KEY WORDS:** Curling, depression, *Kalanchoe integra*, noradrenaline, opioid, serotonin

Depression is a very common debilitating psychiatric disorder characterized by symptoms that hamper a person's ability to work, sleep, study, eat, and enjoy formerly pleasurable activities.<sup>[1,2]</sup> It is estimated that more than 120 million people are affected worldwide.<sup>[3]</sup>

Notwithstanding the treatment advances, depression is still a major cause of morbidity and mortality. Current therapies are unable to offer full remission. Most patients (40%) are refractory to current depression pharmacotherapy and this serves as a testament to the limitations of the current medications.<sup>[4,5]</sup> Furthermore, no antidepressant with rapid and sustained effect has been approved clinically, although there are reports of the availability of these agents.<sup>[6,7]</sup> Considering that high suicidal rates among depressed patients are positively correlated with the delay in remission of symptoms, the unavailability of an approved drug with rapid onset, sustained effect, and less severe side effects that caters for the refractory conditions paints rather a gloomy picture.<sup>[6,8]</sup> These reasons accentuate the need for more vigorous drug research that will lead to the discovery of the "wonder antidepressant."

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The medicinal potential of plants and their usefulness in primary healthcare cannot be ignored. Medicinal plants are important repositories of bioactive agents that can be employed in the management of treatment-refractory conditions like depression.<sup>[9]</sup> *Kalanchoe* sp. has been used since 1921 in traditional medicine as an antipsychotic agent.<sup>[10]</sup> Previous studies have shown the antidepressant activity of *Kalanchoe pinnata* leaf extract,<sup>[11,12]</sup> while Shashank demonstrated the antidepressant effect based on locomotor activity in mice using actophotometer.<sup>[13]</sup> The leaves of *Kalanchoe integra* have wide ethnomedical uses in Ghana and the plant has been found to possess central nervous system depressant effect.<sup>[14]</sup> Though the plant has been reported as possessing central active properties, its possible effect in depression has not been investigated.

The study, therefore, investigated the effect of the aqueous leaf extract of *K. integra* in animal models of depression subjected to the forced swimming test (FST) and tail suspension test (TST).

## Materials and Methods

### Materials

Fresh leaves of *K. integra* were collected from the botanical garden of the University of Ghana in October and sent to the Botany Department, University of Ghana for identification, authentication, and storage at the herbarium. A voucher specimen (IAGSP-001) was subsequently deposited in the herbarium at the Botany Department.

The fresh leaves (1 kg) were carefully washed under tap water and blended using Sanyo SM (G300) blender. The leaf extract was then strained using muslin cloth and freeze-dried to yield 150 g (15%) of the extract (also known as KIE). The powdered samples were stored at 4°C and used within 7 weeks after production.

### Animals

Male ICR mice were obtained from Noguchi Memorial Institute for Medical Research, Accra, Ghana, and housed at the animal facility of the Department of Pharmacology, KNUST, Kumasi, Ghana. The animals were housed in groups of five in stainless steel cages (34 × 47 × 18 cm) with soft wood shavings as bedding, fed with normal commercial pellet diet (GAFCO, Tema, Ghana), given water *ad libitum*, and maintained under laboratory conditions. All animals used in these studies were treated in accordance with the Guide for the Care and Use of Laboratory Animals.<sup>[15]</sup>

### Chemicals

Fluoxetine hydrochloride (Prozac<sup>®</sup>) was from Eli Lilly and Co., Basingstoke, England. Desipramine hydrochloride,  $\alpha$ -methyl paratyrosine (AMPT), reserpine, and *p*-chlorophenylalanine (pCPA) were purchased from Sigma-Aldrich Inc. (St. Louis, MO, USA).

### Tail suspension test

The TST was carried out as described earlier,<sup>[16]</sup> with slight modifications given by Berocosso *et al.*<sup>[17]</sup> Mice were allowed to acclimatize to the room for 3.5-4 h before the test. Groups of 10 mice ( $n = 7$ ) were treated with KIE (30, 100, or 300 mg/kg, p.o.), fluoxetine (FLX) (3, 10, or 30 mg/kg, p.o.), and desipramine (DSP) (3, 10, or 30 mg/kg, p.o.) or water (vehicle). One hour after oral administration of the test drugs, mice were individually suspended by the tail from a horizontal bar (distance from the floor = 30 cm) using an adhesive tape (distance from the tip of tail = 1 cm). Immobility (the absence of all movements except for those required for respiration), curling (active twisting movements), and swinging (vertical movement of the paws and/or side-to-side movement of body) behaviors were recorded for 5 min. The predominant behavior in each 5-s period of the 5 min was scored and the means computed. Mice that climbed up on their tails during the test session were gently pulled down and testing continued, but those that continued to climb up on their tails were excluded from the study.

### Forced swimming test

The FST was based on that described by Porsolt *et al.*<sup>[18]</sup> Mice were randomly assigned to 10 groups of seven animals each. Water (vehicle), extract (30, 100, or 300 mg/kg, p.o.), or the standard reference drugs FLX (3, 10, or 30 mg/kg, p.o.) and DSP (3, 10, or 30 mg/kg, p.o.) were administered. One hour after oral administration of the test drugs, mice were gently placed individually in transparent cylindrical polyethylene tanks (25 cm high, 10 cm internal diameter) containing water (25-28°C) up to a level of 20 cm and left for 5 min. Each session was recorded by a video camera suspended approximately 100 cm above the cylinders. An observer scored the immobility (when mouse floated upright in the water and made only small movements to keep its head above water), swimming (active horizontal movements across water), and climbing (active vertical movement by the walls of the cylinder) behaviors during the 5 min test, from the videotapes with the aid of the public domain software JWatcher Version 1.0 (University of California, Los Angeles, USA and Macquarie University, Sydney, Australia; available at <http://www.jwatcher.ucla.edu/>).

### Involvement of noradrenergic systems

Mice were pre-treated with reserpine and/or AMPT in order to investigate the possible role of noradrenergic system in the actions of KIE.<sup>[19]</sup> The doses of AMPT and reserpine were chosen on the basis of the work done by others.<sup>[19]</sup> To deplete newly synthesized pools of noradrenaline and serotonin, mice were treated with a single dose of AMPT (400 mg/kg, i.p.) 3.5 h before behavioral testing. To deplete the vesicular pools of noradrenaline and dopamine, mice were treated with a single dose of reserpine (1 mg/kg, s.c.) 24 h before behavioral testing. In an effort to deplete both the vesicular and cytoplasmic pools of noradrenaline and dopamine, mice were pre-treated with a combination of reserpine (1 mg/kg, s.c., 24 h before

behavioral testing) and AMPT (200 mg/kg, i.p., 3.5 h before behavioral testing), respectively.

### Involvement of serotonergic systems

Mice were randomly assigned to 20 groups ( $n = 7$ ). pCPA (200 mg/kg, i.p.) was administered once daily for three consecutive days to some of the animals. On the fourth day, group 1 received saline without pre-treatment; group 2 received pCPA after pre-treatment; groups 3-5 received KIE (30, 100, or 300 mg/kg) without pre-treatment; groups 6-8 received KIE (10, 30, and 100 mg/kg, p.o.) after pre-treatment; groups 9-11 received FLX (3, 10, and 30 mg/kg, p.o.) alone; groups 12-14 received FLX (3, 10, and 30 mg/kg, p.o.) after pre-treatment; groups 15-17 received DSP (3, 10, and 30 mg/kg, p.o.) alone; and finally groups 18-20 received DSP (3, 10, and 30 mg/kg, p.o.) after pre-treatment. After the tail suspension sessions, mice were taken through the FST.

### Statistical analysis

GraphPad Prism for Windows version 5.03 (GraphPad Software, San Diego, CA, USA) was used for all data and statistical analyses.  $P < 0.05$  was considered statistically significant. In all the tests, a sample size of 10 animals ( $n = 7$ ) was used. Differences in means were analyzed by analysis of variance (ANOVA) followed by Newman-Keuls' *post hoc* test.

## Results

### Effect of KIE on mean immobility, swimming, and climbing scores in the FST

Extract treatment significantly reduced the immobility score (34%; 100 mg/kg), increased the swimming score (28%; 100 mg/kg), and had a minimal effect on the climbing score. FLX, like the extract, decreased immobility and increased the swimming behavior, but had no effect on the climbing behavior. In contrast, DSP decreased immobility and swimming behavior, but increased the climbing behavior [Table 1].

### Effect of KIE on mean immobility, swinging, and curling scores in the TST

In the TST, *K. integra* extract decreased the immobility score by 65% and increased both swinging score (by approximately 52%) and curling behavior (by 69%) (30 mg/kg). FLX also decreased immobility and increased swinging, but had no effect on curling behavior. DSP decreased immobility and increased the curling behavior, but had no effect on swinging [Table 2].

### Involvement of the noradrenergic system

#### *Depletion of monoamine stores with reserpine*

Pre-treatment of mice with reserpine (1 mg/kg) failed to reverse the decline in immobility induced by extract treatment, but reversed this behavioral effect of FLX and DSP. Following

reserpine treatment, however, the swimming score was decreased in extract-, FLX-, and DSP-treated mice. The climbing score was increased in extract-treated mice, but was decreased in DSP-treated mice compared to non-reserpine-treated mice. Climbing behavior was not affected by the administration of FLX [Table 3].

#### *Inhibition of noradrenaline synthesis with AMPT*

AMPT treatment failed to reverse the decline in immobility seen in extract-treated mice. It, however, potentiated the climbing behavior while reducing the swimming behavior induced by the KIE. The effect of FLX on the decline of immobility was not reversed compared to vehicle treatment, but that of DSP was totally reversed. Swimming score decreased in FLX-treated mice, but was still significantly higher when compared to vehicle treatment; climbing score was decreased. In contrast, the swimming score for DSP was increased by AMPT treatment, though this was not significant when compared to non-AMPT-treated groups; climbing scores were significantly reduced [Table 4].

#### *Depletion of both newly synthesized noradrenaline and vesicular stores*

Concomitant pre-treatment with reserpine and AMPT did not reverse the decline in immobility induced by the extract. The increase in swimming behavior induced by the extract was reversed, while the climbing behavior was potentiated by the administration of reserpine and AMPT. The pre-treatment reversed the declined in immobility induced by FLX and DSP. The increase in swimming score induced by FLX was reversed, while the decrease in swimming induced by DSP was not reversed. Moreover, the increase in climbing score caused by DSP and FLX was reversed by the pre-treatment [Table 5].

#### *Effect of pCPA treatment on behavior in the TST*

Three-day pre-treatment with pCPA reversed the decline in immobility (partially) and the increase in curling and swinging behaviors caused by the extract. The pre-treatment reversed the behavioral effects of FLX by reversing the decline in immobility and increase in swinging (partial). pCPA treatment did not reverse the decline in immobility induced by DSP, but reduced the swinging and curling scores [Table 6].

## Discussion

The results from the present study indicate that *K. integra* has antidepressant-like effects in two widely used animal models for investigating the antidepressant effect of test compounds, the TST and FST. In both models, the extract caused a decline in immobility behavior while increasing various active behaviors like swimming, climbing, curling, and swinging.

Decline in immobility is used as the principal index for the antidepressant effect of test substances in these models. Indeed, virtually all antidepressants used medically induce a diminution

**Table 1: Effect of KIE on mouse behavior in the forced swimming test**

CTRL	KIE (mg/kg)			FLX (mg/kg)			DSP (mg/kg)		
	30	100	300	3	10	30	3	10	30
Immobility	34.14±1.12	24.86±2.04***	22.43±0.68***	24.71±1.30***	22.43±1.25***	22.00±1.70***	31.43±2.22	20.00±0.76***	19.86±1.10***
Swimming	17.71±0.99	20.00±1.69	26.29±0.84***	20.86±0.88	24.57±1.25**	26.57±1.29***	7.71±1.13*	12.29±0.87**	11.43±0.43**
Climbing	8.28±0.97	15.14±1.06*	12.00±0.79	14.43±1.48	11.43±0.65	10.86±0.80	20.86±3.20***	27.71±0.68***	28.71±0.99***

Effects of the extract (KIE; 30-300 mg/kg), fluoxetine (FLX; 3-30 mg/kg), and desipramine (DSP; 3-30 mg/kg) treatment on the mean immobility counts, swimming counts, and curling counts in the FST. Data are presented as mean±SEM. Significantly different from control: \**P*<0.05, \*\**P*<0.01, \*\*\**P*<0.001 by Newman-Keuls' test

**Table 2: Effect of KIE on mouse behavior in the tail suspension test**

CTRL	KIE (mg/kg)			FLX (mg/kg)			DSP (mg/kg)		
	30	100	300	3	10	30	3	10	30
Immobility	35.71±0.78	22.86±3.00***	24.29±1.66***	11.71±2.33***	18.00±2.05***	11.86±1.44***	25.00±1.13**	20.57±1.88***	14.29±0.64***
Swinging	18.71±1.27	24.43±3.86	23.71±2.76	40.00±3.33***	19.57±1.13	39.29±2.13***	8.86±1.16	11.86±1.70	22.43±1.34
Curling	5.00±0.90	12.71±2.49**	12.00±1.56*	7.43±0.65	2.28±0.42	2.86±0.51	26.14±0.67***	26.14±2.41***	23.29±1.34***

Effects of the extract (KIE; 30-300 mg/kg), fluoxetine (FLX; 3-30 mg/kg), and desipramine (DSP; 3-30 mg/kg) treatment on the mean immobility counts, swinging counts, and curling counts in the TST. Data are presented as mean±SEM. Significantly different from control: \**P*<0.05, \*\**P*<0.01, \*\*\**P*<0.001 by Newman-Keuls' test

**Table 3: Effect of reserpine pre-treatment on mice in the forced swimming test**

CTRL	KIE (mg/kg)			FLX (mg/kg)			DSP (mg/kg)		
	30	100	300	3	10	30	3	10	30
Immobility	48.14±1.12	48.14±1.46	24.86±2.04***	2.29±0.68	22.43±0.68***	1.86±0.60††	24.71±1.30***	3.71±0.71†	22.00±1.70***
Swimming	17.71±0.99	8.14±1.42	20.00±1.69	3.57±0.48††	26.29±0.84***	4.00±0.53†††	20.86±0.88*	4.86±0.99†	28.86±1.34***
Climbing	8.28±0.97	3.00±0.62	15.14±1.06*	53.71±1.13†††	12.00±0.79	53.71±0.99†††	14.43±1.48	51.43±0.81†††	9.14±0.86

Effects of reserpine (1 mg/kg) pre-treatment on the mean immobility counts, swimming counts, and climbing counts of groups treated with oral doses of extract (KIE; 30-300 mg/kg), fluoxetine (FLX; 30 mg/kg), and desipramine (DSP; 30 mg/kg) in the FST. Data are presented as mean±SEM. Significantly different from untreated group: \**P*<0.05, \*\**P*<0.01, \*\*\**P*<0.001 by Newman-Keuls' test; †significantly different from pre-treated group: †*P*<0.05, ††*P*<0.01, †††*P*<0.001

**Table 4: Effect of AMPT pre-treatment on mice in the forced swimming test**

CTRL	KIE (mg/kg)			FLX (mg/kg)			DSP (mg/kg)		
	30	100	300	3	10	30	3	10	30
Immobility	34.14±1.12	39.43±1.88	24.86±2.04***	14.71±1.46†††	22.43±0.68***	17.43±0.10††††	24.71±1.30***	24.29±0.64†††	22.00±1.70***
Swimming	17.71±0.99	6.14±0.88	20.00±1.69	12.43±0.48	26.29±0.84***	13.29±0.84†††	20.86±0.88	8.00±1.18	28.86±1.34***
Climbing	8.28±0.97	11.57±0.57	15.14±1.06***	32.86±1.74†††	12.00±0.79*	29.29±1.11†††	14.43±1.48***	27.71±0.71†††	9.14±0.86

Effects of  $\alpha$ -methyl paratyrosine (AMPT; 400 mg/kg) pre-treatment on the mean immobility counts, swimming counts, and climbing counts of groups treated with oral doses of extract (KIE; 30-300 mg/kg), fluoxetine (FLX; 30 mg/kg), and desipramine (DSP; 30 mg/kg) in the FST. Data are presented as mean±SEM. Significantly different from untreated group: \**P*<0.05, \*\*\**P*<0.001 by Newman-Keuls' test; †significantly different from pre-treated group: †*P*<0.05, ††*P*<0.01, †††*P*<0.001

**Table 5: Effect of reserpine/AMPT combination in the forced swimming test**

	KIE (mg/kg)						FLX (mg/kg)		DSP (mg/kg)			
	30		100		300		30		30			
	Untreated	Treated	Untreated	Treated	Untreated	Treated	Untreated	Treated	Untreated	Treated		
Immobility	34.14±1.12	34.29±1.14	24.86±2.04***	34.29±0.97	22.43±0.68***	20.86±0.51###	24.71±1.30***	23.57±0.48###	22.00±1.70***	31.29±0.68	19.86±1.10***	34.29±0.82
Swimming	17.71±0.99	18.29±1.15	20.00±1.69	14.57±0.75###	26.29±0.84***	11.00±0.62###	20.86±0.88	8.86±1.01###	28.86±1.34***	16.29±0.94###	11.43±0.43***	11.43±0.61###
Climbing	8.28±0.97	6.43±0.57	15.14±1.06***	10.57±0.57#	12.00±0.79*	27.29±0.68###	14.43±1.48***	26.57±1.19###	9.14±0.86	12.00±0.82###	28.71±0.99***	12.29±0.68###

Effects of both AMPT (200 mg/kg) and reserpine (1 mg/kg) pre-treatment on the mean immobility counts, swimming counts, and climbing counts of groups treated with oral doses of extract (KIE; 30-300 mg/kg), fluoxetine (FLX; 30 mg/kg), and desipramine (DSP; 30 mg/kg) in the FST. Data are presented as mean±SEM. Significantly different from untreated group: \*  $P < 0.05$ , \*\*  $P < 0.01$ , \*\*\*  $P < 0.001$  by Newman-Keuls' test; significantly different from pre-treated group: ###  $P < 0.001$ .

**Table 6: Effect of pCPA pre-treatment in the tail suspension test**

	KIE (mg/kg)						FLX (mg/kg)		DSP (mg/kg)			
	30		100		300		30		30			
	Untreated	Treated	Untreated	Treated	Untreated	Treated	Untreated	Treated	Untreated	Treated		
Immobility	35.71±0.78	33.0±0.82	22.86±3.00***	17.57±1.07###	24.29±1.66***	22.28±0.75###	11.71±2.33***	20.86±0.51###	11.86±1.44***	31.43±1.96	14.29±0.64***	16.00±0.53###
Swinging	18.71±1.27	6.43±0.61	24.43±3.86***	7.71±0.75	23.71±2.76***	8.86±1.14	40.00±3.33***	9.86±0.96	43.71±1.96***	11.14±1.28	22.43±1.34***	10.43±0.75
Curling	5.00±0.90	1.86±0.26	12.71±2.49***	2.00±0.38	12.00±1.56***	1.57±0.30	7.43±0.65	2.14±0.40	4.14±0.67	2.57±0.43	23.29±1.34***	7.34±0.51###

Effects of pCPA (200 mg/kg) pre-treatment on the mean immobility counts, swinging counts, and curling counts of groups treated with oral doses of extract (KIE; 30-300 mg/kg), fluoxetine (FLX; 30 mg/kg), and desipramine (DSP; 30 mg/kg) in the TST. Data are presented as mean±SEM. Significantly different from untreated group: \*  $P < 0.05$ , \*\*  $P < 0.01$ , \*\*\*  $P < 0.001$  by Newman-Keuls' test; significantly different from pre-treated group: ###  $P < 0.001$ .

in immobility in rodents, while other drugs fail to give the same response.<sup>[20,21]</sup> KIE was able to cause a reduction in immobility behavior which is suggestive of an antidepressant-like effect.

In the FST, substances that increase the swimming score without altering the climbing behavior are purported to be acting via the serotonergic pathway, while those that increase the climbing score without affecting swimming are sensitive to the noradrenergic pathway.<sup>[22,23]</sup> Since the extract induced increases in both swimming and climbing scores, we hypothesized that it might be acting via both serotonergic and noradrenergic pathways.

In the TST, the extract demonstrated its antidepressant effect by decreasing the immobility score and increasing both curling and swinging behaviors. Curling behavior is reported to be indicative of opioidergic activity.<sup>[17]</sup> The role of opioid as putative antidepressants is supported by the clinical effectiveness of  $\mu$ -opioid receptor agonists such as tramadol, oxycodone, oxymorphone, etc., in the treatment of refractory depression.<sup>[24-26,17]</sup> It is likely the antidepressant effect of the extract is partly dependent on its effect on opioidergic systems.

The impact of depletion of noradrenaline and serotonin on the behavioral effect of the extract was investigated. Pre-treatment of mice with reserpine potentiated the decline in immobility induced by the extract, reversed the increase in swimming, and further increased the climbing score. Reserpine is an irreversible inhibitor of the vesicular monoamine transporter 2 (VMAT-2) which is located chiefly within the CNS and is responsible for transferring monoamines from the cytoplasm into secretory vesicles.<sup>[27,28]</sup> Treatment with reserpine, therefore, leads to depletion of vesicular monoamine stores-both serotonin and noradrenaline.<sup>[29]</sup> The failure to reverse the decline in immobility and increase in climbing score may suggest that depletion of vesicular stores of noradrenaline does not affect the action of the extract. It may also suggest that the extract acts directly on post-synaptic neurons, competitively inhibits the effect of reserpine, or the extract does not act on the noradrenergic pathways. That reserpine treatment reversed the swimming behavior while maintaining its decline in immobility may suggest that the antidepressant effect of the extract may be partially dependent on release of serotonin from the vesicles.

AMPT reduces synthesis of noradrenaline and dopamine by reversibly inhibiting the rate-limiting enzyme, tyrosine hydroxylase, thereby blocking the conversion of tyrosine to l-dopa.<sup>[30]</sup> Pre-treatment with AMPT did not reverse decline in immobility or increase in climbing for mice that received the extract. This further confirms that the antidepressant effect of the extract may not be dependent on noradrenergic pathways. Thus, the increase in climbing score when the extract was administered alone may not be as a result of direct action on noradrenergic pathways. The results were not different when mice were pre-treated with both AMPT and reserpine.

The possible contribution of serotonin or its pathway on the antidepressant effect of the extract was investigated in the TST by pre-treating mice with pCPA for 3 days. pCPA is a selective

irreversible inhibitor of tryptophan hydroxylase, the enzyme responsible for serotonin (5-HT) synthesis.<sup>[31]</sup> The decline in immobility induced by the extract was partially reversed, suggesting that serotonin contributes to the antidepressant effect of the extract. In the absence of pCPA, the extract increased both curling and swinging behaviors. These behaviors were, however, reversed by pCPA pre-treatment. According to Berrococo *et al.*, swinging and curling behaviors are mediated by increase in monoaminergic and opioidergic neurotransmission, respectively.<sup>[17]</sup> The observed phenomenon suggests that the antidepressant effect of the extract involves a complex interplay between serotonergic pathways and opioidergic neurotransmission. This is not surprising because the extract induced the Straub tail effect in mice (a rodent behavior sensitive to opioids) in a preliminary screening that was carried out (unpublished data).

Although this study was limited by our inability to look at other possible mechanisms by which the extract may act, e.g. glutamatergic neurotransmission, and also conduct *in vitro* studies to ascertain whether or not the extract acts specifically on receptors in the serotonergic and opioidergic pathways, we can conclude that KIE has antidepressant-like potential.

## Conclusion

The study has provided evidence that *K. integra* leaf extract has antidepressant effect which is mediated by a complex interplay of serotonergic, opioidergic, and noradrenergic systems, when administered orally within the dose range.

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