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In vitro and *In vivo* antidepressant activity of *Sarcostigma kleinii* (wight & arn.) family-*Icacinaceae*

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Abstract
Depression, or major depressive disorder, is a mental health condition marked by a feeling of sadness, isolation and despair that affects how a person thinks, feels and functions. Lack of sufficient scientific evidence indicating the utility of this plant in the treatment of major depressive disorder prompted us to investigate activity of the plant. In *in vitro* activity neuroprotective effect of ethyl alcoholic extract at concentrations 6.25, 12.5, 25, 50 and 100 µg/ml on IMR32 (Neuroblastoma cells) were determined by MTT assay and Reactive Oxygen Species measurement using flourascence analysis. In *In-vivo* pharmacological screening method anti-depressant activity of extract at doses of 200mg/kg and 400 mg/kg was assessed by Sucrose preference test, Locomotor activity test, Forced swim test, and Tail suspension test using female Swiss Albino mice. The results of *in vitro* screening showed that percentage of cell viability significantly increased when treated with a concentration of 12.5 µg/ml extract when compared with other treated concentrations. The decrease in ROS generation in cells showed that it can be neuroprotective due to increased cell viability. *In-vivo* pharmacological screening data supported antidepressant potential of extract of *Sarcostigma kleinii* by these models of depression. The present study exhibits anti-depressant potential of Ethyl alcoholic extract of *Sarcostigma kleinii* (EAESk*)* in depression like symptoms in mice exposed to unpredictable chronic mild stress.

Keywords: ethyl alcohol extract of *sarcostigma kleinii* (EAESK*),* imr32 (neuroblastoma cells), reactive oxygen species (ros), major depressive disorder (MDD), unpredictable chronic mild stress (UCMS)

Introduction

Depression

Depression, or major depressive disorder, is a mental health condition marked by a feeling of sadness, isolation and despair that affects how a person thinks, feels and functions. The condition may significantly interfere with a person's daily life and may prompt thoughts of suicide. Depression isn't the same as sadness, loneliness or grief caused by a challenging life experience, such as the death of a loved one but a condition from which not able to come out by oneself. Depression can affect people of all ages, races and socioeconomic classes, and can strike at any time. The condition is found twice in women due to the hormonal changes than in men [1]. Antidepressant drugs are having many adverse effects which may lead to toxicity. Antidepressants help in altering mood by affecting naturally occurring neurotransmitters in brain. There are several categories of antidepressants [2], but doctors often start with a class of drugs called selective serotonin reuptake inhibitors (SSRIs) which inhibit reuptake of serotonin, a signaling chemical (neurotransmitter) deficiency of which was found to be involved in depression, and may try other medications if the patient's condition didn't improve. This class of medication includes fluoxetine (Prozac), escitalopram (Lexapro) citalopram (Celexa) etc. To overcome adverse effects natural medicines can be used for treatment of depression which will have very less side effects. *Sarcostigma kleinii* Wight & Arn. (Erumathali, Odal, Vattodal, Velloda in Malayalam) [3] Is one of the potential medicinal plants, widely used as a source of drug in the treatment of several diseases. This is seen in Evergreen and semi- evergreen forests, also in sacred groves and in all districts of Kerala. The plant's bark and leaves are bitter, acrid, thermogenic, anthelmintic, digestive, carminative, diuretic, anaphrodisiac, depurative, vulnerary and stomachic [4]. The entire plant was recognized as valuable drug and frequently used by many of the ancient traditional medical systems. The leaf extract showed the highest total phenolic content and total flavonoid content and the best antioxidant activity [5]. It showed presence of flavonoids, phenols, alkaloids, tannins, glycosides and triterpenoids.

Materials and Methods

Collection, Authentication and extraction of plant leaves

Fresh leaves of *Sarcostigma kleinii* were collected from MG University Kottayam, Kerala in July 2015.The plant was identified and authenticated by Dr. Rogimon P. Thomas, Asst. Professor. Department of Botany, C.M.S. College, Kottayam, Kerala, India. Specimen No. CMS 1754.The collected leaves were washed under running tap water to remove dust particles from the surface. Then the leaves were dried under shade and powdered mechanically. The powdered leaves were extracted using ethanol by soxhlet extraction method for 24hrs.

In vitro neuroprotective effect

IMR32 (Neuroblastoma cells) cell line was purchased from NCCS Pune was maintained in Dulbecco's modified eagles media (HIMEDIA) from National Centre for Cell Sciences (NCCS), Pune, India and maintained Dulbecco's Modified Eagles medium (DMEM) (Sigma Aldrich, USA).
The cell line was cultured in 25 cm^2 tissue culture flask with DMEM supplemented with 10% FBS, L-glutamine, sodium bicarbonate and antibiotic solution containing: Penicillin (100U/ml), Streptomycin (100µg/ml), and Amphoteracin B

(2.5µg/ml). Cultured cell lines were kept at 37ºC in a humidified 5% CO_2 incubator (NBS Eppendorf, Germany).
The viability of cells were evaluated by direct observation of cells by Inverted phase contrast microscope and followed by MTT assay method [6].

Cells seeding in 96 well plate
Two days old confluent monolayer of cells were trypsinized and the cells were suspended in 10% growth medium, 100µl cell suspension ($5x10^3$ cells/well) was seeded in 96 well tissue culture plate and incubated at 37ºC in a humidified 5% CO_2 incubator.

Preparation of compound stock
1mg of the sample was weighed and completely dissolved in 1mL DMEM using a cyclomixer. The extract solution was filtered through 0.22 µm Millipore syringe filter to ensure the sterility. H_2O_2 (150µM) was added to induce toxicity.

Cytotoxicity Evaluation
After attaining sufficient growth that is 70% confluency, H_2O_2 (150µM) was added to induce toxicity and incubated for one hour, prepared extracts in 5% DMEM were five times serially diluted by two fold dilution (100µg, 50µg, 25µg, 12.5µg, 6.25µg in 500µl of 5% DMEM) and each concentration of 100µl were added in triplicates to the respective wells and incubated at 37ºC in a humidified 5% CO_2 incubator.

Cytotoxicity Assay by Direct Microscopic observation
Entire plate was observed at an interval of each 24 hours; up to 72 hours in an inverted phase contrast tissue culture microscope (Olympus CKX41 with Optika Pro5 CCD camera) and microscopic observation were recorded as images. Any detectable changes in the morphology of the cells, such as rounding or shrinking of cells, granulation and vacuolization in the cytoplasm of the cells were considered as indicators of cytotoxicity.

Cytotoxicity Assay by MTT Method
15 mg of MTT (Sigma, M-5655) was reconstituted in 3 ml PBS until completely dissolved and sterilized by filter sterilization.
After 24 hours of incubation period, the sample content in wells were removed and 30µl of reconstituted MTT solution was added to all test and cell control wells, the plate was gently shaken well, then incubated at 37ºC in a humidified 5% CO_2 incubator for 4 hours. After the incubation period, the supernatant was removed and 100µl of MTT Solubilization Solution (DMSO) was added and the wells were mixed gently by pipetting up and down in order to solubilize the formazan crystals. The absorbance values were measured by using microplate reader at a wavelength of 540 nm (Laura B. Talarico *et al*., 2004).
The percentage of growth inhibition was calculated using the formula

$$\% \text{ of viability} = \frac{\text{Mean OD Samples x } 100}{\text{Mean OD of control group.}}$$

***In vitro* ROS measurement using flourascence analysis**
After attaining sufficient growth that is 70% confluency, H_2O_2 (150µM) was added to induce toxicity and incubated for one hour, after incubation 12.5µg/mL concentration of sample (Ethyl Acetate Extract) from a stock of 1mg/ml was added and incubated for 24 hours. The cells were washed with PBS to remove serum and blood contaminants, 50 µl of Dichloro Fluorescein Diacetate (DCFDA) was added and incubated for 30 minutes. After incubation excess dye was washed with PBS and fluorescence was imaged[7] in a fluorescent microscope (Olympus CKX41 with optika pro5 CCD camera) and fluorescence was measured using a fluorimeter at 470 nm excitation and emission at 635nm (Qubit 3.0, Life technologies, USA) and expressed in arbitrary units.

***In vivo* Pharmacological Evaluation**
Acute oral toxicity study was performed by Sabu N S, *et al*. (2015) [8] using female Swiss Albino mice (20-25g weight). The toxicity study was carried out as per the guidelines set by OECD 423 and animals were observed for mortality and behavioral changes and found that at 2000 mg/kg, the extract neither produced mortality nor the signs of morbidity. Hence, the dose 400 mg/kg (1/5th of 2000 mg/kg) was selected for further studies.
The mouse procedure is, more rapid and less costly than that with rats, mouse being smaller animals easy to handle and thus more suitable for the primary screening of antidepressant drugs. The Swiss albino mice are grouped into five groups, each containing 6 mice. Mice exposed to UCMS received p. o vehicle, fluoxetine, test extract administered on day 7 and were stopped the day after the end of UCMS (day 28). The sucrose preference was measured before stress administration and every week during the treatment period. Behavioral test was evaluated after 21 days treatment.

Sucrose preference test (SPT)
Anhedonia was measured by preference for a sucrose solution over water [9], using a two bottle free choice method, each animal was presented simultaneously with two bottles, one containing 1% sucrose solution (w/v), the other containing tap water. Blunted sucrose intake in this test is proposed to reflect impaired sensitivity to reward and model anhedonia, a core symptom of depression. Fluid intake was monitored for 1 h, both bottles were removed and measured after 1 h. Mice were denied food and water for about 20 h before each sucrose preference test, were then conducted weekly throughout the UCMS period. Sucrose preference (%) = (sucrose consumption in ml ×100) ÷ (sucrose consumption in ml +water consumption in ml)

Locomotor activity test (LA)
The locomotor activity test was carried on the subsequent day after the last sucrose preference test. The locomotor activity test [10] is used to measure spontaneous activity in rodents. The actophotometer is a closed chamber. Interruption of beams by the locomotion of animal was recorded using the sensors which were connected to a counter. A single mouse was placed in the center of the box for 30 s adaptation, and then allowed to freely explore the area for 5 minutes. The action of extract on spontaneous locomotor activity was measured automatically using Actophotometer during the 5 minutes. After each test, the floor was cleaned thoroughly with alcohol solution to eliminate possible bias due to odors left by previous mice.5% Gum acacia was positive control and UCMS + 5% Gum acacia was negative control.

Forced swim test (FST)

A depressed state can be induced in mice by forcing them to swim in a narrow cylinder from which they cannot escape. After a brief period of vigorous activity the mice adopt a characteristic immobile posture, reflects a state of behavioral despair or helplessness which is readily identifiable. The forced swim test [11] was performed following the locomotor activity test. Each mouse was placed individually in a container filled with water maintained at 25°C ±3°C. Mice were forced to swim in the water for 6 minutes. The duration of immobility during the final 5 minutes of the test was recorded. Mice were dried immediately and returned to their home cages after the swimming test. 5% Gum acacia was positive control and UCMS + 5% Gum acacia was negative control.

Tail suspension test (TST)

A test procedure for antidepressants was in which a mouse is suspended by the tail from a Lever, the movements of the animal being recorded. The total duration of the test (6 min) can be divided into periods of agitation and immobility interpreted as behaviour al despair or depression-like behaviour. The tail suspension test [11] was performed following FST. In the test, mice were individually suspended 50cm above the floor by placing adhesive tape about 1cm from the tip of the tail. Each mouse was suspended for 6min and the total immobility time was measured during the last 4min.Mice were considered immobile only when they passively suspended and remained completely motionless.

Statistical analysis

The statistical analysis of all the pharmacological analysis was carried out using Graph pad instant software version 5 for windows. The values are represented as mean± Standard Error Means (SEM) for six mice data were analyzed by One Way ANOVA using Dunnett's Multiple comparison test.

Results and Discussion

Ethanolic extract of leaves yielded 7.7% and was dark green and sticky semisolid.

***In Vitro* Analysis by Cell Line Study**

Cytotoxicity Assay by MTT Method

MTT Assay of EAESK

Different concentrations of EAESk was compared with control and was analysed by One-way analysis of variance and Dunnett's Multiple Comparison Test (Table 1)

Neuro Protective effect of EAESk at concentrations 6.25, 12.5, 25, 50 and 100 µg/ml on IMR32 (Neuro blastoma cells) were determined by MTT assay. The results showed that percentages of cell viability significantly increased when treated with a concentration of 12.5 µg/ml EAESk was particularly effective when compared with other treated concentrations.

***In Vitro* Ros Measurement Using Dcfda**

The intracellular reactive oxygen species production in H_2O_2 induced cells and the effect of EAESk in the production of ROS was confirmed, visualized (Figure 1) and expressed in terms of fluorescence intensity by staining the cells with DCFDA (Table 2). In this study H_2O_2 induced cells showed maximum fluorescence (8438.87 AU) and those treated with EAESk was found to show reduced fluorescence (3612.44 AU) which indicated reduction in ROS production in respective cells. The decrease in ROS generation in cells treated with EAESk shows that it can be neuroprotective and increased cell viability.

In vitro studies showed that ethanolic extract of *Sarcostigma kleinii* has significant antidepressant activity by reversing increased oxidative stress. Notably, a number of clinical trials revealed that treatment with antidepressants can reverse increased oxidative stress in individuals with MDD.

***In vivo* Pharmacological Evaluation**

Sucrose preference test

The change in sucrose preference in all the groups was measured weekly The results showed that(Table 3) the sucrose preference in UCMS exposed mice decreased remarkably than the positive control group (5%gum acacia).The standard treatment with fluoxetine (20mg/kg/p.o)shows extremely significant increase in the sucrose preference when compared with negative control. There is a dose dependent significant increase was found in the test groups EAESk (200mg/kg/p.o) and (400mg/kg/p.o) when compared with negative control.

Locomotor Activity

The average basal activity scores in the positive control group, negative control group, standard group,and test groups (200mg/kg and 400mg/kg/p. o.) were measured. The treatment with fluoxetine ($p<0.01$) and EAESk 400mg/kg/p. o ($p<0.01$) significantly increased the locomotor activity. (Table 4) It may be due to the antidepressant property of the extract.

Forced swim test

The immobility time of UCMS exposed mice in the forced swim test increased significantly than the positive control (5% gum acacia/p. o) group (Table 5).In this test animals are treated with two doses of EAESk (400 and 200 mg/kg/p. o) showed decreases in their total immobility time. The total immobility time decrease of EAESk 400mg/kg/p. o was significant ($p<0.01$) than EAESk 200mg/kg/p. o when compared with negative control (UCMS+5% gum acacia/p. o).

Tail suspension test

The immobility time of UCMS exposed mice in the tail suspension test The The immobility time of UCMS exposed mice in the tail suspension test increased significantly than the positive control (5% gum acacia/p. o) group (Table 6)..In this test animals are treated with two doses of EAESk (400 and 200 mg/kg/p.o)showed decreases in their total immobility time. The total immobility time decrease of EAESk 400mg/kg/p.o was significant ($p<0.01$) than EAESk 200mg/kg/p.o (p, 0.05) when compared with negative control (UCMS+5% gum acacia/p.o).

In vivo antidepressant study explored the antidepressant potential of ethyl alcohol extract of *Sarcostigma kleinii* by SPT, LA, TST and FST models of depression. Pharmacological screening data supported antidepressant potential of ethyl alcohol extract of *Sarcostigma kleinii*.

Table 1: MTT Assay of EAESk

	Average Absorbance @ 540nm	Percentage Viability
Control(IMR32 cells)	0.9939	100.00
H_2O_2	0.5538	55.72
Sample-EAESk Concentration (µg/ml)		
6.25	0.8088	81.38
12.5	0.8551	86.04
25	0.7707	77.54
50	0.6782	68.23
100	0.6684	67.25

Table 2: Fluorescence Intensity

Sample code	Fluorescence Intensity (AU)
Control(IMR-32 cells)	1761.61
H_2O_2	8438.87
EAESk	3612.44

Table 3:. Effect of EAESk (200 and 400mg/kg) on Sucrose preference test

Unpredictable chronic mild stress 0 week	Positive control (5% gum acacia/p.o)	Negative control(UCMS+5% gum acacia/p.o)	Fluoxetine (20mg/kg/po)	EAESk (400mg/kg/po)	EAESk (200mg/kg/po)
	Volume in ml	Volume in ml	Volume in ml	Volume in ml	Volume in ml
1	85.09 ± 7.54 **	62.92 ± 7.09	83.18 ± 3.38 *	74.53 ± 3.09	69.99 ± 4.30
2	83.62 ± 7.42 *	62.66 ± 7.06	82.18 ± 3.48 *	73.95 ± 3.24	69.03 ± 4.35
3	84.87 ± 7.76 **	62.68 ± 7.25	82.84 ± 3.26 *	74.47 ± 3.21	69.64 ± 4.82
4	85.06 ± 7.65 **	62.56 ± 6.99	82.21 ± 3.09 *	73.91 ± 3.06	68.89 ± 4.48
5	83.26 ± 7.30 *	62.90 ± 7.21	82.81 ± 3.44 *	74.77 ± 3.45	69.53 ± 4.55
6	85.29 ± 7.58 *	63.77 ± 6.96	82.36 ± 3.20 *	74.94 ± 3.50	69.75 ± 5.05

Table 4: Effect of EAESk and fluoxetine treatment in locomotor activity

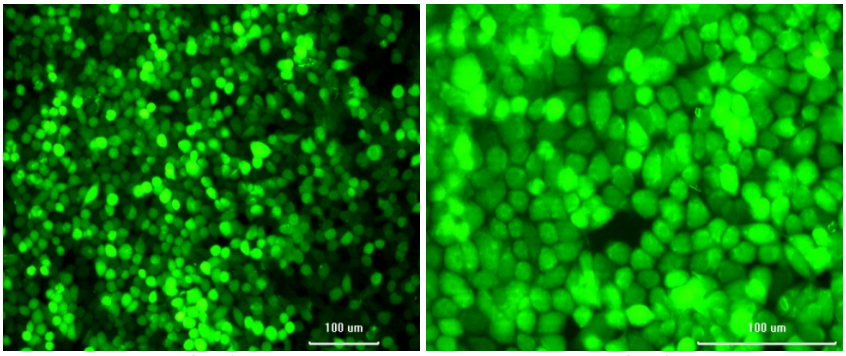
Treatment groups	Score of locomotor activity in sec
Positive control(5% gum acacia/p.o)	607.23 ± 1.77 **
Negative control(UCMS+5% gum acacia/p.o)	210.83 ± 2.31
Fluoxetine (20 mg)	546.34 ± 3.77 **
EAESk (400 mg/kg)	459.99 ± 4.38 **
EAESk (200 mg/kg)	274.31 ± 4.37 **

Table 5: Effect of EAESk and fluoxetine in forced swim test

Treatmentgroups	Immobility time (Sec)
Positive control(5% gum acacia/p.o)	61.58 ± 1.10 **
Negative control(UCMS+5% gum acacia/p.o)	148.47 ± 1.49
Fluoxetine (20 mg/kg)	104.63 ± 1.17 **
EAESk (400 mg/kg)	119.03 ± 0.80 **
EAESk (200 mg/kg)	129.88 ± 1.01 **

Table 6: Effect of EAESk and fluoxetine in tail suspension test

Treatment groups	Immobility time(Sec)
Positive control(5% gum acacia/p.o)	74.76± 1.64 **
Negative control(UCMS+5% gum acacia/p.o)	156.33 ± 1.82
Fluoxetine (20 mg)	107.15 ± 1.38 **
EAESk (400 mg/kg)	118.77 ± 1.45 **
EAESk (200 mg/kg)	130.58 ± 1.04 **



H_2O_2 10X H_2O_2 20X

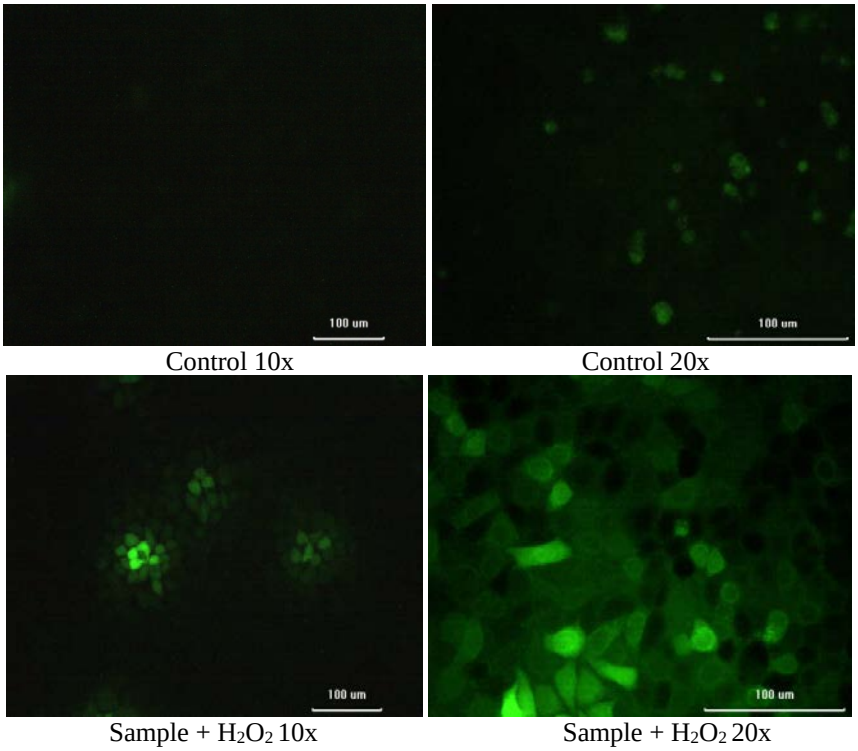


Fig 1: Images for *Invitro* ros measurement using DCFDA

Conclusion

With these preclinical results it can be concluded that Ethyl Alcoholic Extract of *Sarcostigma kleinii* is effective in the treatment of major depressive disorder. The results of *in vitro* screening shows that percentage of cell viability significantly increased when treated with extract. The present study also exhibits anti-idepressant potential of ethyl alcohol extract of *Sarcostigma kleinii* in depression like symptoms in Mice exposed to unpredictable chronic mild stress (UCMS).

Conflicts of Interest

No conflicts of interest

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Data Availability

Not available

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Referances

1. Gholipour LB, Writer S. Depression: Causes, Symptoms and Treatments. Livescience, 2017.
2. KD Tripathi. Essentials of Pharmacology 7thed. New Delhi: jaypee Brothers medical publishers (p) limited, 2013, 454.
3. Indiabiodiversity.org/species/show/248959IndiaBiodiversity Portal. By Dr. NSaidharan (Dr. BPPalFellow), Kerala Forest Research Institute, Peechi. warrier PK, Nambiar VPK, Ramankutty C, Vasudevan Nair R. Indian medicinal plants: a compendium of 500 species, Hyderabad: Orient Longman,1996:5:76-79.
4. Arunachalam K, Parimelazhagan T, Saravanan S. Phenolic content and antioxidant potential of *Sarcostigma kleinii* Wight. & Arn. Journal Food and Agricultural Immunology,2011:(2):161-170.
5. Ramachandran R, Saraswathy M. Up-regulation of nuclear related factor 2 (NRF2) and antioxidant responsive elements by metformin protects hepatocytes against the acetaminophen toxicity. toxicology Research,2014:(3):350.
6. Paul Held. Laboratory Manager, Applications Dept, Bio Tek Instruments, Inc. An Introduction to Reactive Oxygen Species Measurement of ROS in Cells White Paper Bio Tek Instruments, 2015.
7. Sabu NS, Jayachandran TP, P Sri Ganesan. Evaluation of anxiolytic activity of aerial part sof *sarcostigma kleinii* wight and arn.Asian Journal of pharmaceutical and clinical research,2015:18(2):412-416.
8. Belovicova K, Bogi E, Csatlosova K, Dubovicky M. Animal tests for anxiety-like and depression-like behavior in rats. Interdisciplinary Toxicology,2017:10(1):40-43.
9. Clarke RL, Smith RF, Justesen DR. Methods & Designs.An infrared device for detecting locomotor activity.Behavior Research Methods, Instruments, &Computers,1985:17(5):519-525.
10. Porsolt RD, Bertin A, Jalfre M. Behavioral despair in mice: a primary screening test for antidepressants. Arch Int Pharmacodyn Ther,1977:229(2):327-336.