



Journal of Pharmaceutical and Biomedical Analysis Letters

Journal Home Page: www.pharmaresearchlibrary.com/jpbmal



Research Article

Open Access

Anti-Depressant Activity of *Alstonia Venenata* R.Br Stem Bark Extracts in In-vivo Methods

C. Pradeep Kumar*, T. Sravanthi, Dr. Vijaya Kuchana, CH. Maheswara Reddy

Teegala Krishna Reddy College of Pharmacy, Hyderabad, Telangana, India

ABSTRACT

The ethanolic extract of *Alstonia venenata* R.Br stem bark was subjected for phytochemical investigation and LD₅₀ studies. It was found that ethanolic extract contained reducing sugars, proteins, Aminoacids, flavonoids, phenolic compounds and tannins. Phenolic compounds and flavonoids are responsible for anti-depressant activities. The extract was tested for their lethal effect up to the dose level of 1000mg/kg. No mortality was observed in mice. The administration of extract at doses of 50mg/kg, 100mg/kg, by oral administration, produced a significant ($P < 0.05-0.01$) anti-depressant effect in mice by Tail Suspension Test and Forced Swim Test of 50mg/kg by oral administration, significantly ($P < 0.05-0.01$) produce. Further studies are necessary to confirm and extend the results.

Keywords: *Alstonia venenata* R.Br stem bark, soxhlet apparatus, Mice, Tail Suspension Test and Forced Swim Test.

ARTICLE INFO

CONTENTS

1. Introduction	218
2. Materials and Methods	219
3. Results and discussion	221
4. Conclusion	222
5. References	222

Article History: Received 10 September 2014, Accepted 15 December 2014, Available Online 18 January 2015

*Corresponding Author

C. Pradeep Kumar
Teegala Krishna Reddy College of
Pharmacy, Hyderabad, Telangana, India
Manuscript ID: JPBMAL2337



PAPER-QR CODE

Citation: C. Pradeep Kumar, et al. Anti-Depressant Activity of *Alstonia Venenata* R.Br Stem Bark Extracts in In-vivo Methods. *J. Pharm, Biomed. A. Lett.*, 2015, 3(1): 218-223.

Copyright © 2015 C. Pradeep Kumar, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution and reproduction in any medium, provided the original work is properly cited.

1. Introduction

Depression is considered as an affective disorder characterized by change in mood. It is a severe psychiatric [1] or mental illness characterized by excessive sadness.

Depression is mainly two types. i) Major Depression: It is diagnosed when five of at least nine symptoms are present most of the day nearly every day [2] for at least two weeks. ii) Bipolar or manic depression: In mania the mood is elated or irritable over activity is usually unproductive.

Pathophysiology: It is associated with low levels of Nor-adrenalin and 5-Hydroxy tryptamine physiologically; large [2] amounts of these neurotransmitters liberated at the nerve

Anti-Depressants:

Classification:

- A. Reversible inhibitors of MAO-A (RIMAs):
Meclobemide, Clorgyline.
- B. Tricyclic antidepressants (TCAs):²
 - a. NA^+ , 5-HT Reuptake inhibitors:
imipramine, Doxepin, Dothiepin, Clomipramine.
 - b. Predominantly NA Reuptake inhibitors:
Desipramine, Nortriptyline, Amoxapine, Clomipramine.
- C. Selective serotonin reuptake inhibitors (SSRIs):
Fluoxetine, fluvoxamine, Paroxetine, Sertraline, Citalopram, Escitalopram.

2. Materials and Methods

Family: Apocynaceae

The plant material was collected from Tirupati.

Chemical constituents: Plant is a rich source of indole alkaloids. Major alkaloids in the stem bark are alstovenine, venenatine, 3-dehydroalstovenine, reserpine (0.003-0.3%), venoxidine and kopsinine.

Therapeutic uses: Analivegam is a medicinal plant commonly used for snake bites by the traditional and tribal physicians of India. The root of Analivegam is used for the treatment of skin diseases, leprosy and other venomous bites.

Preparation of extract: *Alstonia venenata* R.Br stem bark was shade dried and made into coarse powdered which was passed through a # 40 mesh sieve to get uniform particle size and was extracted using Petroleum ether, and ethanol by continuous hot percolation process using soxhlet apparatus.

Soxhlet extraction: It is an automatic, continuous process; The extraction time is less than 24 hours for a 500gm sample. The disadvantage is that some compounds like carotenoids may decompose due to high temperature during heating.

Petroleum ether extraction process:

Drug: Menstrum ratio : 150gms: 700ml
Number of cycles : 13 cycles
Percentage yield : 7.6

Drug preparation:

The drug was prepared by dissolving the extract in 2% W/V Tween 80.

Test for reducing sugars:

Fehling's test:

1ml Fehling's A and 1ml Fehling's B solutions was mixed and boiled for one minute. Add equal volume of test solution. Heated in boiling water bath for 5 minute. First yellow, then brick red precipitate was observed, indicates presence of reducing sugars.

Benedict's test:

Equal volume of Benedict's reagent and test solution in test tube were mixed. Heated in boiling water bath for 5

minutes. A red color solution was observed, indicates presence of reducing sugars.

- D. Atypical anti-depressants: Trazodone, Mianserin, Mirtazapine, Amineptine, Bupropion, Duloxetine.

Tricyclic Antidepressants: Pharmacological actions of CNS, ANS, CVS

Mechanism of TCAs:

TCAs inhibit the reuptake of monoamines (5-HT and NA) into respective neurons. This causes an increase [2] in the monoamine concentration in the synaptic gap in the CNS and periphery leading to their enhanced neurotransmission. Thus the inhibition⁴ of this 5-HT and NA reuptake produces anti-depressant effects. They also block adrenergic, H_1 and muscarinic receptors leading to adverse effects.



Figure 1: Plant Name: *Alstonia venenata* R.Br.

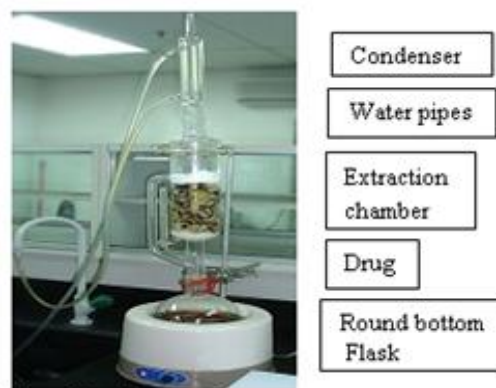


Figure 2: Soxhlet apparatus

Ethanol extraction process:

Drug: Menstrum ratio : 150gms:700ml.
Number of cycles : 14 cycles.
Percentage yield : 6.5

minutes. A red color solution was observed, indicates presence of reducing sugars.

Test for proteins:

Biuret test (General test):

To 3ml test solution add 4% NaOH and few drops of 1% CuSO_4 solution. Slightly violet color was observed, indicates slightly presence of proteins.

Test for amino acids:

a) Test for ninhydrin:

Heat 3ml of test solution and 3 drops of 5% ninhydrin solution in boiling water bath for 10min. Purple colour was observed.

b) Test for Cysteine:

To 5ml test solution add few drops of 40%NaOH and 10% lead acetate solution. Boil the solution black ppt of lead sulphate is not formed.

Test for cardiac glycosides:

Legal's test (test for cardenoloides):

To extract, add 1ml pyridine and 1ml of sodium nitroprusside. Red color was not observed, indicates absence of cardiac glycosides.

Test for flavonoids:

Lead acetate test: 2ml extract were treated with few drops of 5% lead acetate solution. A yellow precipitate was not observed, indicates absence of flavonoids. Heat test solution with zinc and HCL, pink to red colour was not observed.

Shinoda test: To dry powder or extract, add 5ml of 95% ethanol, few drops of conc.HCL and 0.5gms of magnesium turnings. Orange colour was observed, indicates presence of flavonoids.

Test for tannins and phenols:

- To 2ml of extract were treated with few drops 5%FeCl₃ solution. A deep blue – black color was observed, indicates presence of phenols.
- To 2ml of extract were treated with few drops lead acetate solution. A white precipitate was observed, indicates presence of tannins.
- To 2ml of extract were treated with few drops dilute iodine solution transient red colour was observed, indicates presence of tannins.
- To 2ml of extract were treated with few drops potassium dichromate solution red colour ppt was observed, indicates presence of tannins.

Test for starch:

Tannic acid test: Mix 3ml test solution and few drops of tannic acid solution gives buff colour ppt, indicates presences of starch.

Test for alkaloids: The extract were treated with dilute HCL and filtered. The filtrate was treated with various alkaloidal agents.

Dragendorff's test: 2-3ml filtrate was treated with Dragendorff's reagent, orange brown precipitate was observed, indicates presence of alkaloids.

Hager's test: 2-3ml filtrate was treated with Hager's reagent, yellow precipitate was observed, indicates presence of alkaloids.

Wagner's test: 2-3ml filtrate was treated with Wagner's reagent, reddish brown precipitate was observed, indicates presence of alkaloids.

Test for saponin glycosides.

Foam test: Shake the drug extract vigorously with water. Foam was not observed, indicates absence of saponin glycosides.

Test for steroid:

Salkowski reaction:

To 2ml of extract, added 2ml chloroform and 2ml con.H₂SO₄. Shake well. Chloroform layer was not observed indicates absence of steroid.

Pharmacological Screening (In-vivo Methods):

Acute toxicity study of *Alstonia venenata* R.Br stem bark extract:

Acute toxicity study was performed for ethanolic extract of roots of *Alstonia venenata* R.Br. According to BLIND Screening (IRWIN 1959) Female mice selected by random sampling technique were employed in this study. Female mice were used because literature surveys of conventional LD₅₀ tests show that females are generally slightly more sensitive for the study.

The animals were fasted prior to dosing. Ethanolic extract of stem bark of *Alstonia venenata* R.Br. was administered orally to different groups at the dose levels of 5, 50, 300, and 1000 mg/kg body weight. The animals were observed the behavioural, neurological and autonomic effects studies 24hrs for mortality with special attention during first 3hr and intermittently for 14 days.

During acute toxicity study, no mortality was observed in animals treated with ethanolic extract of stem bark of *Alstonia venenata* R.Br. up to a high dose of 1,000mg/kg body weight.

Pharmacological screening of *Alstonia venenata* R.Br stem bark extract for Antidepressant Activity:

Grouping of animals:

Adult swiss albino mice were divided in to four groups, either of sex.

Control group (6)

Standard group [6]

Test group -1 [6]

Test group- 2 [6]

Total number animals required 24.

Weight of animals between 20 to 30 Gms.

Doses for each group

Control group: 2%W/V Tween 80 10ml/kg body weight. p. o. Route.

Standard group: Fluoxetine 25mg/kg body weight. P.o. Route.

Test group 1 : ethanolic extract of *Alstonia venenata* R.Br. stem Bark 50mg/kg body weight. p. o. route.

Test group 2 : ethanolic extract of *Alstonia venenata* R.Br. stem Bark 100mg/kg body weight. p. o. Route.

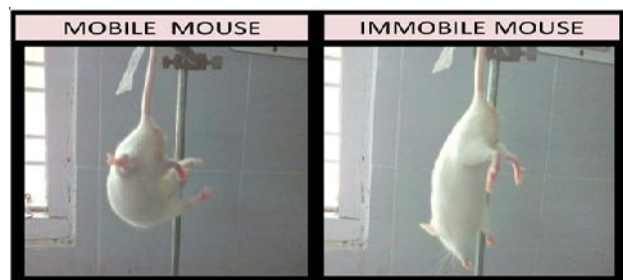
Tail suspension test method:

Procedure: [17]

The method originally described by Steruet et al (14). The total duration of immobility induced by tail suspension was measured according to the method. Mice were divided into 4 groups and 6 animals in each group.

Mice were visually isolated were suspended 58cm above the floor by adhesive tape placed approximately 1-2cm from the tip of the tail. Immobility time was manually recorded during a 5 min period. Mice were considered immobile only when they hung passively or stayed completely motionless.

Conventional antidepressants decrease the immobility time in the test. The animals were treated with the extracts (50,100), fluoxetine (25mg/kg) vehicle, and 45min before the test.

**Figure 3:** Tail suspension test**Forced swim test:****Procedure:** [17]

The FST is the most widely used pharmacological model for assessing antidepressant activity. This method is based on the observation of animals exposed to a situation of forced swimming, in which they become passive and immobile after a period of vigorous activity, producing only

the movements required to keep their heads above the water. The FST was carried out on mice according to the method of Porsolt et al., Swimming sessions were conducted by placing the animals in individual plexiglass cylinders (40cm high, 20cm diameter) containing 20cm of water at $24 \pm 1^\circ\text{C}$. All animals were forced to swim for 6 min, and the time spent in immobility during the last 5min of a 6min observation period was recorded manually by the competent observer. The animals were treated with the extracts (50,100 mg/kg, p.o), fluoxetine (25mg/kg, p.o) or vehicle, 45 min before the test.

Statistical analysis: [16,17,18]

Statistical analysis was carried out using Prism graph pad 6 software. All results were expressed as Mean \pm S.E.M. The statistical analysis of all the results was done using one way analysis of variance (ANOVA) followed by Dunnett's test.

3. Results and Discussion

Preliminary phytochemical analysis of Stem bark of *Alstonia venenata* R.Br extract:

The revealed results of the preliminary phyto chemical analysis of Stem bark of *Alstonia venenata* R.Br extract results were shown below.

Table 1: Preliminary phytochemical analysis

S. No	Compound	Test	Ethanollic extract
1	Reducing agents	Fehling's & Benedicts	+
2	Test for proteins	Biuret	+
3	Test for Amino acids	Ninhydrin	+
4	Test for Amino acids	Cysteine	—
5	Test for Cardiac glycosides	Legal	—
6	Test for flavanoids	Shinoda	+
7	Test for flavanoids	Lead acetate	+
8	Test for Tannins	Lead acetate & 5% FeCl ₃	+
9	Test for Starch	Tannic acid	+
10	Test for Alkaloids	Dragendorff's & Hager's & Wagner's	+
11	Test for Saponin Glycosides	Foam	—
12	Test for Steroids	Salkowski	—

Tail Suspension Test:

Ethanollic extract of stem bark of *Alstonia venenata* R.Br significantly ($P < 0.05$, $P < 0.01$) decrease the immobility time as compared to standard group.

Table 2: Effect of ethanollic extract of stem bark of *Alstonia venenata* R.Br on Tail Suspension Test

Group no.	Drug treatment	Dose	Immobility period, mean \pm S.E.M (n=6) sec
sI	Control (p.o)	0.2% w/v Tween 80 solution	166 \pm 5.2
II	Test (p.o)	50mg/kg	123 \pm 2.9 ***
III	Test (p.o)	100mg/kg	105 \pm 3.5 ***
IV	Standard (p.o)	25mg/kg	133 \pm 2.3***

n=6. The observations are mean \pm SEM. * $P < 0.05$, *** $P < 0.0001$, as compare to control. (ANOVA followed by Dunnett's test). Test = ethanollic extract of stem bark of *Alstonia venenata* R.Br.

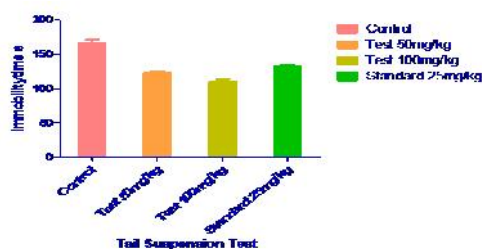


Figure 4: Effect of ethanolic extract of stem bark of *Alstonia venenata* R.Br on Tail Suspension Test

Table 3: Effect of ethanolic extract of stem bark of *Alstonia venenata* R.Br on Tail Suspension Test

Group no.	Drug treatment	Dose	Immobility period, mean± S.E.M (n=6) sec
I	Control (p.o)	0.2% w/v Tween 80 solution	179±3.9
II	Test (p.o)	50mg/kg	137±4.1 ***
III	Test (p.o)	100mg/kg	120±4.0***
IV	Standard (p.o)	25mg/kg	150±5.3 ***

n=6. The observations are mean ± SEM. * $P < 0.05$, *** $P < 0.0001$, as compare to control. (ANOVA followed by Dunnett's test). Test = ethanolic extract of stem bark of *Alstonia venenata* R.Br.

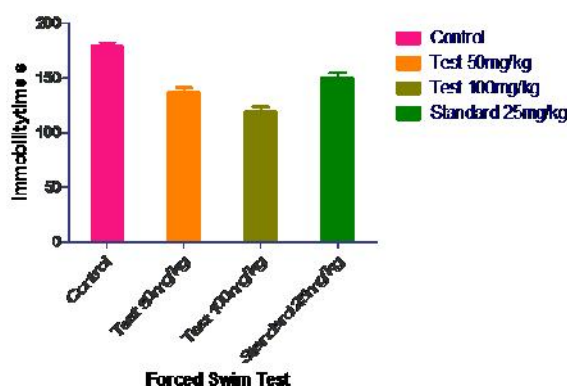


Figure 5: Effect of ethanolic extract of stem bark of *Alstonia venenata* R.Br on Forced Swim Test

n=6. The observations are mean ± SEM. * $P < 0.05$, *** $P < 0.0001$, as compare to control. (ANOVA followed by Dunnett's test). Test = ethanolic extract of stem bark of *Alstonia venenata* R.Br.

4. Conclusion

The ethanolic extract of *Alstonia venenata* R.Br stem bark was subjected for phytochemical investigation and LD₅₀ studies. It was found that ethanolic extract contained reducing sugars, proteins. Aminoacids, flavonoids, phenolic compounds and tannins. Phenolic compounds and flavonoids are responsible for anti-depressant activities. The extract was tested for their lethal effect up to the dose level of 1000mg/kg. No mortality was observed in mice. The

administration of extract at doses of 50mg/kg, 100mg/kg, by oral administration, produced a significant ($P < 0.05$ - 0.01) anti-depressant effect in mice by Tail Suspension Test and Forced Swim Test of 50mg/kg by oral administration. It is concluded that, the ethanolic extract of stem bark of *Alstonia venenata* R.Br. Possess anti-depressant activities. It is not possible to elucidate the action mechanism through which *Alstonia venenata* R.Br. exerts its effects. Further studies are necessary to confirm and extend the results.

5. References

1. Satoskar, "Pharmacology and therapeutics", twentieth edition, pp. 141- 180 and 609-623.
2. K.D. Tripathi, "Essentials of medical pharmacology" 6th edition, pp 453- 468 and 609 - 648.
3. Lippincott Williams & Wilkins. Review of Pharmacology" 3rd Edition, pp. 158- 168 and 323 -329
4. F.S.K. Barar, Essentials of pharmacotherapeutics", Fourth edition, pp. 105- 130 and 534-538.
5. C.P. Khare, Springer reference, Indian medicinal plants, first edition, 2007, pp.39.

6. TURNER book of screening methods volume-II pp.22-33.
7. S.K.Kulkarni, Hand book of experimental pharmacology, 3rd edition, **1999**, pp. 122- 135.
8. Dr. S. Sardana, Dr O.P. Sharma, Fundamentals of Pharmacognosy. First edition, **2009-2010**, pp. 110-117.
9. Sutha S, Kalpana Devi V and Mohan VR, GC-MS Determination of Bioactive Components of *Alstonia venenata* R.Br. Research Journal of Pharmaceutical, Biological and Chemical Sciences ISSN 0975-8585. June **2012**, pp.291-296.
10. Williams Scott J. and Thankamani Formulation of a Natural Medium for the Induction of Callus and their bioactivity in *Alstonia Venenata* R. Br, Research journal of Biotechnology, vol-2, May **2007**.
11. Moin A. Khan and M. Badruzzaman Siddiqui Size variation in the vascular cambium and its derivatives in two *Alstonia* species, Nov **2006**, pp. 531-538.
12. Steru L, Chermat R, Thierry B and Simon P. The tail suspension test: A new method for screening antidepressants in mice. *Psychopharmacology* **1985**, 85: 367-370.
13. Porsolt RD, Le Pichon M and Jalfre M. Depression: a new animal model sensitive to antidepressant treatments. *Nature*, **1977**, 266: 730-732.
14. Fisher RS. Animal models of the epilepsies. *Brain Res. Rev.*, **1989**, 14: 245-278.
15. Porsolt R D, Bertin A, Jalfre M. Behavioural despair in mice: a primary screening test for antidepressants. *Archives Internationales de Pharmacodynamie et Therapie*, **1977**, 229: 327–336.
16. Dinesh kumar, Jitender singh, Anupama baghotia, Sunil kumar, Anticonvulsant effect of the ethanol extract of *Caesalpinia pulcherrima*(L) SW, Fabaceae leaves. *Brazilian journal of pharmacognosy*, pp 1410-1411.
17. Ambawade SD, Kasture VS, Kasture SB. Anticonvulsant activity of roots and rhizomes of *Glycyrrhiza glabra*. *Indian J Pharmacol*, **2002**, 34: 251–55.
18. Sayyah M, Mandgary A. Anticonvulsant effect of *Ferula gummosa* root extract against experimental seizures. *Iran Biomed J*, **2003**, 7(3): 139 – 43