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Observation on Depression Relieving Properties of Hydro Alcoholic Extracts of Aniba Riparia in Experimental Mice

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A B S T R A C T
In the present study, *Aniba riparia* significantly increased the frequency of 5-HTP induced head twitches, Clonidine induced aggression and L-DOPA induced hyperactivity and aggressive behavior indicating its enhanced activity on serotonergic, noradrenergic and dopaminergic pathways respectively. Our results also confirm the involvement of serotonergic, noradrenergic and dopaminergic pathways in depression. Pretreatment with *Aniba riparia*. Results from behavioral experiments indicate that the antidepressant activity of *Aniba riparia*, might be due to the facilitatory effect on serotonergic, noradrenergic and dopaminergic systems apart from the antioxidant activity. The results from the present study confirm the antidepressant activity of *Aniba riparia*, since it reduced the immobility in both FST and TST.
Key Words: *Aniba riparia*, antidepressant activity

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C O N T E N T S

1. Introduction

Recently, the notice to the herbal medicine research and the medicinal plant effects on human health to treat different neurological diseases like depression has increased. Using of medicinal plants had been coming from early times. As a rule, they are used to control mental problems and the soothing agents, antidepressant effects, anticonvulsants anxiolytic and others. Depression has become a common psychological illness in recent years. According to an investigation by the World Health Organization International Consortium of Psychiatric Epidemiology (WHO-ICPE), 6.3−15.7% of the world's population has been estimated to get depression once in their life. The studies were shown that depression will be the second important disease after cardiovascular disease in the world by the year 2020. Although a wide variety of antidepressant drugs are available to treat depression, most of the synthetic

drugs are not without side effects. Therefore, the search for regularly eaten foods with an antidepressant activity seems to be an essential approach to finding an effective antidepressant treatment without side effects. Recently, new research indicated that using of medicinal plants has increased in psychiatry. In Iranian and other traditional medicines, an antidepressant effect has been indicated for some medicinal plants. The oil obtained from A. riparia wood was not predominantly composed of terpenoids, but a type of lipid component.

The major component identified, γ-palmitolactone, in the essential oil of Aniba riparia wood has been evaluated and applied in cosmetics. This compound, also known as γ-hexadecalactone, is used as fragrance component. Although the total content of components of A. riparia oil is not very similar to the composition of Aniba essential oils previously reported, several of them are common. For example, benzyl benzoate (1.3%) is also present in A. riparia and A. riparia essential oils. This compound is considered a characteristic feature of the genus Aniba, and is commercially used as a topical medication against several parasitoses.

Although a minor component of this species (0.9%), the monoterpene linalool, usually present in high proportion in rosewood A. rosaeodora, has anti-inflammatory, antioxidant, and inhibitory activities, as well as bactericidal effects the t-cadinol, a common component in the oil of A. hostmanniana, has shown bactericidal effect on Staphylococcus aureus; the δ-cadinene, also a main component of A. hostmanniana, is anti-inflammatory and sedative. The chamazulene possesses anti-inflammatory and antioxidant activity.

The 1-nitro-2-phenylethane, a vasorelaxant major component in Aniba canelilla, was not found in A. Riparia. The causes of depression are decreased brain levels of monoamines like noradrenaline, dopamine and serotonin. Therefore, drugs restoring the reduced levels of these monoamines in the brain either by inhibiting monoamine oxidase or by inhibiting reuptake of these neurotransmitters might be fruitful in the treatment of depression.

Aim of the present study was to evaluate the antidepressant activity of aniba riparia in experimental models of depression using mice. They are shrubs or trees up to 25 m high, hermaphrodites. The leaves are alternate, entire, and elliptical or narrowly elliptical.

The inflorescences are paniculate and axillary, the flowers are arranged in cymes essentially, and those strictly opposite side are small. The fruit is a berry-like drupe dispersed mostly by birds. Fruits are 3 cm long and 1.5 cm wide, with deep domes, and warty. Many species have a valuable timber in yellow wood, others have the wood and bark pleasantly scented. The oils extracted from certain species are used as ingredients in the manufacture of perfumes.

2. Materials and Methods

Materials: Reduced nicotinamide adenine dinucleotide (NADH), Glutathione reduced are bought from Sisco Research Laboratories Pvt. Ltd, Mumbai, India. Hydrogen peroxide, Ethanol, 2,4-dinitro phenylhydrazine (DNPH), Dipotassium hydrogen phosphate, Potassium dihydrogen phosphate are bought from Merck, Mumbai, India. Azathioprine is bought from RPG Life sciences Pvt, Ltd, Hyd. Ascorbic acid is bought from Finar chemicals, Ahmedabad, India. Normal saline is bought from Claris life sciences. Ltd., Ahmedabad, India.

Methods

Collection and Authentification of Plant Material:

The fruits of *Aniba riparia* were collected from local market and authenticated by Dr.K.Madhava chetty department of botany, Sri Venkateshwara University, Tirupathy.

Extraction of Plant Material:

The fruits are grinded in to a coarse powder with the help of suitable grinder and sun dried.

Cold Extraction (hydro alcoholic Extraction):

In this work the cold extraction process was done with the help of water and alcohol. About 200gms of powdered material was taken in a clean, flat bottomed glass container and soaked in 750 ml of alcohol and 250ml of water. The container with its contents were sealed and kept for period of 7 days accompanied by continuous shaking with the shaker. The whole mixture then went under a coarse filtration by a piece of a clean, white cotton wool.

Evaporation of Solvent:

The filtrates (hydroalcoholic extract) obtained were evaporated using Rotary evaporator in a porcelain dish. They rendered a semi solid concentrate of creamy colour final product. The extract was kept in vaccum dissecator for 7 days.

Preliminary Phytochemical Screening:

Preliminary phytochemical screening of the *aniba riparia* extract was carried out for the analysis of Alkaloids, Carbohydrates, Tannins, Saponins, Steroids, Phenols, and Flavonoids as per the standard methods.

Animals:

Healthy Adult Male mice of 5 weeks old with Average weight in the range of 20-30gms were selected. Animals are housed 4 per cage in temperature controlled (27 ^{0}C ±3 ^{0}c) room with light/dark cycle in a ratio of 12:12 hrs is to be maintained. The Animals are allowed to acclimatize to the environment for seven days and are supplied with a standard diet and water *ad libitum*. The prior permission was sought from the Institutional Animal Ethics Committee (IAEC) for conducting the study.

Acute toxicity studies:

The Acute oral toxicity test of the extracts was determined prior to the experimentation on animals according to the OECD (Organization for Economic Co-operation and Development) guidelines no 423. Female Albino wistar mice (20-30 g) were taken for the study and dosed once with 2000 mg/kg of the extract. The treated animals were monitored for 14 days to observe general clinical signs and symptoms as well as mortality. No mortality was observed till the end of the study revealing the 2000 mg/kg dose to be safe. Thus, 1/5, 1/10 and 1/20 doses of 2000 mg/kg i.e. 100 mg/kg and 200 mg/kg 400mg/kg were chosen for

subsequent experimentation.

***In Vivo* Models of Depression Employed in the Study**

1. Forced swimming test (FST)
2. Tail suspension test (TST)
3. 5-HTP induced head twitches in mice
4. Clonidine-induced aggression in mice
5. L-DOPA-induced hyper activity and aggressive behavior in mice (LHA)

1. Forced swimming test (FST):

Procedure:

The procedure was described by Porsolt et al. (1978) was used. Swimming sessions were conducted by placing mice in individual glass cylinders (45 cm high×20 cm in diameter) containing (25±2 °C) water 38 cm deep so mice could not support themselves by touching the bottom with their feet Two swimming sessions were performed between 12:00 h and 19:00 h an initial 15 min pretest followed 24 h later by a 6 min test"

"Doses were given once daily for 7 days On the 7th day mice were subjected to 15 min pretest After 15 min in the water the mice were removed and allowed to dry in a heated enclosure (32 °C) before being returned to their home cages They were again placed in the cylinder 24 h later and the total duration of immobility was measured during a 6 min test Floating behavior during this 6 min period had been found to be reproducible in different groups of mice An animal was judged to be immobile whenever it remains floating passively in the water in a slightly hunched but upright position its nose just above the surface The total immobility time for the period of 6 min was recorded with the help of stopwatch"

2. Tail suspension test (TST):

"Doses are given once daily for 7 days On the 7th day 1hr after the administration of the test and standard drugs mice were suspended on the edge of a table 50 cm above the floor by the adhesive tape placed approximately 1 cm from the tip of the tail Immobility time was recorded during a 6 min period[29]Animal was considered to be immobile when it did not show any movement of body and hanged passively"

3. 5-HTP induced head twitches in mice:

"Doses were given once daily for 7 days On the 7th day 1hr after the administration of the test and standard drugs mice were treated with 5-HTP (100 mg/kg i.p.) and the numbers of head twitches performed by each mice was counted by staggering method using three 2 min periods (19–21 min) (23–25 min) (27– 29 min) after 5-HTP administration and number of head twitches were scored live by a blind observer"

4. Clonidine-induced aggression in mice:

"The method of Morpurgo (1968) was used Mice were divided into 5 groups of 8 each (n=8) each group contain 4 pairs of mice two pairs from each sex (each pair contained same sex of mice) Doses were given once daily for 7 days On the 7th day Clonidine was given 1 h after the administration of the test and standard drugs The animals were then caged in bell shaped glass jar with a floor area of approximate 16 cm^{2}The biting/fighting episodes were recorded live by a blind observer over a period of 30 min in each pair"

5. L-DOPA induced hyper activity and aggressive behavior in mice (LHA):

"Mice were treated with L-DOPA (100 mg/kg i.p.) and the experiment was performed according to the method of Mice were divided into 5 groups of 8 each (n=8) each group contain 4 pairs of mice two pairs from each sex (each pair contained same sex of mice). Doses were given once daily for 7 days On the 7th day L-DOPA was given 1 h after the administration of the test and standard drugs Stages of activity and aggressive behavior were recorded live every 10 min for 30 min after L-DOPA administration by the blind observer The different parameters of observation were piloerection, salivation increase in motor activity irritability reactivity jumping squeaking and aggressive fighting The scores were graded in the following manner"

"0-No effect 1-Piloerection slight salivation slight increase in motor activity 2-Piloerection salivation marked increase in motor activity and irritability 3-Piloerection profuse salivation marked increase in motor activity reactivity jumping squeaking and aggressive fighting"

Statistical Analysis:

Results were expressed as mean ± S.E.M Statistical analysis was performed using one-way analysis of variance (ANOVA) If the overall *P*-value was found statistically significant ($P < 0.05$).

3. Results and Discussion

% Yield value of hydro alcoholic Extract from fruits of HAEAR was found to be 06%

Phytochemical Screening Methods:

Hydro alcoholic extract of HAEAR had showed positive results for Alkaloids, Carbohydrates, tannins, Phytosterols, but negative results for saponins, Glycoside.

Acute toxicity studies:

As per (OECD) draft guidelines 423 Female albino mice were administered HAEAR and doses was be selected in the sequence (1.75- 5000) using the default dose progression factor, for the purpose of toxicity study. Animals are observed individually at least once during the first 30 minutes after dosing, periodically during the first 24 hours and daily thereafter, for a total of 14 days,. In all the cases, no death was observed within 14 days. Attention was also given to observation of tremors and convulsions, salivation, diarrhoea, lethargy, sleep and coma. Overall results suggested the LD_{50} value as 2000 mg/kg. Hence therapeutic dose was calculated as 1/10th and 1/20th i.e. 100mg/kg, 200 mg/kg and 400 mg/kg of the lethal dose for the purpose of antidiabetic investigations.

1. Forced Swim Test (FST):

The results (Table. 6) showed that both *HAEAR* (100, 200 and 400 mg/kg, p.o.) and imipramine (15 mg/kg, i.p.) significantly decreased the duration of immobility time in a dose dependent manner in FST model. Post-hoc analysis showed that the *HAEAR* (100, 200 and 400 mg/kg) and Imipramine (IMP) treated groups were significantly different ($p<0.001$) from the vehicle treated group (Fig. 1).

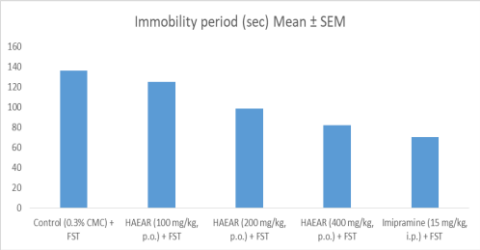

Fig 1: Effect of *HAEAR* (100, 200 and 400 mg/kg, p.o.) and Imipramine (IMP; 15 mg/kg) on forced swim test (FST) in mice. Each column represents mean ± S.E.M. of immobility period (sec), n = 6. *= p<0.001 compared to control

2. Tail Suspension Test (TST):

The results (Table. 7) showed that both *HAEAR* (100, 200, 400 mg/kg, p.o.) and imipramine (15 mg/kg, i.p.) significantly decreased the duration of immobility time in a dose dependent manner in TST model. Post-hoc analysis showed that the *HAEAR* (100, 200 and 400 mg/kg) and IMP treated groups were significantly different (p<0.001) from the vehicle treated group (Fig. 2).

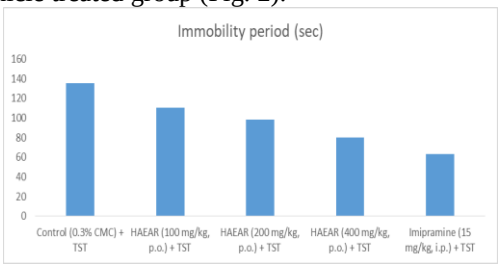

Fig 2: Effect of *HAEAR* (100, 200 and 400 mg/kg, p.o.) and Imipramine (IMP; 15 mg/kg) on tail suspension test (TST) in mice. Each column represents mean ± S.E.M. of immobility period (sec), n = 6. a = p<0.001 compared to control

5-HTP induced head twitches in mice:

Table.8. illustrates the effect of *HAEAR* and IMP on 5-HTP-induced head twitches in mice. Post-hoc analysis revealed that three doses of *HAEAR* (100, 200 and 400 mg/kg, p<0.01, p<0.001) significantly increased the 5-HTP-induced head twitches in comparison to control group. Further, the dose of 400 mg/kg was more effective than 100, 200 mg/kg. Similarly, IMP treated group showed significant increase (p<0.001) in the 5-HTP-induced head twitches compared to control. However, the effect of 400 mg/kg of *HAEAR* was significantly higher than IMP (p<0.001) (Fig. 3).

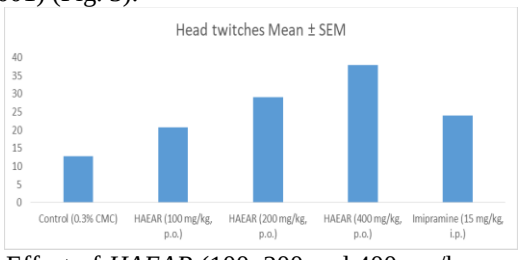

Fig 3: Effect of *HAEAR* (100, 200 and 400 mg/kg, p.o.) and Imipramine (IMP; 15 mg/kg) on 5-HTP-induced head twitches in mice. Each column represents mean ± S.E.M. of number of head twitches, n = 6. a = p<0.01, b = p<0.001, compared to control

L-DOPA induced hyperactivity and aggressive behavior in mice: The effect of *HAEAR* and lorazepam on L-DOPA-induced hyperactivity and aggressive behavior is shown in Table 4. Post-hoc analysis revealed that three doses of *HAEAR* (100, 200 and 400 mg/kg, p<0.001) significantly increased the L-DOPA-induced hyperactivity and aggressive behavior (LHA) in comparison to control group (Fig. 4).

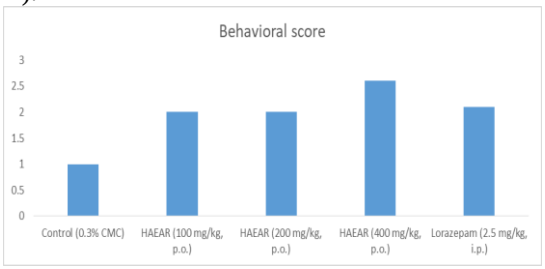

Fig 4: Effect of *HAEAR* (100, 200 and 400 mg/kg, p.o.) and Lorazepam (2.5 mg/kg) on L-DOPA-induced hyperactivity and aggressive behavior in mice. Each column represents mean ± S.E.M. of number of head twitches, n = 6. a = p<0.001, compared to control

Clonidine induced aggression in mice:

Table. 10. indicates the effect of *HAEAR* (100, 200 and 400 mg/kg, p.o.) and lorazepam (LA; 2.5 mg/kg) on the latency to first attack and the number of bouts in the clonidine induced aggressive behavior in mice. Post-hoc analysis showed that *HAEAR* (p<0.001) significantly increased the latency to first attack and decrease the no. of bouts compared to control (Fig. 5).

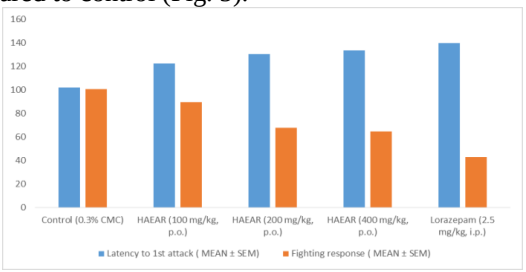

Fig 5: Effect of *HAEAR* (100, 200 and 400 mg/kg, p.o.) and Lorazepam (2.5 mg/kg) on clonidine induced aggression in mice. Each column represents mean ± S.E.M, n = 6. a = p<0.01, b = p<0.001 compared to control.

4. Conclusion

The results from the present study confirm the antidepressant activity of *aniba riparia*, since it reduced the immobility in both FST and TST. In the present study, *aniba riparia* significantly increased the frequency of 5-HTP induced head twitches, Clonidine induced aggression and L-DOPA induced hyperactivity and aggressive behavior indicating its enhanced activity on serotonergic, noradrenergic and dopaminergic pathways respectively. Our results also confirm the involvement of serotonergic, noradrenergic and dopaminergic pathways in depression. Results from behavioral experiments indicate that the antidepressant activity of *aniba riparia*, might be due to the facilitatory effect on serotonergic, noradrenergic and dopaminergic systems apart from the antioxidant activity.

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