Evaluation of Antidepressant-like Effect of Morus Mesozygia Extract in Mice: the Monoaminergic Involvement

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***Abstract*:-*Morus mesozygia* is a deciduous tree found in Nigeria and native to tropical Africa. This study was carried out to investigate the antidepressant-like effect of ethanol extract of *Morus mesozygia* (EEMM) leaves in Mice. The acute toxicity of EEMM was determined and the motor activity was assessed using the open field. The antidepressant activity of EEMM (2.5, 5, 10 mg/kg, i.p.) was investigated using forced swimming test (FST) and tail suspension test (TST). The possible mechanisms for antidepressant action were assessed with reserpine (2.5mg/kg) induced depression tests and antagonist pretreatment using parachlorophenylalanine (p-CPA 100 mg/kg, an inhibitor of serotonin synthesis), Ciproheptadine (3 mg/kg, a serotonin 5-HT2 receptor antagonist), Metergoline (4 mg/kg, a non-selective serotonin receptor antagonist), Prazosin (62.5 µg/kg, a α1 adrenoceptor antagonist), Yohimbine (1 mg/kg, α2-adrenoceptor antagonist), Propranolol (5mg/kg, a β adrenoceptor antagonist), Haloperidol (0.2mg/kg, a non-selective dopamine receptor antagonist), or Sulpiride (50 mg/kg, a dopamine D2 receptor antagonist). The results showed, the median lethal dose (LD_{50}) of *Morus mesozygia* was 2449 mg/kg, a significant dose dependent reduction in immobility time in FST and TST. It was also found that EEMM significantly antagonized hypothermia, ptosis and diarrhea induced by reserpine. The antidepressant-like activity in FST was blocked by pretreatment with p-CPA, Prazosin, Propranolol, Haloperidol and Sulpiride, whereas, pretreatment with Cyproheptadine markedly reduced the immobility time of mice in FST. In Conclusion, the results of this investigation provide evidence that confirm the significant antidepressant-like activity of EEMM in mice and it involves the monoaminergic system which is probably mediated through reuptake inhibition.**

***Keywords:* *Morus mesozygia*, Depression, Monoamine neurotransmitters, Acute toxicity, Serotonin Receptors.**

I. INTRODUCTION

In traditional Chinese medicine *Morus alba* (white mulberry) a plant member of the genus Morus native to China has been extensively used to treat brain and nervous disorders (1). *Morus mesozygia* is the only member of the genus Morus native to Tropical Africa and it is commonly referred to as African mulberry in English (2). In traditional folklore medicine the local population uses the roots, the stem and the leaves of *M. mesozygia* to treat arthritis, asthenias, debility, stomach trouble, dermatitis, rheumatism, malnutrition, veneral disease, fever and malaria, and as a pain killer (3). *Morus mesozygia* is a source of three flavonoids (artocarpesin, artochamin C and kushenol E) and four arylbenzofuran (moracin M, moracin C, moracin L and mulberofuran F) derivatives (4). Previous studies on *M. mesozygia* revealed antimicrobial activity (5), antioxidant activity (6) antiplasmodial and cytotoxicity activity (7). Generally, medicinal plants are a potential rich source of novel compounds for different diseases where therapeutic needs are still unmet. Depression for example is a life threatening illness characterized by low mood and aversion to activity and lack of disease-modifying treatments for depression represents a very significant unmet medical need and makes the discovery of novel antidepressants and alternative mechanisms a high priority (8). There is a high prevalence globally, reaching about 21% of the population and can occur at any age from childhood to late life (9). Depression alone accounts for 12.3% of the global burden of disease, impairing people's quality of life, reduce productivity and increase disability and mortality (10). A derangement in the monoamine neurotransmitters circuits within the brain accounts for the underlying pathophysiology of depression (11). Classically, Reserpine, an old antihypertensive agent that depletes monoamine stores was also found to produce depressive symptoms in a subset of patients (12). Hence, the therapeutic mechanisms for antidepressants primarily target the neural monoaminergic systems while other new alternative mechanisms are explored (13). Despite the enormous research into the pathophysiology and pharmacology of depression, the available conventional antidepressants are limited for their long therapeutic delays (at least 3-4 weeks) and low remission rates of about 30% (14, 15, 16). These have encouraged the search for more effective agents and better understanding of interplay in the mechanisms responsible for alterations in central monoamine function and antidepressant's mechanism of activity (17). Numerous antidepressant compounds are available, which presumably act via different mechanisms involving the serotonergic, noradrenergic and/or dopaminergic systems (9). The purpose of this study is to explore the antidepressant activity of *Morus mesozygia* and gain further insight into the involvement of monoaminergic neural system as an effective target for depression.

Plant Material

Fresh leaves samples of *Morus mesozygia* were collected in Ibadan at the University of Ibadan from a registered Morus mesozygia tree in the University of Ibadan botanical garden. The taxonomical identification and authentication of the plant was done at the herbarium section of the Forestry Research Institute of Nigeria (FRIN), Ibadan, Nigeria. A voucher specimen with identification number FHI:110387 was deposited and compared with the reference specimen.

Preparation of Plant Materials

The leaves were air dried for three weeks at room temperature under shade. Hundred (100) grams of the air-dried leaves were pulverized and macerated with 50% ethanol (1.75 L) for 48 hours. The extract was decanted, filtered and concentrated under reduced pressure at the University of Ibadan central laboratory. The dried extract was stored in a dessicator and subsequently reconstituted in distilled water at appropriate concentrations for the various experiments.

Laboratory Animal

Male Swiss albino mice (20-25 g) were used. All the animals were maintained in standard environmental conditions, with free access to food and water. Different groups of mice were used for each experimental task. The experimental procedures adopted in this study were in accordance with the United States National Institutes of Health Guidelines for Care and Use of Laboratory Animals in Biomedical Research (18).

Drugs and Chemicals

Reserpine (Pfizer Inc., New York, NY, USA)., Imipramine (Shanghai Zhongxi Pharmaceutical Co., Ltd. Shanghai, China). Yohimbine (an α2-adrenoceptor antagonist), pCPA (an inhibitor of serotonin synthesis), Ciproheptadine (a serotonin 5-HT2 receptor antagonist), Metergoline (4 mg/kg, a non-selective serotonin receptor antagonist), Prazosin (an α1 adrenoceptor antagonist), Propranolol (a β adrenoceptor antagonist), Haloperidol (a non-selective dopamine receptor antagonist), Sulpiride (a dopamine D2 receptor antagonist), were purchased from Sigma-Aldrich (St. Louis, MO, USA)

Acute Toxicity Test

The method described by Lorke (1983) was used to determine the LD_{50}, which is the index of acute toxicity (19). Male Swiss Albino mice (20 - 25 g) were used. This method involved an initial dose finding procedure, in which the animals were divided into three groups of three animals. Doses of 10, 100 and 1000 mg/kg were administered intraperitonealy (i.p.), one dose for each group. The treated animals were monitored for 24 hours mortality and general behavior. From the results of the above step, four different doses of (500, 1000, 2000 and 3000 mg/kg) were chosen and administered intraperitoneally respectively to four groups of one mouse per group. The treated animals were monitored for 24 hours. The LD_{50} was then calculated as the geometric mean of the highest dose showing no death and the lowest dose showing death.

Forced Swimming Test (FST)

FST is a behavioral test for the detection of antidepressants and was performed in this study as previously described (20). The mice were placed individually in plexiglas cylinders (40 cm in height, 18 cm in diameter) filled with water (25 °C) up to 15 cm. A 2-minutes pre-swimming period was followed later by a 4-minutes test period during which the total immobility time was recorded. Mice were considered immobile when they made no further attempts to escape, except for necessary movements to keep their heads above the water. The absence of hind leg movement was recorded as immobility time by stopwatch during the exposures.

The water in the cylinders was changed before every trial and mice were towel dried and returned to their housing conditions after the swimming session. Each experimental group consisted of 5 mice. All experiments were performed between 8:30 a.m and 12:30 a.m. The animals were divided into five groups. Group 1 was given distilled water (0.2 mL/20g, i.p), group 2 - 4 received EMM (2.5, 5, and 10 mg/kg, i.p) respectively and group 5 received Imipramine (25 mg/kg) as the reference drug, all treatments were administered 15 minutes before being tested in the FST.

Tail Suspension Test (TST)

TST is a commonly employed behavioral model for screening antidepressant-like activity in mice (21). For the test, the mouse was individually suspended 15 cm above the floor by the tip of the tail (approximately 1 cm) adhered to a lever. Each animal under test was both acoustically and visually isolated from other animals during test. The total testing period was 6 min. After the first 2 min following suspension of the animal by the tail; the duration of immobility was manually recorded using a stopwatch during the next 4 min of the test. Animal was considered to be immobile when it did not show any body movement, hung passively and completely motionless. The test was conducted in a quiet room to avoid disturbances to animals and all experiments were performed between 8:30 am-12.30 pm daily to avoid changes in biological rhythm. Each experimental group consisted of 5mice. The animals were divided into five groups, Group 1 received distilled water (0.2 mL/20 g, i.p), Group 2 - 4 received EMM (2.5, 5, and 10 mg/kg, i.p) respectively and group 5 received Imipramine (25 mg/kg), all treatments were administered 15 minutes before being tested in the TST.

Locomotor Activity in the Open Field

In order to rule out any unspecific locomotor effect of EEMM on its antidepressant effect, motor activity was measured in an open field apparatus consisting a white plexiglas box (28 cm × 28 cm × 25 cm) with a painted black grid dividing the floor into 16 (7 × 7 cm) equal squares. The animals were divided into five groups (n = 5). Group 1 was given the vehicle (0.2 mL/20 g distilled water), while group 2 - 4 were given EEMM (2.5, 5, and 10 mg/kg, i.p) respectively and group 5 received Imipramine (25 mg/kg). Thirty minutes after a single i.p.

injection of extract or standard drug, the animals were placed singly in the center of the box; the number of squares crossed with all four paws was counted for 5 min. The cage was cleaned with 70% ethanol at 5 minute interval when the animal is removed (22).

Reserpine Antagonism in Mice

Hypothermia, ptosis, and diarrhea were induced in reserpine-treated experimental animals and this was used in this study as previously described (23). The mice were administered reserpine (2.5 mg/kg, i.p.) 10 min after treatment. The treatment was performed in five groups of male mice (n=5). Group 1 was given distilled water (0.2 ml/20 g, i.p.), while groups (2-4) were given different doses of EEMM (2.5, 5, and 10 mg/kg, i.p) respectively and group (5) received Imipramine (25 mg/kg, i.p.) as reference drug. Three parameters; the degree of palpebral ptosis, rectal temperature and diarrhea were recorded at 1 h, 2 h, 3h and 4 h, respectively, after the administration of reserpine. The average results over the time intervals was calculated and presented. The degree of palpebral ptosis was evaluated according to the following rating scale: 0, eyes open; 1, eyes one-quarter closed; 2, eyes half closed; 3, eyes three-quarters closed and 4, eyes completely closed. The recording of the body temperature was carried out using a thermoprobe. The probe of the thermometer was inserted 1.5 cm into the rectum. The pre-drug recording served as the reference point for the determination of temperature changes (24).

EEMM Antidepressant-like Mechanistic Studies

In another set of experiment conducted, probe for the possible mechanisms of EEMM antidepressant-like activity was done by pre-treating animals (intraperitoneally) with neurotransmitter blockers 15 minutes prior extract administration and then subjected to FST (25). The following transmitter receptor blockers were used: p-CPA (100 mg/kg, serotonin synthesis inhibitor), Ciproheptadine (3 mg/kg, 5-HT2 receptor antagonist), Metergoline (4mg/kg, non-selective serotonine receptor antagonist), Prazosin (62.5 µg/kg, α1-adrenoceptor antagonist), Yohimbine (1 mg/kg, α2-adrenoceptor antagonist), Propranolol (5 mg/kg, β-adrenoceptor antagonist), Haloperidol (0.2mg/kg, dopamine receptor antagonist), Sulpiride (50 mg/kg, i.p., dopamine D2 receptor antagonist) and EEMM (10 mg/kg) was administered 15 mins later following each pretreatment. Each test group consists of 5 male mice, and alteration of EEMM effect on immobility time in FST was recorded using a stopwatch as described previously.

Data Analysis

All data were presented as Mean ± SEM. The results were analyzed by One way Analysis of Variance (ANOVA) and post hoc tests (Student's-Newman-Keuls) was carried out to determine the source of significant main effect using GraphPad Prism Biostatistics software version 5.00.

The level of significance for all tests was set at $p < 0.05$ (i.e. at 95% confidence interval).

II. RESULTS

Acute Toxicity

The ethanol extracts of *Morus mesozygia* leaves did not produced any sign of toxicity till the intra-peritoneal dose of 3000 mg/kg. The median lethal dose (LD_{50}) of EEMM in mice was found to be 2449 mg/kg i.p. body weight.

Effect of EEMM on FST and TST in Mice

EMM (2.5-10 mg/kg i.p) and Imipramine (25mg/kg i.p) significantly decreased the immobility period in FST [F (4, 20)= 314.2, p<0.05, Fig. 1]] and TST [F (4,20)= 172.0, p<0.05, Fig. 2].

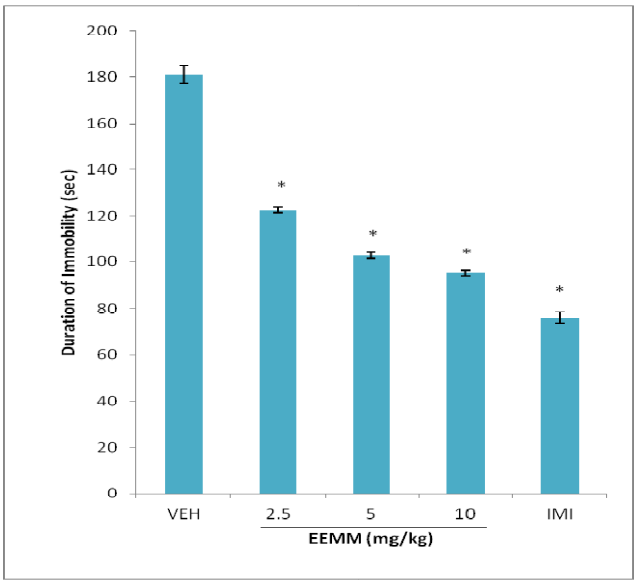

Fig. 1: Effect of EEMM on immobility periods of mice in FST (*P < 0.05)

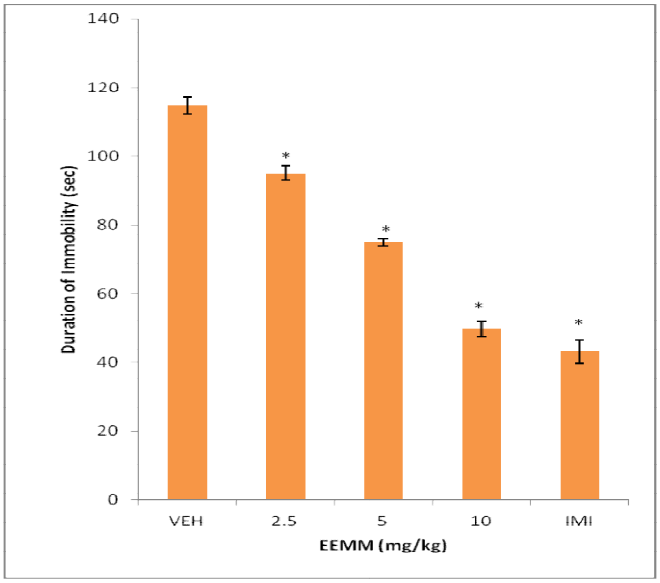

Fig. 2: Effect of EEMM on immobility periods of mice in TST (*P < 0.05)

Effect of EEMM on Locomotor Activity in Open Field

The administration of EEMM (2.5, 5 or 10 mg/kg, i.p.) showed a significant reduction [F (5, 24) = 137.7, P < 0.05] in the locomotor activity of mice when compared to vehicle (Table 1).

Table 1: Effect of EEMM on locomotor activity in open field

Locomotor Activity Test		
Treatment (mg/kg)		**Number of Square Crossing**
VEH		**89.60±1.631**
EEMM	**2.5**	**57.60±1.568***
	5	**51.60±2.657***
	10	**46.60±1.661***
IMI	**25**	**81.60±1.625**

Values represent mean ± S.E.M for 5 animals per group. *P < 0.05 compared to Control (ANOVA followed by Newman Keuls test).

VEH: Vehicle; **EEMM**: Ethanol Extract of *Morus mesozygia;* **IMI**: Imipramine

Effect of EEMM on Reserpine Antagonism in Mice

As shown in Table 2, the vehicle treated animals administered reserpine (2.5 mg/kg i.p) showed marked alterations of ptosis, hypothermia, and diarrhea. However, administration of EEMM at 2.5–10 mg/kg significantly antagonized these symptoms induced by reserpine. At the same time, Imipramine at 25 mg/kg also antagonized all these effects.

Table 2: Effect of EEMM on Reserpine-induced hypothermia, ptosis and diarrhea in mice

Treatment	**Rectal temperature**	**Scores of Ptosis**	**Diarrhea**
Vehicle	**32.40±0.21**	**3.67±0.33**	**6.67±0.88**
EEMM (2.5mg/kg)	**34.47±0.15***	**2.00±0.0***	**3.00±0.58***
EEMM (5mg/kg)	**34.80±0.16***	**1.67±0.33***	**1.67±0.88***
EEMM (10mg/kg)	**35.20±0.06***	**1.00±0.0***	**1.67±0.33***
Imipramine (10mg/kg)	**35.27±0.24***	**0.67±0.33***	**1.33±0.33***

*P < 0.05 as compared to vehicle control

VEH: Vehicle; **EEMM**: Ethanol Extract of *Morus mesozygia;* **IMI**: Imipramine

Effect of Antagonists Pre-treatment on EEMM Activity in FST in Mice

While pretreatment with Metergoline had no effect, Cyproheptadine further potentiate the effects of EEMM on immobility period in FST, but other pretreatment antagonized the effect of EEMM (10 mg/kg) [p<0.01(Table 3)], suggesting their neurotransmitter system involvement in EEMM antidepressant-like action.

Table 3: Pretreatment with Antagonist

Treatment	**Immobility time (secs)**
VEH	**181.0± 3.8**
EEMM (10 mg/kg)	**95.4± 1.2**
SUL (50 mg/kg) + **EEMM** (10 mg/kg)	**173.2± 9.3****
HAL (0.2mg/kg) + **EEMM** (10 mg/kg)	**175.8± 5.4****
CIP (3 mg/kg) + **EEMM** (10 mg/kg)	**16.0± 7.2***
MET (4 mg/kg) + **EEMM** (10 mg/kg)	**63.2± 8.1***
pCPA (100 mg/kg) + **EEMM** (10 mg/kg)	**175.0± 1.0****
PRZ (62.5 µg/kg) + **EEMM** (10 mg/kg)	**170.4± 5.8****
PNL (5mg/kg) + **EEMM** (10 mg/kg)	**137.2± 5.3****

**P < 0.05 (reversal) significance between EEMM + Antagonists and EEMM alone , *P < 0.05 (further depression) as compared to EEMM alone treated mice.

VEH: Vehicle; **EEMM**: Ethanol Extract of *Morus mesozygia;* **SUL**: Sulpiride; **HAL**: Haloperidol; **CIP**: Ciproheptadine; **MET**: Metergoline; **pCPA**; **PRZ**: Prazosin; **PNL**: Propranolol

III. DISCUSSION

We must not assume that since an herb has been used for thousands of years that the herb is necessarily safe and truly effective for its claimed indication(s), thus, toxicity studies for medicinal plants are now a growing concern. From the method described by Lorke (1983), the acute lethal dose (LD_{50}) of the ethanol extract of *M. mesozygia* (intraperitoneal route) was calculated to be 2445 mg/kg and the value obtained is not toxic to the animal. Aderibigbe*et al.* (2010) described that from this model of acute toxicity study, the higher the value of the LD_{50} for a substance, the relatively safe the substance is assumed to be (26). Therefore, *Morus mesozygia* is considerably safe because of its high LD_{50} value. The different doses used for this present study were chosen using geometric mean based on the high LD_{50}.

In the present study, EMMM was evaluated for antidepressant activity using acute models. The observed results provided evidences for the antidepressant-like activities of EEMM. The FST and TST are commonly used acute behavioral despair models in rodents to predict antidepressant potential by measuring the decrease in immobility periods (27). The immobility displayed by rodents when subjected to an unavoidable and inescapable stress has been hypothesized to reflect behavioral despair which in turn may reflect depressive disorders in humans. It has been suggested that mice forced to swim in a restricted space from which they cannot escape are induced to a characteristic behavior of immobility (20). This behavior reflects a state of despair which can be reduced by several agents which are therapeutically effective in human depression. The “tail suspension test” was described as a facile means of evaluating potential antidepressants (21). Clinically effective antidepressants reduce the immobility that

mice display after active and unsuccessful attempts to escape when suspended by the tail. In both FST and TST, treatment with EEMM significantly reduced the duration of immobility in animals in dose dependent manner, suggesting EEMM to have antidepressant properties. It is interesting to note that in this study; smaller doses were effective, what can indicate a high potency of this extract. The decrease in immobility period in FST and TST produced by the extract resembles the effect obtained with leaves extract of plants such as *Cassia Occidentalis*, *Hibiscus Tiliaceus,* and *Citrus Limon*, (28, 29, 30).

In the acute model tests, EEMM did not increase the locomotor activity of the mice as compared to their respective controls, so this indicated that EEMM did not show CNS stimulant activity, and the antidepressant-like activity of EEMM is specific and not false positive. Measure of movements of mice in activity cage ("open field") has been used by many authors to establish neurobehavioral properties of test compounds and the same was valid for EEMM (31, 32, 33).

To elucidate the possible mechanisms underlying the antidepressant-like activity of EEMM, the reserpine reversal test was conducted. As reserpine (2.5 mg/kg i.p) can irreversibly block the vesicular monoamine transporter and inhibit the vesicular uptake of monoamines, such as 5-HT, NE, and DA, the depletion of monoamine stores may stimulate the reuptake of monoamines and produce ptosis, hypothermia, and diarrhea (23). Chronic oral administration of EEMM (2.5–10 mg/kg) significantly antagonized the clinical observations induced by reserpine, which indicates that EEMM can inhibit the reuptake of the neurotransmitter and thereby increase the amount of monoamines in synaptic clefts.

Involvement of the monoaminergic neurotransmitter system in the antidepressant activity of EEMM was evaluated by pretreatment with antagonists. The effects of EEMM on immobility period in FST were reversed by pretreatment with Haloperidol, Sulpiride, Propranolol, Atropine, Prazosin, implicating the involvement of dopaminergic and adrenergic system in EEMM antidepressant activity. Pre-treatment of animals with pCPA significantly inhibits EEMM effect, suggesting the involvement of serotonergic system in modulation of EEMM antidepressant activity, however, pre-treatment with Metergoline, a serotonin receptors 1, 2, 5, 7 antagonist and Ciproheptadine, serotonin receptor 2A antagonist did not reverse the effects of EEMM. It was inferred that involvement of the serotonergic system may be mediated via serotonin receptor 4 which has been identified as mediator of rapid antidepressant activities (34), thus, EEMM may have early onset of antidepressant activity compared to conventional antidepressants.

Through pharmacological characterization, antidepressants that are Selective reuptake inhibitors have been seen to have both primary pharmacological target(s), i.e. NE and/or 5-HT transporters (NET and SERT, respectively) and secondary receptor or transporter targets, but having more selective affinity for the primary target. For example, among the SSRIs paroxetine shows affinity for the NET and the muscarinic cholinergic receptor (35). Therefore, the activity of EEMM as a reuptake inhibitor may be primary and secondary at the receptor targets.

A key observation in this study was the significant potentiation of EEMM activity on FST by pre-treatment with Cyproheptadine. Involvement of serotonin receptor 5-HT2A in the pathophysiology of depression was indicated by the increased densities of cortical 5-HT2A receptors observed in postmortem examination of depressed patients (36) and this receptor site has been suggested as a viable target for antidepressant (37). Frequent therapeutic failure of antidepressant agent led to introduction of augmentation therapy years ago, which is defined as the addition of a second agent to an existing antidepressant to achieve improved clinical response. Commonly used strategies are augmentation of TCA drugs with Li^{+}, or SSRIs with pindolol. Therefore, from the observation in this study, 5HT2A antagonists should be investigated for their potentiating effects in antidepressant therapy, particularly in combination with reuptake inhibitors.

IV. CONCLUSION

The antidepressant activity of EEMM as demonstrated both in FST and TST showed that EEMM has a valid antidepressant property, and its antidepressant activity is mediated by the alteration of dopaminergic, adrenergic and serotonergic system, which is partially through receptor interactions and through inhibition of reuptake in the synaptic clefts as the primary pharmacological target.

However, further studies are needed to isolate the active principles responsible for the observed activity.

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